TRANSESTERIFICATION OF PALM OIL BY AN IMMOBILIZED Rhizomucor miehei LIPASE

[1]A.Z. Hazlina and [2]H.M. Ghazali
[1]Department of Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Mengabang Telipot, 21030 K. Terengganu, Terengganu
[2]Department of Biotechnology, Faculty of Food Science and Biotechnology
Universiti Putra Malaysia, 43400 Serdang, Selangor

Abstract: Enzymatic transesterification of RBD palm oil was studied. Transesterification was done by reacting RBD palm oil with an immobilized R. miehei lipase in water-saturated hexane at various reaction temperatures. Analyses were done on the transesterified oils triglyceride composition and its melting characteristics using HPLC and DSC, respectively. Transesterification increased the concentration of MMM, OOL, MMP, OOO and PPS at reaction temperatures of 50°C and 60°C. At 30°C and 40°C, MMM, OOL, MMP was observed to increase. The degree of transesterification was between 18% and 46%, which coincided with the degree of hydrolysis between 1.9% and 2.5%. Percentage of triglycerides retained after transesterification was between 83% and 85%. Lowest transesterification rate was noted when palm oil was transesterified at 30°C. Generally, the heating thermogram of palm oil transesterified at different reaction temperatures showed three different regions compared to the non-transesterified palm oil. One new peak was found to be sandwiched between the low-T and high-T peaks. However, this peak was not observed in palm oil transesterified at 60°C. One peak at the high-T region was also observed for heating thermogram of oil transesterified at 60°C, compared to two peaks formed for the other reaction temperatures studied. The transesterified oils started to melt at between -25.74°C and -24.53°C and totally melted at between 43°C and 45.5°C. The non-transesterified oil started to melt at -24.65°C and totally melted at 43.2°C.

Keywords: RBD palm oil, Lipozyme IM60, Transesterification, HPLC, DSC

INTRODUCTION

The development of methods to improve the nutritional and functional properties of fats and oils is of great interest to food processors. One of such method is by transesterification. Transesterification is the exchange of acyl groups between two esters. It has an ability to alter the chemical composition and the physical characteristics of fats and oils. In transesterification, enzymes are more favoured than chemical catalysts. Apart from able to react under mild conditions, they also show specificity, which can be exploited to produce 'tailor-made' fats and oils. One type of enzyme that has this property is the 1,3-specific lipase. For instance, Rhizomucor miehei lipase, which is 1,3-specific, has been applied in many studies including in the synthesis of structured lipids [4, 10]; production of cocoa-butter equivalents [8, 16] and in the production of biodiesel fuel from vegetable oils [12-13].

Palm oil is semi-solid at ambient temperature of 30°C due to presence of a mixture of triglycerides with different melting points and solubilities [9]. It also contains an equal amount of saturated and unsaturated fatty acids. This unique composition contributes to the unique physical properties of palm oil, which are usually exploited in many food applications. The composition of fatty acids attached to a triglyceride molecule also determines the thermal behaviour of the triglyceride. The more saturated the fatty acids attached, the faster the triglyceride crystallises [3]. Tan and Che Man [14] had compared the composition of palm oil with its thermal properties using differential scanning calorimetric analysis.

In this paper, we report on the effect of lipase-catalysed transesterification on the chemical composition as well as the melting characteristic of palm oil. Products formed after transesterification were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC), differential scanning calorimeter (DSC) and slip melting point (SMP).

MATERIALS AND METHODS

Materials. Refined, bleached and deodorized (RBD) palm oil was purchased from Kempas Edible oil (M) Sdn. Bhd., Johor. Rhizomucor miehei lipase (Lipozyme IM60) obtained in its immobilized form and used as such was purchased from Novozymes Industry (Copenhagen, Denmark). All other chemicals were either of analytical or high performance liquid chromatography (HPLC) grades.

