Acidolysis of Babassu Fat Catalyzed by Immobilized Lipase[1]

L.A. Gioielli*, R.N.M. Pitombo, M. Vitolo, R. Baruffaldi, M.N. Oliveira and **M.S. Augusto**
Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, São Paulo, Brazil

The lipase-catalyzed interesterification of oils and fats gives products that are unobtainable by chemical interesterification methods. Acidolysis of babassu fat and palmitic acid, catalyzed by immobilized lipase (Lipozyme; Novo Industri, Bagsveard, Denmark), was studied. The reactions were performed at 65°C with 5% w/w enzyme for 4 h. The molar proportions of babassu fat/palmitic acid were 1:0.1, 1:0.3 and 1:0.5. At the end of the reaction period, the catalyst particles were removed by filtration, and the residual oil was extracted with organic solvent (diethyl ether). The recovered particles were then reused. The palmitic acid content of babassu fat before and after acidolysis changed from 10 to 22% at a molar proportion of 1:0.5. The equilibrium was attained in about 4 h. The original water and enzymatic activities of Lipozyme were maintained after acidolysis. Water sorption isotherms of the immobilized enzyme were determined at 25, 35 and 45°C. From the temperature dependence of the isotherms, isosteric heats of sorption were evaluated by means of the Clausius Clapeyron equation. Monolayer moisture content was calculated by means of the B.E.T. and Guggenhein–Anderson–De Boer analyses.

KEY WORDS: Babassu fat, biotechnology, fat acidolysis, immobilized lipase, interesterification, isosteric heats of sorption, monolayer moisture content, palmitic acid, water activity, water sorption isotherms.

The enzymatic interesterification of fats and oils presents, in relation to the chemical process, the following advantages: (i) specificity; (ii) mild process conditions; and (iii) lowered waste treatment costs (1,2).

The catalyst is activated by addition of a small amount of water. Control of the amount of water in the reaction system is important for the orientation of the process and in the determination of the final reaction products (3). Under such conditions, the amount of water in the reaction system is limited, the fat hydrolysis can be minimized, and the interesterification catalyzed by lipase is the dominant reaction (1). The water activity (A_w) of the enzyme can be controlled by equilibrating the lipase in an environment of known relative humidity (3). The use of immobilized lipase allows the interesterification on an industrial scale (1).

The rates of triacylglycerol interesterification with some substances, when catalyzed by immobilized *sn*-1,3-specific lipase, occur in the sequence: long-chain alcohol > free fatty acids > triacylglycerol > methyl ester > glycerol (4). It is better to use the free fatty acid form with the triacylglycerol or the methyl ester form for the purpose of incorporating some specific fatty acid in a fat or oil.

The objectives of the research were to investigate the incorporation of palmitic acid into babassu fat in an acidolysis reaction catalyzed by immobilized lipase; and to determine the water sorption isotherms of the immobilized enzyme.

[1]Paper presented at the International Meeting on Fats and Oils Technology, Universidade Estadual de Campinas, Brazil, 1991.

*To whom correspondence should be addressed at Faculdade de Ciências Farmacêuticas de Universidade de São Paulo, Av. Prof. Lineu Prestes 580 Bloco 16, Caixa Postal 66083, CEP 05389-970, São Paulo - SP, Brazil.

MATERIALS AND METHODS

The refined babassu fat and the palmitic acid (97.4% 16:0) were obtained from Refinadora de Óleos Brasil (São Paulo, Brazil), and Riedel (Hannover, Germany), respectively. The immobilized lipase (Lipozyme) was generously donated by Novo Industri (Bagsveard, Denmark) and had an activity of 25 BIU/g (1 BIU corresponds to 1 μmol of palmitic acid incorporated into triolein per min, at standard conditions). The reagents were supplied by traditional companies (Merck, Darmstadt, Germany; Riedel).

Determination of fatty acid composition. Fatty acid composition determination was achieved in a gas chromatograph equipped with ionization flame detector and electronic integrator, in a DEGS (17%) column with Chromosorb W (80–100 mesh) at 192°C.

Free fatty acids and melting and softening points. This was determined in accordance with the American Oil Chemists' Society's methods Ca 5a-40, Cc 1-25 and Cc 3-25 (5).

Preparation of sorption isotherms. Adsorption isotherms were determined at 25, 35 and 45°C. Triplicate samples were dried in a vacuum oven (0.1 mm Hg and 30°C) for 24 h. Then they were dried to constant weight over phosphorus pentoxide and placed over saturated salt solutions in a dessicator at constant temperature to provide A_w in the range 0–0.90 (6). Equilibrium was reached within 10 d, as evidenced by constant weight after successive weighings of the samples. A standardized procedure was employed for weighing the samples on a Mettler analytical balance. The moisture content of the samples (g/100 g solids) was plotted against water activity for each of the three temperatures tested.

