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REDUCTION IN FREE FATTY ACIDS IN CRUDE PALM OIL BY ENZYMATIC REMEDIATION

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ABSTRACT

Fatty acids present in crude palm oil are normally removed in processing and result in a yield loss. This investigation studied if they could be recovered through an enzymatic condensation reaction. Free fatty acids (FFA) present in crude palm oil at a level of 4.8%-7.2% could be converted back to triacyl glycerols utilising a microbial esterase to perform a condensation reaction with mono and diglycerides present in the oil. The rate of removal of fatty acids could be increased by the addition of glycerol to provide more sites for attachment. However, the addition of glycerol resulted in an increase in the mono and diglyceride content of the oil as the fatty acid preferentially attached to this molecule. In a glycerol free system, the reduction in fatty acids was achieved when an immobilised form of the enzyme was used. This enzyme did not cause significant interesterification of the palm oil, an advantage for later fractionation. An addition of a small amount of palmitic acid (3% w/w), to the reaction gave a lower residual level of mono and diglycerides compared to a reaction with only the intrinsic level of FFA.

Keywords: lipase, fatty acids, palm oil.

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INTRODUCTION

Crude palm oil normally contains free fatty acids which need to be removed as part of the refining process. Levels can vary according to harvesting practices, including the length of time that passes between collection of the fruit bunches, their handling and their processing at the extraction and refining locations. Different mechanisms for the generation of these fatty acids have been described by Bock (2012) and include lipase activity coming from microorganisms that colonise the fruit bunches and more importantly, cellular lipase activity coming from damaged fruits.

Several lipase producing microorganisms have been isolated from palm fruit bunches and have been shown to be capable of producing enzymes which can hydrolyse palm oil, Hiol *et al*. (1999). Fruit damage creates a double problem because not only will endogenous lipase become activated but also the protective covering (exocarp) loses its integrity and no longer protects the oil containing mesocarp from microbial colonisation.

Removal of these fatty acids results in a loss of yield in the subsequent deacidification and deodorisation processes in the physical refining of the oil. Alternatively, if sodium hydroxide neutralisation is used, then the generated soaps will also entrain oil, producing a greater loss of oil. However, if the fatty acids can be re-combined with the mono and diglycerides left in the oil as a result of the lipolysis, then, the possibility exists to reverse the yield loss and recover the oil.

Bhattacharya and Bhattacharya (1989) evaluated *Rihizomucour miehei* lipase for the biorefining of high acid rice bran oil and observed that reduced pressure and glycerol addition improved the reduction of free fatty acids (FFA). De (2006) reviewed the different chemical, physical and enzymatic processes that could be used to deacidify rice bran oils and concluded that in the non-enzymatic processes, loss of yield was inevitable. The study also compared the use of a lipase with the autocatalytic reaction between FFA and monoglycerides and concluded that the enzymatic

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reaction was more efficient and could be carried out at a lower temperature. However, as the enzymes used were predominantly 1.3 specific the regenerated material would be found in the oil in the form of diglycerides, which may not be optimum. The presence of partial glycerides and chlorine containing compounds has been associated by some authors with increased levels of glycidyl esters and 3-MCPD (Matthaüs *et al*., 2011) therefore, the reduction in these components could be beneficial. However, as there are some disputes about this linkage, this is not taken into account in any evaluation of the overall benefit of this process.

MATERIALS AND METHODS

Samples of crude palm oil were obtained from palm oil mills in Malaysia and were described as of standard quality by the suppliers. In order to avoid changes in the FFA level upon storage, fresh samples were obtained for the individual experiments. Palmitic acid and glycerol were of analytical grade and were obtained from a local laboratory chemical supplier. Liquid and immobilised esterase from *Candida antarctica* B, were Novozymes standard food grade products with the commercial names of Lipozyme Calb L and Lipozyme 435, respectively. The 1.3 specific lipase from *Rhizomucor miehei*, was the Novozymes commercial product, *Palatase* 20 000 litres.

For the reduction of the FFA content of crude palm oil, 100 ml aliquots were heated to 70°C and mixed with differing amounts of lipase in the liquid or immobilised form and varying amounts of glycerol. The oil samples were then transferred into conical flasks and high shear mixing (24 000 rpm) was applied to each oil sample. In the case where immobilised *C. antartica* B enzyme was used, this was added after the high shear mixing to avoid damage to the enzyme particles. The flasks were incubated at 70°C, under vacuum and agitation applied via a magnetic stirrer operating at 350 rpm.

