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Effect of Enzymatic Interesterification on Melting Point of Palm Olein

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Abstract

Immobilized *PS-C 'Amano' II* lipase was used to catalyze the interesterification of palm olein (POo) with 30, 50, and 70% stearic acid in *n*-hexane at 60°C. The catalytic performance of the immobilized lipase was evaluated by determining the composition change of fatty acyl groups and triacylglycerol (TAG) by gas liquid chromatography and high-performance liquid chromatography, respectively. The interesterification process resulted in the formation of new TAGs, mainly tripalmitin and dipalmitostearin, both of which were absent in the original oil. These changes in TAG composition resulted in an increase in slip melting point, from the original 25.5°C to 36.3, 37.0, and 40.0°C in the modified POo with 30, 50, and 70% stearic acid, respectively. All the reactions attained steady state in about 6 h. This type of work will find great applications in food industries, such as confectionery.

Index Entries: Interesterification; *PS-C 'Amano' II* lipase; palm olein; stearic acid; slip melting point.

Introduction

Natural oil/fat rarely satisfy the requirements for its intended use. Therefore, some modification is required, especially of its melting and crystallization characteristics and nutritional properties. The ability to modify an oil/fat to a desired physical and chemical properties is thus of great importance in the food and oleochemical industries.

There are several methods of modification, including hydrogenation and interesterification. Hydrogenation, unless carried to completion,

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converts the unsaturated *cis* fatty acids to their *trans* forms *(1)*. This detracts from the process because *trans* fatty acids are increasingly implicated in several disease etiologies, including thrombogenesis, which leads to coronary heart disease *(2–4)*.

This problem has generated a demand for nonhydrogenated, low (or even no) *trans* fatty acid solid fats and spurred interest in alternative processing methods, such as interesterification, or the exchange of fatty acids between triacylglycerols (TAGs) *(5)*. Exchanging the fatty acid positions on the TAG molecules by interesterification can alter the physical properties of the original oil/fat and lead to formation of a new, desired product *(6)*.

Chemical interesterification can also be used but requires a temperature above 100°C and an inorganic catalyst, which may degrade and contaminate the finished product. This treatment is usually done with the oil in a vacuum or under a nitrogen blanket to prevent oxidative degradation *(7)*.

It is therefore not surprising that lipase interesterification is gaining interest. With lipases, interesterification can be carried out at lower temperature, and the reaction is better controlled owing to the high selectivity of enzymes for specific reactions *(8–10)*. Protection by a vacuum or nitrogen blanketing is not necessary *(7)*. Another advantage of enzymatic over chemical interesterification is that it is not necessary to remove the byproducts *(10)*.

In lipase interesterification, the equilibrium of the thermodynamically reversible chemical reaction can be manipulated *(11)*. Low water content is used to veer the reaction toward interesterification from hydrolysis *(12)*.

Enzymatic interesterification is now commonly used commercially to produce high-value products, such as structured TAGs for confectionery. One example is the lipase-catalyzed production of cocoa butter used in the manufacture of chocolate from low-value oils *(13)*.

The objective of the present study was to modify palm olein (POo) by using immobilized lipase to produce fat of desirable melting point characteristics.

Materials and Methods

Chemicals

Malaysian RBD POo was purchased from a local supermarket, and lipase *PS-C 'Amano' II* from *Pseudomonas cepacia* immobilized on ceramic particles (1019 U/g) was obtained from Amano (Nagoya, Japan). TAG standards were purchased from Sigma (St. Louis, MO). All other chemicals used were of the highest purity available.

Interesterification

Ten grams of POo was interesterified with stearic acid in a closed flask with 1 g of immobilized lipase (10%)/g of oil and 0.1 mL of water (10%)/g of immobilized lipase. The amounts of stearic acid in the mixture were 3, 5,

and 7 g, constituting 30, 50, and 70%, respectively, of the POo used. The mixture was agitated on an orbital shaker at 150 rpm and 60°C and allowed to react for up to 12 h before stopping by filtering off the lipase. Each interesterification reaction was done in duplicate.