Transesterification reaction: The reaction mixture was composed of 60% (w/v) RBD palm oil in a water-saturated hexane. Prior to use, palm oil was liquefied in the oven at 40°C. The reaction was started when 1% (w/v) Lipozyme IM60 was added to the reaction mixture. The reaction mixture was then incubated at various reaction temperatures (i.e. 30°-60°C) and agitated in an orbital shaker at 200 rpm for 10 hours. After 10 hours, the reaction was terminated by adding acetone:ethanol (1:1) mixture and Lipozyme was removed by ordinary filtration. Free fatty acids (FFA) formed in the reaction mixture were then neutralised by titration with 0.1M NaOH. Controls were prepared and reacted similarly as described above. However, no Lipozyme was added. All experiments were done in duplicate.

HPLC analysis: The triglyceride of transesterified oil samples and controls were separated on a single commercially packed (250mm X 4.0mm) RP-18 column (Lichrosphere) with a particle size 5 μm (Merck, Darmstadt, Germany). The mobile phase used was a mixture of acetone and acetonitrile (60:40) and the flow rate was set at 1 mL/min. The running time was 50 min. The injection volume used was 10 μL. The triglycerides were identified according to Haryati et al. [7]. Peak areas produced by the data integrator were used to quantify the components based on relative percentages. The degree of transesterification was calculated according to Ghazali et al. [5].

GC analysis: The melting characteristics of transesterified samples and their controls were analysed using a differential scanning calorimeter (DSC 7 Perkin-Elmer Norwalk, CT). It was calibrated with indium and n-decane. Samples from which the FFA and solvents had been prior removed, were weighed into aluminium pans (range from ca. 5 to 10 mg) and lids were crimped into place. The sample and reference (empty) pans were placed in the calorimeter at room temperature, while the cell block of the DSC was cooling to -55°C and flushed with nitrogen. Samples from transesterified oils and their controls were subjected to the following heating temperature program: -50°C isotherm for 5 min and heating from -50°C to 80°C at 5°C/min. The melting points expressed as X_1 (start melting), X_2 (totally melted) and peak temperatures were recorded. The values for X_1 and X_2 were obtained from intersection of the tangents to the slope against the base line.

RESULTS AND DISCUSSIONS

Table 1 shows the triglyceride (TG) composition of palm oil before and after transesterification at various reaction temperatures. It was observed that five TG compositions were increased, namely, MMM, OOL, MMP, OOO and PPS (where M is myristic, P is palmitic, O is oleic, L is linoleic and S is stearic, acids) compared to the non-transesterified oil. However, only MMM, OOL and MMP were increased at lower reaction temperatures studied (i.e. 30°C and 40°C). The degree of transesterification for palm oil transesterified at 30°C, 40°C, 50°C and 60°C was as follows: 18.3%, 33.9%, 42% and 45.5%, which coincided with the degree of hydrolysis of 1.9%, 2%, 2.5% and 2.4%, respectively. Percentage of TG retained after transesterification was between 83% and 85%. A relatively lower transesterification rate was noted when palm oil was transesterified at 30°C. The lower rate observed was due to the difficulty in catalysing solid oils [15] since palm oil is not fully molten at 30°C (i.e. slip melting point of palm oil is at 33.3°C) [11]. However, the rate of transesterification will increase when palm oil is dissolved in suitable solvent. The addition of solvent will decrease the viscosity of the reaction mixtures visibly. Thus, some degree of transesterification and hydrolysis were observed in these transesterified oils. Bloomer et al. [1] found that the conversion rate obtained with Lipozyme in the interesterification of palm oil mid fraction and ethyl stearate was higher at 60°C than at 40°C. At 60°C, the substrate mixture is fully liquid: the reaction rate was high and the formation of saturated triglycerides was minimal without solvent.