Samples were removed at intervals and heated at 85°C for 30 min to inactivate the added enzyme before centrifugation at 3000 g for 15 min to separate the phases. The crude and treated oils were analysed for FFA by titration and for mono, di and triglycerides by gas chromatography.

Fatty acids were determined by titration, following the procedure described in the AOCS Official Method F 9a-44 and the results presented are the mean of two determinations. Gas chromatography (GC) was performed using an Agilent 7890A, fitted with a Quadrex 007-65HT, (65%Phenyl) methylpolysiloxane column and a flame ionisation detector. Results are expressed as area percentages of the samples as analysed.

RESULTS AND DISCUSSION

A preliminary experiment was carried out to examine the influence of enzyme dosage and type on FFA removal. The reduction in FFA was more complete when the non-specific lipase was used and the speed of reduction was related to the enzyme dosage (*Table 1*).

The non-specific lipase reduced the FFA in the oil more quickly due to the ability of this enzyme to attach a fatty acid at any position on the glycerol backbone. The 1.3 specific lipase could not attach the fatty acid at the Sn-2 position and this resulted in a slower and incomplete re-attachment.

The influence of glycerol addition together with the liquid formulated esterase on FFA reduction and level of partial glycerides and triglyceride content is shown in *Tables 2* and *3* respectively. Partial glycerides are expressed as the sum of monoglycerides (MG) and diglycerides (DG). The observation that the addition of glycerol increases the rate of FFA removal is in agreement with the results of Bhattacharyra and Bhattacharya (1989).

When no additional glycerol was added, reduction in FFA was slower than with 1% or 10% addition, indicating that glycerol was a better acceptor of fatty acids than the partial glycerides. As a result of this reaction, levels of partial glycerides increased when additional glycerol was included in the reaction and this was related to the addition rate.

GC analysis of the triglyceride content of the oils indicated that little interesterification of the oil had occurred during the condensation of the fatty acids. In order to try and limit the increase in partial glycerides, the condensation reaction was carried out using the immobilised form of the *C. antartica* lipase. This version is completely free from glycerol and as such the condensation reaction should be only between the FFA and the entrained partial glycerides. Different dosages of the immobilised enzyme were used and FFA, partial glyceride

TABLE 1. REDUCTION OF % FREE FATTY ACIDS (FFA) IN CRUDE PALM OIL FOLLOWING LIPASE ADDITION AND 2% W/W GLYCEROL

Time (hr)	0.1% *C. antarctica* lipase	1% *C. antarctica* lipase	1.0% *R. meihei* lipase
0	4.98	4.98	4.98
1	3.86	1.29	4.13
2	3.79	0.98	3.57
4	3.36	0.69	3.17
6	3.10	0.83	2.61
8	2.37	0.46	NM
10	2.21	NM	2.38
14	1.73	0.29	NM

TABLE 2. CHANGES IN FREE FATTY ACIDS (FFA) AND PARTIAL GLYCERIDES WITH DIFFERENT GLYCEROL ADDITION RATES

Treatment		Reaction time (hr)		
		0	3	7
2% *C. antarctica* lipase	% FFA	4.84	3.96	3.77
	% MG + DG	8.80	9.00	8.60
2% *C. antarctica* lipase + 1% glycerol	% FFA	4.84	2.82	2.23
	% MG + DG	8.80	10.0	10.9
2% C. antarctica lipase + 10% glycerol	% FFA	4.84	0.33	NM
	% MG + DG	8.80	13.8	NM

Note: MG – monoglycerides.
DG – diglycerides.
NM – not measured.

TABLE 3. CHANGES IN MARKER TRIGLYCERIDES FOLLOWING LIPASE REACTIONS

Treatment	Triglyceride marker		
	PPP	POP	POO
Crude palm oil	7.13	29.09	18.72
2% *C. antartica* lipase	6.85	29.60	18.58
2% *C. antartica* lipase + 1% glycerol	7.02	28.81	18.65
2% *C. antartica* lipase + 10% glycerol	7.25	30.26	18.29

Note: P – palmitic acid.
O – oleic acid.

TABLE 4. INFLUENCE OF LIPASE DOSAGE ON FFA AND PARTIAL GLYCERIDES LEVEL IN CRUDE PALM OIL

Treatment	% FFA	% MG + DG
Crude palm oil	5.01	5.97
CPO + 0.5% lipase	4.33	5.01
CPO + 1.0% lipase	3.84	4.35
CPO + 2.0% lipase	3.43	3.66
CPO + 4.0% lipase	3.17	3.26
R^2 (enzyme dosage)	0.79	0.81

Note: CPO – crude palm oil. FFA – free fatty acid.
MG – monoglycerides. DG – diglycerides.

and triglyceride contents determined after 7 hr of reaction time. A reaction time of 7 hr with no intermediate sampling was chosen as previous experiments, not reported here, had shown that opening the reaction flask for sampling allowed for moisture uptake by the oils and hydrolysis reactions to re-start. The results of the FFA and partial glyceride levels in CPO, following incubation for 7 hr at 70°C, are shown in *Table 4*.