Acidolysis reactions. Acidolyses were conducted in 250-mL three-necked flasks with 100 g of reagents and without solvents. The system was previously heated at 65°C in a water bath with magnetic bar agitation. The amount of immobilized enzyme used was 5%, and the system was maintained in N_2 atmosphere for the time of the reaction. The molar proportions of babassu fat and palmitic acid were 1:0.1, 1:0.3 and 1:0.5. The immobilized lipase had an original moisture content of 8.4%. The reaction time varied from 2 to 6 h. After the reaction, the catalyst particles were removed by filtration, and the residual absorbed oil was extracted with diethyl ether in a Soxhlet apparatus. The recovered particles were used again, under the same conditions described above, after they had attained equilibrium within the dessicator with 43% relative humidity at 25°C. The filtrate was neutralized with NaOH solution for the removal of free fatty acids. The refining process was conducted in a hexane miscella.

RESULTS AND DISCUSSION

The results of the fatty acid compositions of the neutralized products after the acidolysis reactions are presented in Figures 1, 2 and 3.

Despite the high initial acidity (from 4.7 to 20.2%) and losses during the process, the yield of neutralized product

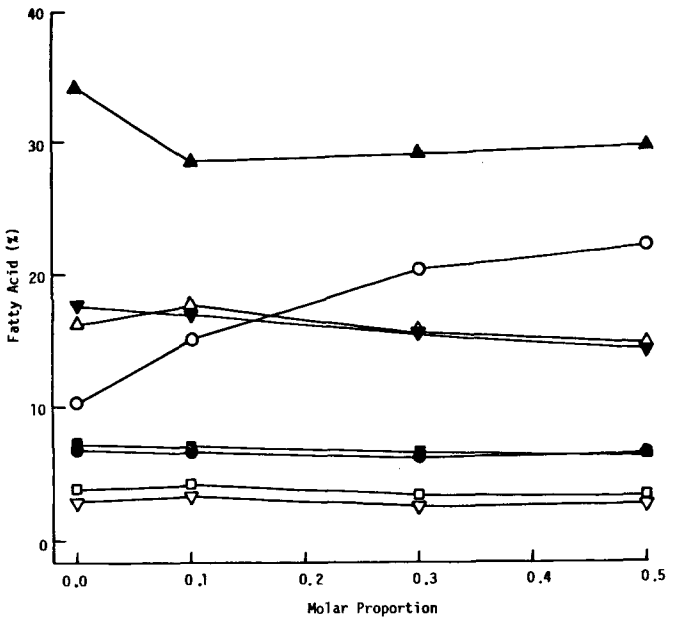

FIG. 1. Fatty acid composition resulting from the lipase-catalyzed interesterification of babassu fat and palmitic acid as a function of the molar proportions of the reactants (time reaction = 4 h); ●, 8:0; ■, 10:0; ▲, 12:0; ▼, 14:0, ○, 16:0, □, 18:0; △, 18:1; ▽, 18:2.

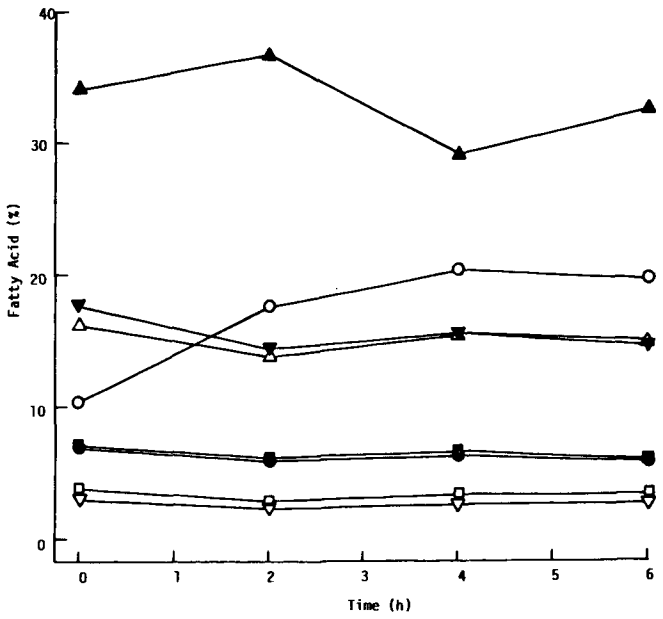

FIG. 2. Fatty acid composition resulting from the lipase-catalyzed interesterification of babassu fat and palmitic acid as function of time (molar proportion 1:0.3); ●, 8:0; ■, 10:0; ▲, 12:0; ▼, 14:0; ○, 16:0; □, 18:0; △; 18:1; ▽, 18:2.

can be considered satisfactory (57.8%). The free fatty acids of the neutral product varied from 0.2 to 0.5%.

