World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:3, No:6, 2009

Modification of Palm Oil Structure to Cocoa Butter Equivalent by *Carica papaya* Lipase-Catalyzed Interesterification

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Abstract**—**Palm oil could be converted to cocoa butter equivalent by lipase-catalyzed interesterification. The objective of this research was to investigate the structure modification of palm oil to cocoa butter equivalent using *Carica papaya* lipase –catalyzed interesterification. The study showed that the compositions of cocoa butter equivalent were affected by acyl donor sources, substrate ratio, initial water of enzyme, reaction time, reaction temperature and the amount of enzyme. Among three acyl donors tested (methyl stearate, ethyl stearate and stearic acid), methyl stearate appeared to be the best acyl donor for incorporation to palm oil structure. The best reaction conditions for cocoa butter equivalent production were : substrate ratio (palm oil : methyl stearate, mol/mol) at 1 : 4, water activity of enzyme at 0.11, reaction time at 4 h, reaction temperature at 45 ^{o}C and 18% by weight of the enzyme. The chemical and physical properties of cocoa butter equivalent were 9.75 ± 0.41% free fatty acid, 44.89 ± 0.84 iodine number, 193.19 ± 0.78 sponification value and melting point at 37-39 ^{o}C.

Keywords**—***Carica papaya* lipase, cocoa butter equivalent, interesterification, palm oil.

I. Introduction

COCOA butter is the natural fat extracted from the cocoa bean and its color is slightly yellowish [1]. Cocoa butter is an important ingredient in the chocolate and related confectionery industries. It is responsible for the different favorable characteristics such as hardness at room temperature, brightness, and fast and complete melting when placed in the mouth [2]. Cocoa butter is composed of three main triacylglycerols (TAGs): 1, 3-dipalmitoyl-2-oleoylglycerol (POP); 1(3)-palmitoyl-3(1)-stearoyl-2-oleoylglycerol (POS) and 1, 3-distearoyl-2-oleoylglycerol (SOS), with oleic acid in the *sn*-2 positions [3]. The typical fatty acid composition of cocoa butter in mole percentage is : 24.4% palmitic acid, 33.6% stearic acid, 37.0% oleic acid, 3.4% linoleic acid and 1.6% others [4]. Cocoa butter contains stearic acid and palmitic acid in a ratio of 1.3: 1.0 [5]. Due to high cost and fluctuations in the supply and demand of cocoa butter, cocoa butter equivalent (CBE) with a TAGs composition similar to cocoa butter is used as an alternative source [6].

Cheap commercial oil that have TAGs with oleic acid in the 2-position can be converted to CBE for adding the value of oils. Palm oil is low cost fat; availability and its chemical composition have a similar to cocoa butter. Therefore, palm oil is suitable raw material for the production of CBE.

Preparation of CBE through enzymatic-catalyzed interesterification has attracted because lipases offer certain advantages over other chemical catalysts. One of these advantages is that it produces fewer by-products. While chemical catalysts will randomize all of the fatty acids in TAGs mixture, 1, 3-specific lipase can incorporate fatty acids into the *sn*-1, 3-positions without changing the fatty acid residues in the *sn*-2-position [7]. Other advantages are lower energy consumption and better product control. Recently, vegetable oils such as Mahua, Kokum and mango fats, palm oil midfraction, teaseed oil, and olive oil have been popularly used to prepare CBE by microbial lipases in batch stirred tank reactor [8]-[11]. However, procedures of lipase-catalyzed interesterification for CBE production have not been practically in industries because of microbial lipase relatively high cost.

Over the last century, latex from *Carica papaya* has been well known for containing papain which is a cysteine protease with numerous industrial applications. The particulate of *C. papaya* latex possesses lypolytic activity with 1(3)-regiospecificity [12]. *C. papaya* lipase has potential as a biocatalyst in lipid transformations such as milk fat modification [13] and synthesis of low-calorie structured TAGs [14]. This latter observation led us to consider that CPL may be a useful inexpensive biocatalyst for the synthesis of CBE. In this study, CBE was prepared by *C. papaya* lipase-catalyzed interesterification of palm oil. Several factors, such as acyl donor sources, reaction time, temperature, initial water of enzyme, amount of enzyme, and substrate ratio were studied.