Table 1: Triglyceride composition of palm oil before (i.e. control) and after transesterification at various reaction temperatures

Triglyceride composition (%)[a]		Reaction temperature			
	Control	30°C	40°C	50°C	60°C
MMM	0.35	0.52	0.57	0.53	0.60
MPL	1.85	1.82	1.70	1.66	1.60
OOL	0.19	0.45	0.49	0.48	0.50
MMP	1.77	2.47	3.12	3.17	3.28
PLO	9.81	9.82	9.78	8.77	8.72
PPL	8.95	8.69	8.64	6.86	6.70
OOO	5.05	4.93	5.06	7.25	7.30
OOP	25.27	25.16	25.09	25.01	31.12
PPO	31.14	30.97	30.98	31.00	25.07
PPP	5.90	5.76	5.45	5.03	5.02
OOS	2.51	2.49	2.70	2.50	2.50
POS	5.50	5.25	4.75	4.88	4.75
PPS	1.15	1.16	1.16	2.29	2.27
SOS	0.57	0.51	0.51	0.57	0.57
% remaining triglyceride	100.00	83.78	85.32	85.14	82.97
Monosaturated triglycerides	37.58	37.47	37.57	36.28	42.34
Disaturated triglycerides	48.01	47.24	46.58	44.97	38.69
Trisaturated triglycerides	9.17	9.91	10.30	11.02	11.17
Triunsaturated triglycerides	5.24	5.38	5.55	7.73	7.80

[a] M, myristic acid; P, palmitic acid; L, linoleic acid; O, oleic acid and S, stearic acid.

The heating thermogram of RBD palm oil is shown in Figure 1A. The thermogram showed four peaks, which corresponded to two major regions. The oil started melting at -24.65°C and completely melted at 43.16°C. The melting thermogram of palm oil showed a considerable broadening and overlapping. According to Che Man et al. [3] and Tan and Che Man [14] the heating thermogram of RBD palm oil showed seven endothermic peaks which could be grouped into a high melting temperature (high-T) peak group and a low melting temperature (low-T) peak group. Haryati et al. [6] only found five such peaks in the heating thermogram of crude palm oil (CPO). However, the heating thermograms of CPO and RBD palm oil were quite similar, with only slight differences in their temperature of the peaks [3]. From the heating thermograms, the polymorphic forms of palm oil could be deduced. Che Man and Swe [2] and Che Man et al. [3] indicated that low-T peaks represent polymorphs β'_2 and α, while the high-T peaks represent polymorphs β'_1 and β_1. Tan and Che Man [14] studied the correlation between the chemical compositions of edible oils with their thermal properties using DSC. They found that in melting curves of oil samples, complex features that were not easily interpretable were noticed. These results illustrate the complex nature of triglycerides in oil samples. They also concluded that DSC provides useful information regarding the nature of the thermodynamic changes that are associated with the edible oils transforming from one physical state to another, which are sensitive to the general chemical composition of the oils. Haryati et al. [6], on the other hand, concluded that the high-T peaks reflected the presence of trisaturated triglycerides, whereas the low-T peaks consisted of triunsaturated, monosaturated and disaturated triglycerides. However, this conclusion was made when the heating thermogram was compared with the cooling thermogram of palm oil. Generally, the heating thermograms of palm oil transesterified at various reaction temperatures showed three distinct regions (Figures 1 B-D). One new peak was found to be sandwiched between the low-T peaks and the high-T peaks (i.e. between 10.5-19.5°C). However, this new peak was absent at reaction temperature of 60°C (Figure 1E). Most of the transesterified oils started to melt and totally melted at lower temperature than non-transesterified oil: between –23.13°C and –24.62°C, and between 41.55°C and 43.08°C, respectively. The DSC data obtained can be used in the fractionation process to separate between the liquid and the solid fractions [3]. For instance, the clear separation of endothermic groups obtained can be used as a guideline in fractionation of these groups and thus, they can be examined further to identify each component.