Determination of TAG Composition

The TAG profiles of the fat before and after interesterification were obtained by reverse-phase high-performance liquid chromatography (HPLC) using AOCS Official Method Ce 5C-93 *(14)*. The HPLC used was a Shimadzu model with a commercially packed RP-18 column (250 × 4 mm) of 5-µm particles (Merck, Darmstadt, Germany). The TAGs were eluted with an acetone/acetonitrile (65:35) mobile phase at a flow rate of 1 mL/min and identified using a refractive index detector. The sample injection volume was 20 µL. TAGs were identified by comparing the retention times with those of TAG standards, such as triolein (OOO), dioleopalmitin (POO), oleodipalmitin (POP), and tripalmitin (PPP). The concentrations were determined by reference to the peak areas of known standards.

Removal of Free Fatty Acids

Free fatty acids from the transesterified fats were removed from the reaction mixture according to the method of Foglia et al. *(15)*.

Preparation of Fatty Acid Methyl Ester

The PORIM official test method p3.4 *(16)* was used. Fifty milligrams of melted and homogenized fat was placed in a 2-mL vial. Then, 0.95 mL of *n*-hexane was added and the mixture shaken. Next 0.05 mL of sodium methoxide was added, and the vial was shaken vigorously for 5 s in a vortex mixer. After 5 min, the clear upper layer of methyl ester was pipeted off for analysis.

Determination of Fatty Acid Composition

The fatty acid composition was determined by PORIM official test method p3.5 *(16)*. The fat was interesterified to fatty acid methyl ester (FAME) and the FAME analyzed in a Hewlett-Packard 5890 II gas chromatograph (Palo Alto, CA) with a polar SP-2340 (Supelco, Bellefonte, PA) capillary column (0.25 mm id × 60 m × 0.2 µm). The detector and injector port temperature was 240°C. The carrier gas was helium at 0.8 mL/min, the column temperature was isothermal at 190°C, and the split ratio was 1:100. The injection volume was 1 µL.

Determination of Slip Melting Point

The slip melting points (SMPs) of the fat before and after interesterification were determined according to AOCS Official Method Cc 1-25 *(14)*. A column of fat was tempered at 10 ± 1°C for 16 h in an open capillary tube. The tube was then heated slowly in a water bath until the fat column

Table 1
Fatty Acyl Profiles of POo and Modified Products (%)[a]

	Palmitic 16:0	Stearic 18:0	Oleic 18:1n-9	Linoleic 18:2n-6	Other
Initial POo	37.9 (0.11)	4.1 (0.15)	42.4 (0.25)	12.9 (0.19)	2.7 (0.26)
POo modified with 30% stearic acid	36.1 (0.18)	13.9 (0.16)	35.6 (0.40)	11.5 (0.15)	2.9 (0.23)
POo modified with 50% stearic acid	36.1 (0.46)	18.4 (0.16)	31.9 (0.29)	10.1 (0.34)	3.5 (0.30)
POo modified with 70% stearic acid	35.8 (0.13)	20.1 (0.23)	30.4 (0.63)	10.4 (0.04)	3.3 (0.95)

[a]%, peak area. Values in parentheses are SDs.

started to rise from the hydrostatic pressure, at which the temperature was taken as the SMP.

Results and Discussion

The fatty acids of TAGs were rearranged (interesterified) by first removing them from their glycerol backbones via hydrolysis and then reattaching them in other positions. Hydrolysis requires water, but too much water will allow the reaction to favor hydrolysis rather than interesterification. In our study, a 10% water level was used based on an optimum level for interesterification *(13,17)*.