II. Materials and Methods

A. Preparation of C. papaya lipase

Papaya latex was obtained by making a longitudinal incision on the unripe fruit (70-100 days) of Thai papaya tree. The latex was then collected and stored at -20^{o}C before

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defrosting at room temperature and then centrifuged at 9,500×g for 15 min. The insoluble particulate was lyophilized and used as *C. papaya* lipase. The water content and water activity of *C. papaya* lipase were determined by a Karl-Fischer 684 coulometer equipped with a 688 KF oven (Metrohm, Switzerland) and a Thermoconstanter TH200 Novasina (Novasina, Switzerland), respectively. The hydrolysis activity of *C. papaya* lipase was investigated by colorimetric method [15].

B. Determination of the fatty acid profiles of glycerides in palm oil

Palm oil (40 mg) was weighted into a small Erlenmeyer flask and then 3 ml of 0.5 M methanolic sodium hydroxide were added. The mixture was heated over a steam bath in hood until a homogeneous solution was obtained. For saponification reaction, BF_3-methanol (5 ml) was added to the reaction mixture and then boiled 2 to 3 minutes. The solution was cooled and transferred into a separatory funnel containing 25 ml of hexane and 20 ml of saturated NaCl solution. The solution was gently shaked well and allowed the layers to separate. The hexane layer, containing the fatty acid methyl esters, was dried with about 1 g of anhydrous $MgSO_4$ and filtered into a small vial. The solution was concentrated on the steam bath until the volume was reduced to 0.5 ml. This solution of fatty acid methyl esters was analyzed by gas chromatography-mass spectrometry. Approximate values of the molecular weights of the oils were calculated using data obtained from the fatty acid profiles [7].

C. Modification of palm oil by interesterification

All the interesterification reactions were catalyzed by *C. papaya* lipase (0.5 g) and carried out in 5 ml vials on a shaking water bath (150 rpm) at 40 ^{o}C. The reaction mixture consisted of 2 mmol palm oil was mixed with 2 mmol methyl stearate and 9 mmol of methyl palmitate. After 24 h, samples (10 mg) were taken and the only modified oil was separated from reaction mixture by Thin-layer chromatography (TLC).

D. Isolation of modified oil by TLC

A single plate of TLC on silica gel plate was applied with a solution of reaction mixture in a row across one side of the silica gel plate, 2 cm from the edge. The plate was developed in hexane-diethyl ether-acetic acid (80:20:1). After solvent front rose to about 1 cm from the top of the plate, the plate was removed and made a small scratch at the solvent level. The chromatogram was allowed to dry and then placed in an iodine chamber for several minutes. The plate was removed and lightly trace with a pencil around red-brown band. The mobility of each band was calculated relative to the solvent front (R_f). Only band of TAG on the TLC plates was scrapped. The separated TAGs were converted to fatty acid methyl ester with BF_3- methanol for further analysis.

E. Optimization of cocoa butter equivalent preparation

Experiments were conducted to study the factors, such as acyl donor sources, reaction temperature, reaction time, and initial water activity of enzyme, amount of enzyme and mole ratio of plam oil: methyl stearate, affecting the TAGs content of CBE.

First, effect of acyl donor sources on TAGs content of CBE was studied. The reaction mixture consisted of palm oil 2 mmol and each 4 mmol of acyl donor source such as methyl stearate, ethyl stearate and stearic acid. These reactions catalyzed by *C. papaya* lipase (0.5 g) were carried out at 40 ^{o}C for 24 h. Effect of reaction temperature (40-55 ^{o}C) and reaction time (0-24 h) were investigated by using 0.5 g *C. papaya* lipase, 2 mmol palm oil and 4 mmol methyl stearate. For effect of initial water activity of enzyme, before starting of the reaction, the enzyme was pre-equilibrated with the water vapor of saturated salt solutions. Pre-equilibration was done at 25 ^{o}C for 5 days. The saturated salt solution used were prepared with LiCl (water activity, a_w = 0.11), CH_3COOK (a_w = 0.23), $MgCl_2$ (a_w = 0.33) and $Mg(NO_3)_2$ (a_w = 0.53). A water activity of the bio-catalyst was determined using a Thermoconstanter TH 200 Novasina. The enzyme (0.5 g) with various a_w was added to the reaction mixture consisted of 2 mmol palm oil and 4 mmol methyl stearate. The reaction was carried out at 45 ^{o}C for 4 h.

To study the effect of amount of enzyme, the enzyme (9-36 wt %) having a_w = 0.11 was added into the same reaction. For effect of mole ratio of substrate, various mole ratio (1:1, 1:2.3, 1:3, 1:4 and 1:5.6) of palm oil : methyl stearate were applied to the reaction that consisted of enzyme 18 wt % and the reaction was carried out at 45 ^{o}C for 4 h.