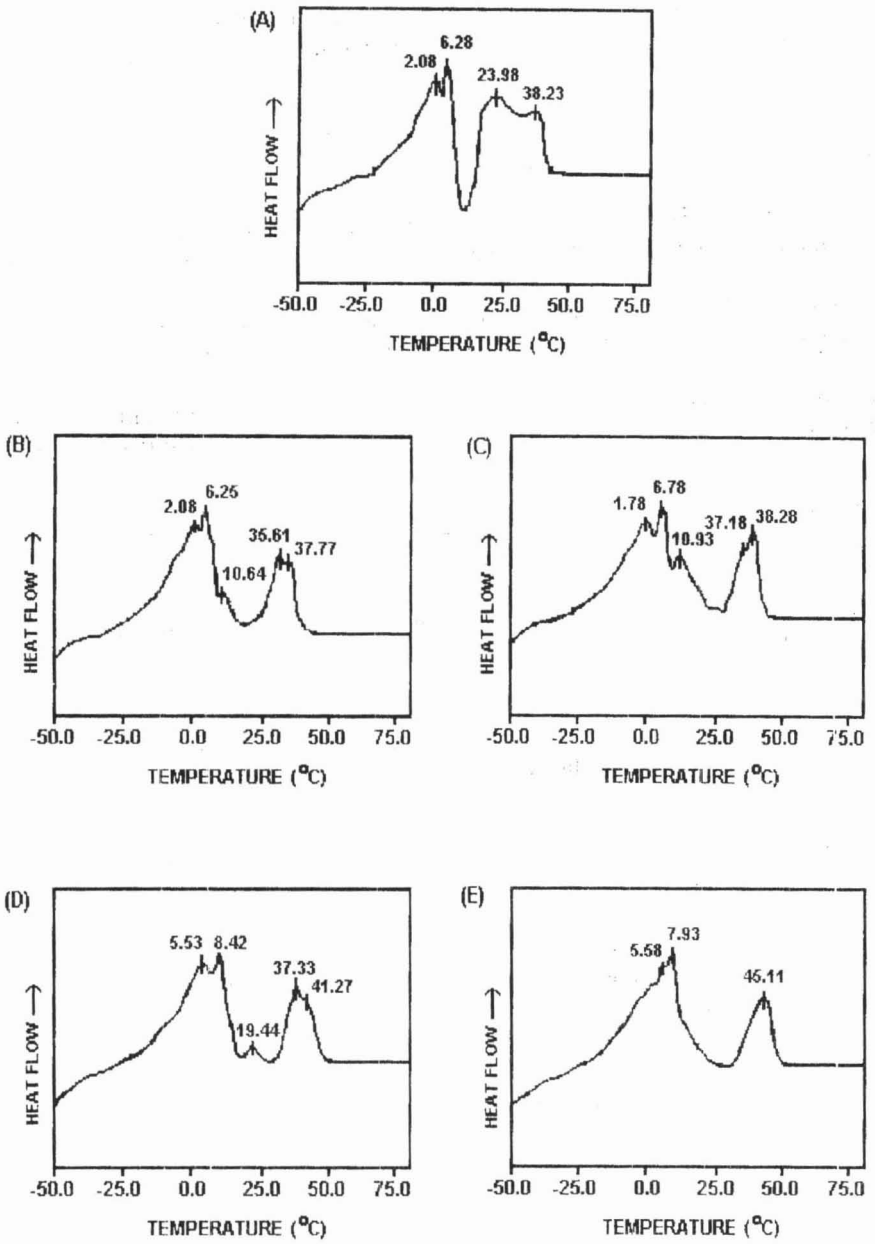

Figure 1: Differential scanning calorimeter heating thermograms of non-transesterified palm oil (A) and palm oil transesterified in hexane at 30°C (B), 40°C (C), 50°C (D) and 60°C (E).

Therefore, we can conclude that transesterification does alter the chemical as well as the physical characteristic of RBD palm oil producing new oil, which has different properties, compared to that of the original oil, non-transesterified oil. Transesterified oil at 60°C, for example, contains 7.3% higher unsaturated TG (Table 1) compared to the non-transesterified oil, making the oil more 'liquid'.