Table 1 shows the fatty acyl groups (after conversion to FAMES) of the POo and modified POo. POo was rich in palmitoyl (C16:0) (37.9%) and oleoyl (C18:1n-9) (42.4%), but low in stearoyl (C18:0) (4.1%). Furthermore, POo relatively contained a high level of linoleoyl (C18:2n-6) (12.9%). In all interesterified products, there was substantial incorporation of stearoyl with concomitant decreases in the palmitoyl, oleoyl, and linoleoyl contents, indicating higher saturation in the modified POo. Palmitoyl fell from 37.9 to 35.8%, oleoyl from 42.4 to 30.4%, and linoleoyl from 12.9 to 10.4%, and stearoyl increased from 4.1 to 20.1% for interesterification reaction with 70% stearic acid. The compositional changes of the fatty acyl groups during enzymatic interesterification of 10:3, 10:5, and 10:7 POo–stearic acid mixture are shown in Figs. 1–3. It is clear that the interesterification reaction attained steady state in about 6 h.

Table 2 summarizes the changes in TAG composition in POo before and after interesterification with stearic acid. The TAG composition changed progressively during the interesterification reaction; PPP and PPS, not initially present in the oil, were formed (Fig. 4). In all the POo–stearic acid blends, there were increases in the concentrations of SOO, POS, and SOS and corresponding decreases in the concentrations of PLL, MLP, POL,

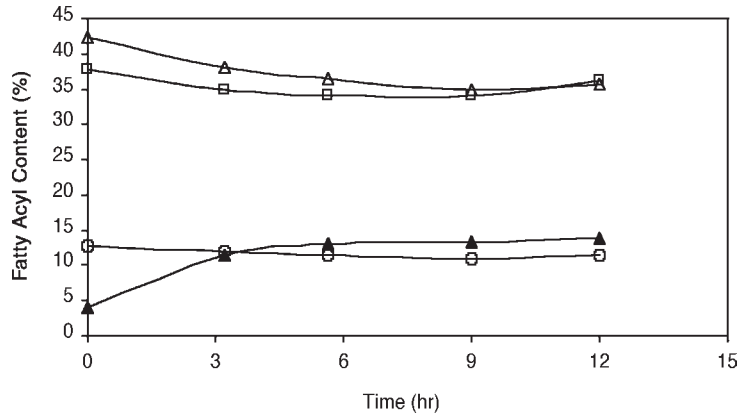

Fig. 1. Fatty acyl profile of products during enzymatic interesterification of 10:3 POo–stearic acid mixture. (□) Palmitic; (▲) stearic; (△) oleic; (○) linoleic.

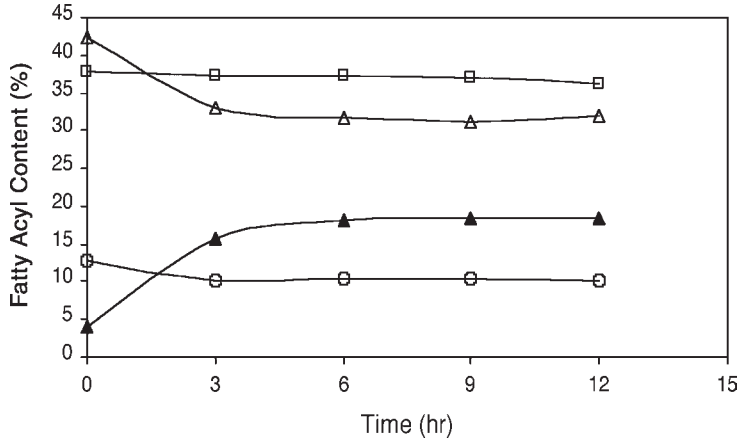

Fig. 2. Fatty acyl profile of products during enzymatic interesterification of 10:5 POo–stearic acid mixture. (□) Palmitic; (▲) stearic; (△) oleic; (○) linoleic.