In this case increasing the enzyme dosage resulted in the simultaneous reduction of FFA and partial glycerides, indicating that the condensation reaction was taking place between these two components. The reduction in FFA and partial glycerides was correlated with the enzyme dosage over the tested range.

Analysis of the triglyceride content of the crude palm oil and the treated oils was made to determine if interesterification had taken place during the condensation reaction and the results for the marker components are shown in *Table 5*. These results indicate that some interesterification had taken place which was related to the dosage of enzyme used. However, this was not to the extent that would have been observed using an enzyme such as the *Thermomyces lanuginosus* lipase which will produce a randomisation of the palm oil. The influence of this apparent limited randomisation on the resulting properties of the oil has yet to be established. As the triglyceride analysis is of the overall areas percentage it is not possible to state whether this change in composition is totally due to interesterification or to a change in composition following the re-synthesis of some triglycerides from the partial glycerides found in the oil.

TABLE 5. INFLUENCE OF ENZYME DOSAGE ON TRIGLYCERIDE COMPOSITION OF CRUDE PALM OIL

Treatment	% PPP	%POP	% POO
Crude palm oil	7.22	30.89	19.78
CPO + 0.5% lipase	8.19	30.41	19.61
CPO + 1.0% lipase	8.10	29.78	19.72
CPO + 2.0% lipase	9.33	29.89	20.02
CPO + 4.0% lipase	9.78	28.81	20.09
R^2 (enzyme dosage)	0.87	0.90	0.47

Note: P – palmitic acid. O – oleic acid. CPO – crude palm oil.

Both lipases were able to reduce the FFA content of crude palm oil and therefore decrease losses in refining. When glycerol was added to the reaction, part of the FFA was attached to the glycerol, resulting in an increase in partial glycerides. The rate of reaction between fatty acids and glycerol was faster than that between fatty acids and diglycerides and therefore in the presence of glycerol

there would be both a synthesis of diglycerides as well as triglycerides. This increase in the level of diglycerides in the presence of glycerol may be a disadvantage if the palm oil is to be used directly for food purposes. However, if the oil is to be used for biodiesel production, these partial glycerides can be readily converted by a chemical or enzymatic methylation catalyst and an extra yield obtained. Enzymatic remediation using liquid lipase plus glycerol could therefore be applied for the treatment of oils which are to be used for biodiesel or the production of oleochemicals.

When glycerol addition is not carried out, the reaction rate is somewhat slower but no increase in partial glycerides occurs. Interesterification of the oils is limited suggesting that the resulting oil can be fractionated and used for food purposes.

In order to maximise the reduction in partial glycerides, one condensation reaction was carried to compare the conversion in the presence of an additional 3% of palmitic acid. By providing more fatty acid, the reaction equilibrium between the partial glycerides and the triglycerides should be changed and the level of diglycerides reduced. When an additional 3.0% of palmitic acid was added to the reaction, partial glycerides decreased from 6.08% in the crude oil to 2.32% compared to 3.66 % in the crude oil treated only with lipase (*Table 6*). By providing an excess of fatty acids the reaction kinetics were altered towards greater condensation with the mono and diglycerides present in the oil.

As the analysis method only reveals percentage area composition, it is not possible to conclude whether these changes are a result of an overall increase in triglycerides, or if limited interesterification has taken place or both. Compared to esterase alone, esterase plus palmitic acid results in a relative increase in palmitic containing triglycerides and a decrease in stearic. This would be expected as palmitic acid is incorporated into the partial glycerides, raising the relative amount of these components. However, as observed in *Table 5*, addition of 4% w/w esterase alone, results in some interesterification and a reduction in the level of POP. But when additional palmitic acid is present, this reduction is largely eliminated.

Three enzyme reactions can therefore be taking place at this time; condensation, resulting in the reduction in partial glycerides and FFA; interesterification and possibly acidolysis; in which palmitic acid replaces some of the stearic acid in the triglycerides. Analysis of the FFA composition of the remaining material should be carried out in a further experiment to determine if acidolysis has occurred. For practical application of this technique the use of palm fatty acid distillate (PFAD) would supply a more balanced source of the required fatty acids, reflecting more accurately the composition of palm oil. The level of FFA and partial glycerides can be reduced by increasing the reaction time or increasing the enzyme dosage.