F. Analysis of fatty acid profile

The acyl composition was determined by a gas chromatograph (GC 6850, Agilent Technologies) fitted with a capillary column (HP-1MS, 30 m × 0.25 mm, 0.25 μm thickness) and equipped with mass spectrometer (MSD 5973(EI), Agilent Technologies). The chromatographic conditions were as follow: on temperature of MS Quadrupole and MS Source were 150 ^{o}C and 230 ^{o}C, helium as a carrier gas at flow rate 1.0 ml/min, and an injector temperature of 250 ^{o}C. Separations were made using the following oven temperature profile: initial temperature 140 ^{o}C, programmed to 240 ^{o}C at 10 ^{o}C/min, and final temperature held for 15 min.

III. RESULTS AND DISCUSSION

A. Preparation of C. papaya *lipase*

Fresh papaya latex was found to contain 80 u of lipase / g of latex. High speed centrifugation (9,500×g) was required to separate the particulate part from the latex. This part of latex

possessed all lipase activities which were found to be 200 u of lipase / g of latex, whereas no activities were found in a cleared solution. The optimum temperature and optimum pH of *C. papaya* lipase in hydrolysis activity of palm oil was 45 ^{o}C and 7, respectively. The lipase activity of dried *C. papaya* lipase on palm oil hydrolysis was 725 u of lipase /g of latex. The crude CPL (a_w = 0.396 and 3.60 % water content) was used as biocatalyst in further interesterification reaction of palm oil without purification because of the tight association of lipase with the particulate fraction [16].

B. Characterization of palm oil

The fatty acid contents of palm oil were determined as a preliminary step in this characterization (Table I). Palm oil contained high levels of oleic acid (48%). Structural analysis of the TAGs in palm oil was then carried out to determine its suitability as starting materials for the production of cocoa butter equivalents. It was found that oleic acid is more concentrated in the 2-position of TAGs. This is indicated that the oil from palm oil is suitable for starting materials as it contain high amounts of oleic acid and the major of this fatty acids in the 2-position. This is an important prerequisite of starting oil in the production of cocoa butter equivalents since the 2-oleic acid is required for the maintenance of the characteristic sharp melting point of cocoa butter [17].

TABLE I
FATTY ACID COMPOSITION OF PALM OIL

Fatty Acid	%
Linoleic acid (C 18:2)	9.52
Myristic acid (C 14:0)	0.99
Oleic acid (C 18:1)	48.1
Palmitic acid (C 18:0)	40.8
Stearic acid (C 18:0)	0.57

C. Transesterification of palm oils for the production of cocoa butter equivalents

Both of stearyl donor and palmityl donor were incorporated into palm oil by lipase-catalyzed transesterification at 45 ^{o}C for 24 h. Methyl stearate, ethyl stearate and stearic acid were used as stearyl donor where as methyl palmitate, ethyl palmitate and palmitic acid were used as palmityl donor. In all case of reaction, the ratios of stearic acid: palmitic acid in the product was less than the typical cocoa butter (Table II) because of the quantity of palmitic acid in starting oil relatively high. The typical cocoa butter contains stearic acid and palmitic acid in a ratio of 1.3: 1.0 [5]. This result showed that palm oil contained significant amounts of palmitic acid making them more suitable starting materials as they require only stearic acid donor to be incorporated into the TAGs.

TABLE II
COMPOSITION OF PRODUCT SYNTHESIZED FROM TRANSESTERIFICATION OF PALM OIL WITH STEARYL AND PALMITYL DONOR

Reaction	Ratio of S:P
Palm oil + methyl stearate + methyl palmitate[a]	0.10 : 1.0
Palm oil + ethyl stearate + ethyl palmitate[b]	0.40 : 1.0
Palm oil + stearic acid + palmitic acid[c]	0.27 : 1.0

[a]The lipase (0.5 g) was added to a reaction mixture containing palm oil (2 mmol), methyl stearate (2 mmol) and methyl palmitate (9 mmol) at 45 ^{o}C for 24 h.
[b]The lipase (0.5 g) was added to a reaction mixture containing palm oil (2 mmol), ethyl stearate (2 mmol) and ethyl palmitate (9 mmol) at 45 ^{o}C for 24 h.
[c]The lipase (0.5 g) was added to a reaction mixture containing palm oil (2 mmol), stearic acid (2 mmol), palmitic acid (9 mmol) and 1 ml hexane at 45 ^{o}C for 24 h.
The ratio of S: P in palm oil was 0.06:1. S = stearic acid; P = palmitic acid.