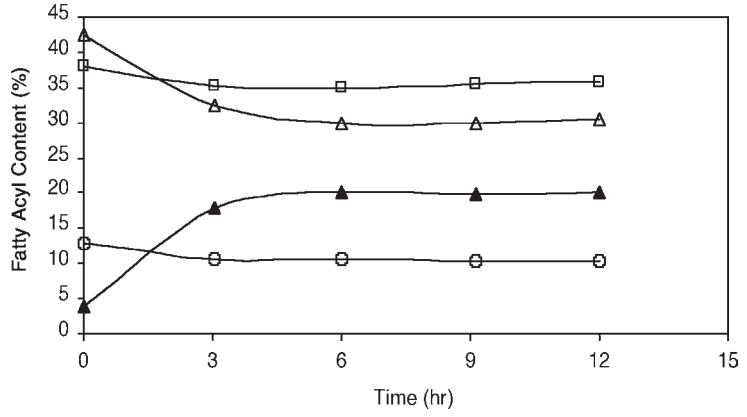

Fig. 3. Fatty acyl profile of products during enzymatic interesterification of 10:7 POo–stearic acid mixture. (□) Palmitic; (▲) stearic; (△) oleic; (○) linoleic.

Table 2
Triglyceride Composition of POo and Modified Products (%)[a]

	POo	10:3 POo: stearic acid mix	10:5 POo: stearic acid mix	10:7 POo: stearic acid mix
OLL	0.6 (0.04)	1.2 (0.13)	0.6 (0.11)	0.5 (0.05)
PLL	3.4 (0.03)	1.9 (0.12)	1.3 (0.18)	1.1 (0.7)
MLP	0.8 (0.01)	0.3 (0.13)	0.4 (0.00)	0.6 (0.11)
OOL	2.2 (0.21)	3.8 (0.16)	2.8 (0.21)	2.0 (0.09)
POL	13.4 (0.40)	9.5 (0.71)	7.9 (0.17)	6.6 (0.68)
PLP	11.8 (0.05)	5.6 (1.33)	6.2 (0.05)	5.6 (0.44)
OOO	4.6 (0.28)	5.3 (0.06)	4.5 (0.96)	4.2 (0.26)
POO	28.2 (0.14)	17.8 (2.25)	14.8 (0.80)	13.6 (1.23)
POP	28.9 (0.58)	19.5 (1.05)	19.1 (0.52)	18.4 (0.55)
PPP	0.0 (0.00)	7.5 (2.03)	7.6 (0.88)	7.8 (1.23)
SOO	0.6 (0.75)	5.3 (0.35)	5.7 (0.35)	6.2 (0.50)
POS	4.9 (0.20)	12.5 (0.10)	15.5 (0.85)	16.6 (0.50)
PPS	0.0 (0.00)	7.2 (0.10)	9.0 (1.22)	10.0 (0.11)
SOS	0.6 (0.01)	2.2 (0.00)	3.1 (0.33)	4.3 (0.13)
Other	0.0 (0.00)	0.4 (0.06)	1.5 (3.01)	2.5 (2.3)

[a]%, peak area. O, oleic; L, linoleic; P, palmitic; M, myristic; and S, stearic acids. Values in parentheses are SDs.

PLP, POO, and POP (in which O, L, P, M, and S are oleic, linoleic, palmitic, myristic, and stearic acids, respectively). The concentration of OLL increased in the 10:3 POo–stearic acid mixtures but did not change in the 10:5 and 10:7 mixtures. The concentration of OOO increased in the 10:3 and 10:5 mixtures but not in the 10:7 mixture, possibly owing to equilibrium constraint. In terms of degree of saturation, comparing POo and the modified POo with 70% stearic acid, the fully saturated TAGs (PPP and PPS) increased from 0 to 17.8% with increases in stearic acid. The monounsaturates (POP, POS, and SOS) increased from 32.8 to 39.3%. The diunsaturates (POO, SOO, PLP, and MLP) fell from 44.2 to 26.0%, the triunsaturates (POL and OOO) from 17.0 to 10.8%, and the polyunsaturates (PLL, OLL, and OOL) from 5.9 to 3.6%. Overall, the combined concentration of polyunsaturates, triunsaturates, and diunsaturates decreased whereas the combined full saturates and monounsaturates increased, making the oil more solid with a higher SMP.