ACKNOWLEDGEMENT

This study was supported by a Malaysian Government (IRPA) grant awarded to H.M. Ghazali.

REFERENCES

1. Bloomer, S., P. Adlercreutz and B. Mattiasson. 1990. Triglycerides interesterification by lipases. 1. Cocoa butter equivalents from a fraction of palm oil. Journal of the American Oil Chemists' Society. 67: 519-524.

2. Che Man, Y.B. and P.Z. Swe. 1995. Thermal analysis of failed-batch palm oil by differential scanning calorimetry. Journal of the American Oil Chemists' Society. 72:1529-1532.

3. Che Man, Y.B., T. Haryati, H. M. Ghazali and B.A. Asbi. 1999. Composition and thermal profile of crude palm oil and its products. Journal of the American Oil Chemists' Society. 76(2):237-242.

4. Fomuso, L.B. and C.C. Akoh. 2002. Lipase-catalysed acidolysis of olive oil and caprylic acid in a bench-scale packed bed bioreactor. Food Research International. 35(1):15-21.

5. Ghazali, H.M., S. Hamidah and Y.B. Che Man. 1995. Enzymatic transesterification of palm olein with nonspecific and 1,3-specific lipases. Journal of the American Oil Chemists' Society. 72(6): 633-639.

6. Haryati, T., Y.B. Che Man and P.Z. Swe. 1997. Effect of repeated heating on thermal behaviour of crude palm oil. Journal of the American Oil Chemists' Society. 74(4):393-396.

7. Haryati, T., Y.B. Che Man, H.M. Ghazali, B.A. Asbi and L. Buana. 1998. Determination of iodine value of palm oil based on triglyceride composition. Journal of the American Oil Chemists' Society. 75(7):789-792.

8. Khumalo, L.W., L. Majoko, J.S. Read and I. Ncube. 2002. Characterisation of some underutilised vegetable oils and their evaluation as starting materials for lipase-catalysed production of cocoa butter equivalents. Industrial Crops and Products. 16(3):237-244.

9. Ong, A.S.H. 1989. Recent developments in the Malaysian palm oil industry. Pp. 285-300 in R.C. Cambie ed., Fats For the Future, Ellis Horwood Ltd.: West Sussex, England.

10. Paez, B.C., A. R. Medina, F.C. Rubio, P.G. Moreno and E.M. Grima. 2002. Production of structured triglycerides rich in n-3 polyunsaturated fatty acids by the acidolysis of cod liver oil and caprylic acid in a packed-bed reactor: equilibrium and kinetics. Chemical Engineering and Science. 57(8):1237-1249.

11. Pantzaris, T. P. 1987. Pocketbook of Palm Oil Uses, PORIM: Kuala Lumpur, pp. 9-14.

12. Shieh, C-J., H-F. Liao and C-C. Lee. 2003. Optimisation of lipase-catalysed biodiesel by response surface methodology. Bioresource Technology. 88(2):103-106.

13. Soumanou, M.M. and Bornscheuer, U.T. 2003. Improvement in lipase-catalysed synthesis of fatty acid methyl esters from sunflower oil. Enzyme and Microbial Technology. 33(1):97-103.

14. Tan, C.P. and Y.B. Che Man. 2000. Differential scanning calorimetric analysis of edible oils: comparison of thermal properties and chemical composition. Journal of the American Oil Chemists' Society. 77(2):143-155.

15. Ucar, T., H.I. Exiz, S.S. Celebi and A. Caglar. 1987. The effects of solvents on the kinetics of free and immobilised lipase. Pp. 381-386 in C. Laane, J. Tramper and M.D. Lilly eds., Biocatalysis in Organic Media. Elsevier Science Publishers B.V.: Amsterdam.

16. Undurraga, D., A. Markovits and S. Erazo. 2001. Cocoa butter equivalent through enzymic interesterification of palm oil midfraction. Process Biochemistry. 36(10):933-939.