The efficiency of water removal is also an important factor and as the condensation reaction continues, the equilibrium is more and more in the direction of hydrolysis. If water removal is not efficient, the FFA will not be able to re-combine with the partial glycerides and form triglycerides. On the laboratory scale, water removal efficiency is affected by the volume contained within the reaction vessel. Increasing the amount of reactants decreases the surface area to volume and can be expected to reduce the efficiency of water removal. This was examined by comparing a condensation reaction with 100, 280 or 500 g of crude palm oil and 4% w/w immobilised esterase. Following condensation with 4% w/w esterase the FFA level in the palm oil had fallen from 4.98% to 4.06%, 3.43% and 2.41% respectively and the sum of mono and diglycerides to 5.2%, 3.7% and 2.8% respectively from a starting level of 6.1%. Thus, water removal efficiency is also a key parameter in promoting the condensation reaction and needs optimisation.

Analysis of the triglyceride composition of these different oils after condensation is shown in *Table 7*. While overall FFA and partial glyceride content was influenced by the surface area to volume ratio, it was

TABLE 6. INFLUENCE OF ADDED FFA ON CONDENSATION REACTION

	Crude palm oil	Treated with lipase	Treated with lipase and palmitic acid
Starting FFA level (titration)	4.98	4.98	7.80
FFA after reaction (titration)	3.43	3.43	5.59
% MAG + DAG	6.08	3.66	2.32
% PPP	7.23	9.72	9.85
% POP	29.18	25.48	28.03
% POO	20.12	19.31	19.41
% POS	4.51	4.25	3.81
% SOS	0.54	0.64	0.37
% SOO	1.76	1.67	1.57

Note: FFA – free fatty acid. DAG – diacylglycerol. S – stearic acid. MAG – monoacylglycerol. P – palmitic acid. O – oleic acid.

TABLE 7. INFLUENCE OF WATER REMOVAL ON FFA REDUCTION

	CPO	Oil amount used (g)		
Oil/ g	-	100	280	500
%FFA (by titration method)	4.98	2.41	3.43	4.06
%MG + %DG (by GC analysis)	6.08	2.76	3.66	5.23
% PPP	7.23	10.38	9.72	10.80
% POP	29.18	25.35	25.48	23.96
% POS	4.51	4.20	4.25	3.63
% POO	20.12	19.64	19.31	17.97
% SOS	0.54	0.77	0.64	0.47

Note: FFA – free fatty acid. DG – diglycerides. GC – gas chromatography. MG – monoglycerides. P – palmitic acid. O – oleic acid. S – stearic acid.

not possible to see a significant connection between triglyceride content and oil amount. However, for the lowest surface area to volume ratio sample the degree of interesterification showed a tendency to be greater. As this would be the sample with the lowest degree of re-incorporation of FFA, these results tend to support the hypothesis that both condensation and interesterification can occur, and the relative importance of these vary according to the conditions of the reaction.

CONCLUSION AND RECOMMENDATIONS

Immobilised and soluble preparations of *C. antartica* lipase have been applied to the condensation of fatty acids with partial glycerides and / or glycerol. In both cases, the FFA content of the oil can be recovered and losses considerably reduced. Interesterification of the palm oil is effectively zero with the liquid preparation and limited compared to *T. lanuginosus* lipase with the immobilised version of the esterase. *C. antartica* is known to have a lower activity rate in carrying out interesterification than the lipase from *T. lanuginosus* (Cowan and Holm, 2008).

Enzymatic remediation could be a promising tool for the improvement of processing yields in palm or other oils where fatty acids are present. Further studies are required to optimise the FFA reduction while at the same time minimising interesterification or at least determining its effect on fractionation of the palm oil. Alternative enzyme formulations should also be considered if the reaction between glycerol and fatty acids should be avoided.

In determining the economy of the reaction, a number of factors need to be taken into account, including the cost of the enzyme and the operation of the process. In addition, there is the differential value between refined palm oil and PFAD. Re-using the enzyme is one means by which the cost of conversion can be reduced. An immobilised enzyme may be separated from a reaction by filtration and recovered or used in a packed bed reactor. In either case, re-use of the enzyme is possible, reducing the overall enzyme cost in the process.

Removing FFA from oils destined for biodiesel production, while at the same time rendering them capable of being converted to methyl esters will simplify and improve the biodiesel process. The avoidance of loss here will also improve the overall economics and sustainability of this biofuel production.

As partial glycerides have been associated with the formation of 3-MCPD and glycidyl esters, their removal before deodorisation could help to reduce the formation of these unwanted compounds.

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