The SMP of POo was determined after the removal of free fatty acids from the reaction mixture. It increased from the original 25.5°C to 36.3, 37.0, and 40.0°C in the modified POo for stearic acid concentrations of 30, 50, and 70%, respectively. This result is in agreement with the finding of Zainal and Yusoff *(7)*, who reported an increase in SMP after interesterification of palm stearin and palm kernel olein with *Rhizomucor miehei* lipase. Lai et al. *(18)* reported a slight decrease in SMP after interesterifying palm stearin and palm kernel olein with *Pseudomonas* lipase.

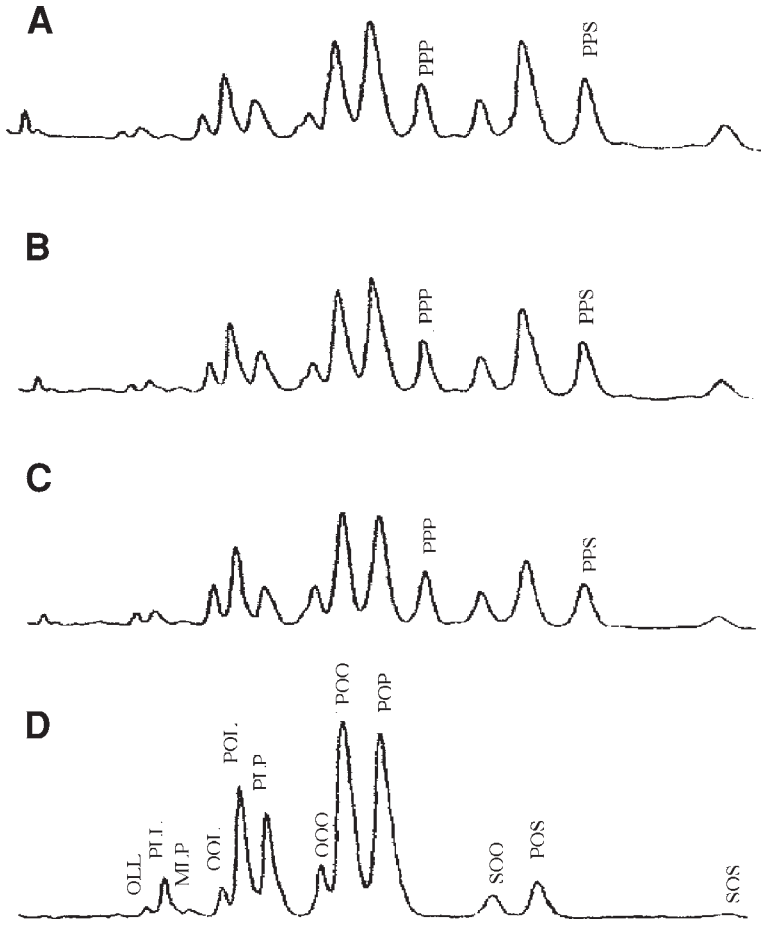

Fig. 4. TAG profiles of POo at beginning **(A)** and after 12 h of enzymatic interesterification reaction with **(B)** 30%, **(C)** 50%, and **(D)** 70% POo–stearic acid mixture. O, oleic; L, linoleic; P, palmitic; M, myristic; and S, stearic acids.

Conclusion

Our study investigated the use of immobilized *Pseudomonas* lipase for interesterification of POo with stearic acid. The catalytic performance of the enzyme was evaluated by determining the changes in fatty acid composition using gas-liquid chromatography and the change in TAG composition and concentrations using HPLC. The effects of enzymatic interesterification of POo and stearic acid resulted in changes in the overall degree of saturation of the TAG components. SMP increased in all the blends from the original 25.5°C, to 36.3, 37.0, and 40.0°C in the modified POo for stearic acid concentrations of 30, 50, and 70%, respectively. All the reactions carried attained steady-state condition in about 6 h. New TAGs, mainly PPP and PPS, were formed during the reaction for all levels of stearic acid used.

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