The immobilized enzyme absorbed 68.0% fat by weight, after filtration. After extraction with organic solvent, it was possible to recover 84.4% of the original lipase. However, the immobilized lipases could be used again without washing or hydration (7) because the fat was the same.

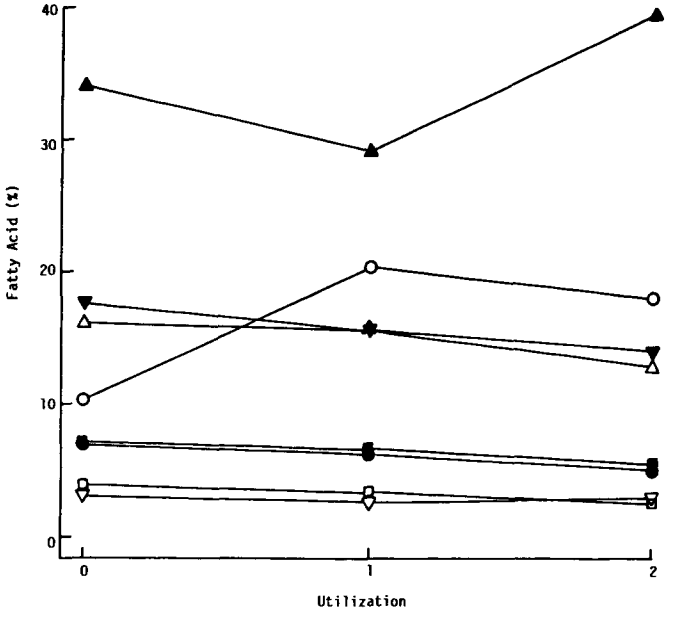

FIG. 3. Fatty acid composition resulting from the lipase-catalyzed interesterification of babassu fat and palmitic acid at first and second utilization of the enzyme (molar proportion 1:0.3, time reaction = 4 h); ●, 8:0, ■, 10:0; ▲, 12:0; ▼, 14:0; ○, 16:0; □, 18:0; △, 18:1; ▽, 18:2.

An increase (5.9%) in free fatty acids occurred during the acidolysis process. This indicates the occurrence of simultaneous hydrolysis and interesterification of triacylglycerols. In the interesterification course, the hydrolysis reaction, which forms free fatty acids and partial acylglycerols, is unavoidable (3,8). The free fatty acids and the monoacylglycerols can be eliminated from the fat by chemical or physical refining. The diacylglycerols are not efficiently eliminated by conventional industrial refining techniques (8).

The palmitic acid incorporation did not alter the melting and softening points as compared to the original babassu fat. This is probably a consequence of eutectic interactions between the triacylglycerols that are rearranged by the process.

The incorporation of palmitic acid, as a process evaluation parameter of babassu fat, showed that it increased 113.5% (Fig. 1) when the proportion was 1:0.5. In the proportion of 1:0.3, the increase was lower (97.1%). This proportion was chosen in the next reactions because the excessive content of free fatty acids in the blend makes the neutralization process difficult. The macroporous support (anion exchange resin) with positive charges tends to attract the carboxylic acids and brings high substrate concentrations near the enzyme (9). The neighborhood of the enzyme was saturated with palmitic acid, and the increase in free fatty acid in the blend did not really affect the interesterification. The maximum palmitic acid content obtained in the interesterified product was 22.2%. Comparable results were obtained in other acidolysis reactions catalyzed by *sn*-1,3-specific lipases (1), where stearic acid incorporated in the interesterified product varied from 15.6 to 28.9%.

According to product information by the enzyme supplier: (i) optimal temperature range is 60–70°C; (ii) the substrate should be saturated with water prior to reaction

(0.1–0.5%); (iii) the enzyme/substrate ratio should be 10% (w/w); and (iv) the reaction time in a discontinuous process is 1–4 h. In the original form, the enzyme has 8–10% moisture (7). Lipase fixation in macroporous anion exchange resin increases the enzymatic activity. The activity of Lipozyme is 40 times higher than that of the free lipase (10).

The reaction time of 4 h was chosen because the palmitic acid content in the interesterified product did not alter significantly after 4 or 6 h of reaction (Fig. 2). The reaction time of 4 h is suggested by the manufacturer for batch processes, but in this study, the amount of enzyme used was half of the amount suggested (5% w/w).