D. Effect of acyl donor

Source of acyl donor plays an important role in the interesterification reaction of oils. In this research, only stearyl donors were incorporated in palm oil because of that oil contained palmitic acid at concentrations near the desired level. Methyl stearate, ethyl stearate and stearic acid were applied to the interesterification of palm oil which catalyzed by *C. papaya* lipase. Among the acyl donors used only methyl stearate was an appropriated stearyl donor for interesterification reaction with palm oil catalyzed by *C. papaya* lipase (Table III) since the product contained stearic and palmitic acid in a ratio of 1.37: 1.0 which similar to cocoa butter [5]. While different lipase such as that from *Rhizopus arrhizus* preferred to catalyzes the interesterification reaction of palm oil with stearic acid or ethyl stearate in CBE preparation [18]-19].

TABLE III
EFFECT OF ACYL DONORS ON FATTY ACID PROFILES OF COCOA BUTTER EQUIVALENT SYNTHESIZED BY *C. PAPAYA* LIPASE

Acyl donor source	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
Methyl stearate	26.9	36.8	30.4	6.0	1.3:1
Ethyl stearate	24.5	52.1	23.4	-	2.1:1
Stearic acid	30.2	24.4	37.7	7.7	0.8:1

The lipase (0.5 g) was added to a reaction mixture containing palm oil (2 mmol) and various acyl donors (4 mmol) at 45 ^{o}C for 4 h. Palm oil composition L : 9.5%; M : 1.0%; O : 48.1%; P : 40.8%; S : 0.6%. L = linoleic acid; M = myristic acid; O = oleic acid; P = palmitic acid; S = stearic acid.

E. Effect of reaction temperature

Temperature also exerts an important influence on enzymatic interesterification. As shown in Table IV, the optimum temperature for interesterification was 45 ^{o}C. With an increase of reaction temperature, in the range of 50-55 ^{o}C, the ratio of stearic acid: palmitic acid decreased respectively. There are several possible reasons to explain this phenomenon: (1) substrates, methyl stearate can be well dissolved at the temperature more than 40 ^{o}C and lead to a low

viscosity reaction mixture in which interesterification can be carried out quickly [20]; (2) activation energy of the interesterification reation is 30.4 j/mol [21]; (3) high temperatures inactivate the lipase [20], which leads to a strong decrease in reaction rate when the temperature is beyond 45 ^{o}C. The temperature of 45 ^{o}C was adopted in further CBE preparation.

TABLE IV
EFFECT OF REACTION TEMPERATURES ON FATTY ACID PROFILES OF COCOA BUTTER EQUIVALENT SYNTHESIZED BY *C. PAPAYA* LIPASE

Reaction temperature (^{o}C)	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
40	32.5	28.9	35.1	3.5	0.9:1
45	26.9	36.8	30.4	6.0	1.3:1
50	31.6	34.8	32.1	1.6	1.1:1
55	31.3	28.2	36.5	4.0	0.9:1

The lipase (0.5 g) was added to a reaction mixture containing 2 mmol palm oil and 4 mmol methyl stearate at various temperatures for 4 h.

F. Effect of reaction time

Reaction time was set from 0 to 24 h to evaluate the time effect on the fatty acid profiles of CBE at 45 ^{o}C using palm oil and methyl stearate as substrates (Table V). The content of palmitic acid decreased rapidly from 62.2% in palm oil to desired level (26.9%) at 4 h. Meanwhile, level of stearic acid increased rapidly from 5.2% in palm oil to 36.8%. Incorporation of stearic acid to desired levels was achieved in shorter reaction times of about 4 h. With an increased of reaction time, the content of stearic acid was incorporated over the demand level. Therefore, on the basis of the above finding, reaction time of 4 h appeared to be optimal and was used in the following experiments.

TABLE V
EFFECT OF REACTION TIMES ON FATTY ACID PROFILES OF COCOA BUTTER EQUIVALENT SYNTHESIZED BY *C. PAPAYA* LIPASE

Reaction time (h)	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
0	62.2	5.2	32.2	0.5	0.08:1
4	26.9	36.8	30.4	5.9	1.3:1
8	24.4	39.3	31.5	4.9	1.6:1
24	19.4	44.3	30.1	0.2	2.3:1

The lipase (0.5 g) was added to a reaction mixture containing palm oil (2 mmol) and methyl stearate (4 mmol) at 45 ^{o}C.