Muderhwa *et al.* (8) observed that the transesterification conversion rate for Lipozyme assumed a maximum value when the A_w of the enzyme was 0.43. For A_w values higher than 0.5, the conversion rate decreases progressively and becomes null above 0.9. The results show that the enzyme is in an ideal condition to promote transesterification at a moisture content of nearly 10%, which corresponds to a A_w of 0.43.

Recycling of the enzyme did not change the palmitic acid content much in the interesterified product compared with its first utilization (Fig. 3). Therefore, activity was barely affected by the recovery process. According to Macrae (1), it is possible to use the same catalyst in ten discontinuous, consecutive interesterification reactions. The stirred-batch reactor is the type of reactor most commonly employed in bench-scale and industrial-scale applications (11).

The sorption isotherms of Lipozyme at 25, 35 and 45°C are shown in Figure 4. Each point of the curves represents the mean value of three replications. The shapes of the isotherms are characteristic of type II isotherms within the classification of Brunauer *et al.* (12), which is often observed in polymeric substances such as protein, starch and cellulose.

Goderis *et al.* (3) showed that the presence of the enzyme itself is irrelevant to the determination of the water adsorption isotherms. The equilibrium moisture content of the enzyme decreased markedly as the temperature was raised from 25 to 45°C. The sorption data of Lipozyme were used to estimate the binding energy as a function of moisture content at the three temperatures. The total heat of sorption vs. moisture content is shown in Figure 5.

From the results of heat of sorption, it is possible to estimate the effect of a temperature shift on the change in A_w at constant moisture content by using the Clausius Clapeyron equation and the excess heat of sorption values. This allowed us to know the A_w of the immobilized enzyme when the esterification was carried out at 65°C (for 0.084 g H_2O/g solids, the A_w is 0.58 at 65°C). Labuza *et al.* (13) showed that the Clausius Clapeyron equation predicts the shift in A_w with temperature quite well, while the isotherm at the actual shift temperature showed a higher value. At the higher temperature, the ten days of storage in creating the actual isotherm at 65°C could decrease the sorption capacity of the material due to chemical reactions.

The values of the monolayer moisture content at each temperature, as calculated by the GAB (Guggenhein-Anderson-De Boer) and BET (Brunauer-Emmett-Teller) equations (13), are presented in Table 1. The results show that the monolayer moisture content (W_m) is almost not affected by increasing the temperature from 25 to 45°C for the BET model. Once the moisture content at the "monolayer" (W_m) was known, the solid surface area of the immobilized enzyme could be determined, and this value is not affected by the temperature. This means that there is no significant alteration of the water sorption site that is thermally induced.

The GAB equation has an advantage compared to the BET model, because it offers an objective method for drawing sorption isotherms up to 0.80 A_w, whereas the

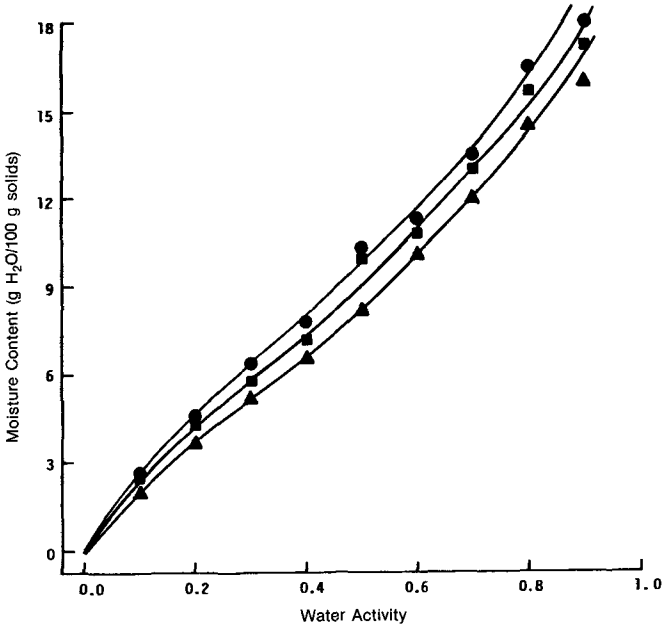

FIG. 4. Water adsorption isotherms of Lipozyme at 25 (●), 35 (■) and 45°C (▲).

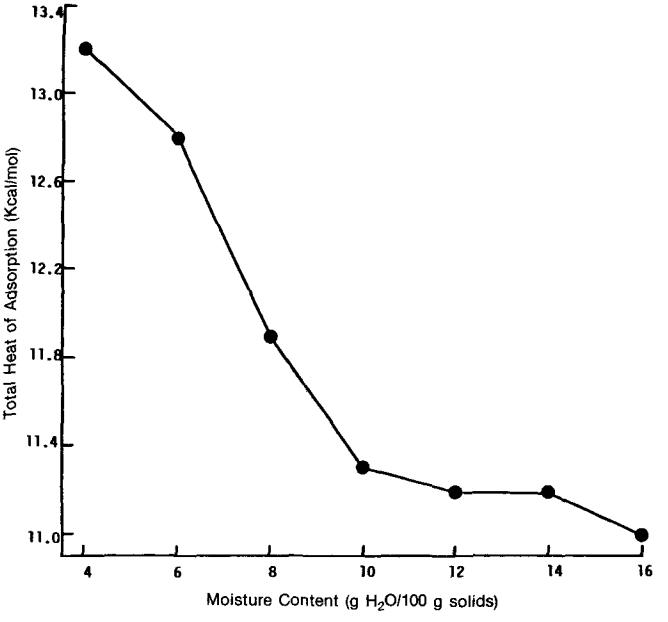

FIG. 5. Variation of total heat of adsorption of Lipozyme with moisture content.

TABLE 1

Moisture Adsorption of Lipozyme as Determined by the Guggenhein–Anderson–De Boer (GAB) and Brunauer–Emmett–Teller (BET) Models[a]

			Parameters of the equations					
Equation	T (°C)	A_w	C′	C	K	W_m (%)	RMS (%)	R
GAB	25	0.100–0.800	—	6.04	0.566	9.42	6.42	—
	35	0.100–0.800	—	5.79	0.590	8.62	7.41	—
	45	0.100–0.800	—	4.92	0.621	7.70	8.58	—
	65[b]	0.283–0.843	—	4.27	0.656	6.38	14.44	—
BET	25	0.10–0.50	6.31	—	—	5.93	—	0.995
	35	0.10–0.50	5.96	—	—	5.64	—	0.980
	45	0.10–0.40	4.89	—	—	5.26	—	0.990
	65[b]	0.10–0.65	8.63	—	—	3.81	—	0.990

[a]A_w, Water activity; C′, C and K are characteristic constants; W_m, monomolecular layer moisture content (g water/100 g solids); RMS, mean square root; R, correlation coefficient.
[b]Sorption data obtained by the Clausius Clapeyron equation.

BET model is limited to 0.50 A_w. The results obtained show that the BET model fits the sorption data for Lipozyme quite well.

After all reactions, the Lipozyme was washed with solvent and then put in a dessicator (0.432 A_w) at 25°C; and there was no gain or loss of moisture, showing that during all processes the water still stayed bound to the catalyst.

ACKNOWLEDGMENT

This work was supported in part by research grants from Financiadora de Estudos e Projetos (FINEP).

REFERENCES

1. Macrae, A.R., *J. Am. Oil Chem. Soc. 60*:291 (1983).
2. Posorske, L.H., *Ibid. 61*:1758 (1984).
3. Goderis, H.L., G. Ampe, M.P. Feyten, B.L. Fouwe, W.M. Guffens, S.M. Van Cauwenbergh and P.P. Tobback, *Biotechnol. Bioeng. 30*:258 (1987).
4. Schuch, R., and K.M. Murkherjee, *J. Agric. Food Chem. 35*:1005 (1987).
5. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by R.O. Walker, American Oil Chemists' Society, Champaign, 1980.
6. Guenspan, *J. Res. Natl. Bur. Stand. 81*:89 (1976).
7. Posorske, L.H., G.K. Lefebvre, C.A. Miller, T.T. Hansen and B.L. Glenvig, *J. Am. Oil Chem. Soc. 65*:922 (1988).
8. Muderhwa, J.M., M. Pina and J.G. Graille, *Oléagineux 43*:427 (1988).
9. Bloomer, S., P. Adlercreutz and B. Matiasson, *J. Am. Oil. Chem. Soc. 67*:519 (1990).
10. Graille, J., M. Pina and D. Montet, *Oléagineux 43*:181 (1988).
11. Malcata, F.X., H.R. Reyes, H.S. Garcia, C.G. Hill, Jr. and C.H. Amundson, *J. Am. Oil Chem. Soc. 67*:890 (1990).
12. Brunauer, S., L.S. Deming, W.E. Deming and E. Teller, *J. Am. Chem. Soc. 62*:1772 (1940).
13. Labuza, T.P., A. Kaanane and Y. Chem, *J. Food Sci. 50*:385 (1985).

[Received July 15, 1993; accepted March 20, 1994]