G. Influence of the initial water activity

C. papaya lipase was pre-equilibrated separately at a desired water activity (a_w) using saturated salt solutions (from 0.11 to 0.53). After 5 days, *C. papaya* lipase was mixed with reaction medium and the synthesis of CBE was followed. Table VI showed the effect of initial a_w on the composition of CBE. Interesterification of palm oil with methyl stearate performed best when *C. papaya* lipase had initial a_w = 0.11. They decreased when the initial water activity rose. It can be explained by the role of water during synthesis. In lipase-catalyzed interesterification, hydrolysis and esterification take place separately. As a reactant in the hydrolysis step and a product in the esterification step, water shifts the enzymatic interesterification equilibrium [20]. A small amount of water is needed to maintain enzyme activity. However, at high water activity value, the equilibrium of the reaction was shifted towards hydrolysis, leading to higher production of by-products such as diacylglycerols and fatty acids. To avoid producing by-products, *C. papaya* lipase with initial a_w = 0.11 was chosen as biocatalyst in further experiment.

TABLE VI
EFFECT OF INITIAL WATER ACTIVITY ON THE FATTY ACID PROFILES OF COCOA BUTTER EQUIVALENT

Initial water activity	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
0.11	26.9	36.8	30.4	6.00	1.3:1
0.23	38.7	29.8	31.5	0	0.8:1
0.33	49.5	20.4	30.1	0	0.4:1
0.53	51.2	16.7	32.1	0	0.3:1

The lipase (0.5 g) with various water activities was added to a reaction mixture containing palm oil (2 mmol) and methyl stearate (4 mmol) at 45 ^{o}C for 4 h.

H. Effect of amount of the C. papaya lipase

The amount of *C. papaya* lipase added was related to the synthesis of CBE (Table VII). Increased enzyme load would improve the incorporation of acyl donors in interesterification under certain conditions. The best amount of CPL was 18% by weight for interesterification of palm oil with methyl stearate. Increasing the amount of lipase above 18 wt% had no significant effect on substrate conversion.

TABLE VII
EFFECT OF ENZYME QUANTITY ON THE FATTY ACID PROFILES OF COCOA BUTTER EQUIVALENT FROM INTERESTERIFICATION CATALYZED BY *C. PAPAYA* LIPASE

Quantity of enzyme (Wt %)	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
9	44.6	20.3	30.1	5.0	0.5:1
18	26.9	36.8	30.4	6.0	1.3:1
27	28.1	38.5	30.1	3.3	1.3:1
36	28.1	38.5	30.5	2.8	1.3:1

The various amount of lipase with water activity = 0.11 was added to a reaction mixture containing palm oil (2 mmol) and methyl stearate (4 mmol) at 45 ^{o}C for 4 h.

İ. Effect of substrate ratio

To optimize the effect of palm oil: methyl stearate mole ratio on fatty acid content of CBE, various ratios of palm oil: methyl stearate (1:1, 1:2.3, 1:3, 1:4, and 1:5.6) were applied to the reaction and the interesterification reaction was carried out at 45 ^{o}C for 4 h. Apparently, higher in methyl stearate levels increased the ratio of stearic acid: palmitic acid (Table VIII).

The fatty acid profiles of CBE similar to that in cocoa butter, when the ratio of palm oil: methyl stearate was 1:4 and 1:5.6. Because of methyl stearate relatively high cost, the mole ratio of palm oil: methyl stearate for cocoa butter equivalent production was optimized to be 1:4.

TABLE VIII
EFFECT OF PALM OIL: METHYL STEARATE RATIO ON THE SYNTHESIS OF COCOA BUTTER EQUIVALENT

Ratio of palm oil: methylstearate (mol: mol)	Fatty acid (%)				Ratio of S:P
	C16:0	C18:0	C18:1	C18:2	
1:1	46.8	23.4	29.8	0	0.5:1
1:2.3	34.2	27.7	31.6	6.5	0.8:1
1:3	29.8	30.2	31.4	8.6	1.0:1
1:4	26.9	36.8	30.4	6.0	1.3:1
1:5.6	28.1	38.5	30.1	3.3	1.3:1

The lipase (18 wt %, a_w = 0.11) was added to a reaction mixture containing various ratios of palm oil: methyl stearate at 45 ^oC for 4 h.

IV. CONCLUSION

This study has shown that palm oil can be modified to cocoa butter equivalent by *C. papaya* lipase-catalyzed interesterification. The reaction conditions were optimized to be 45 ^oC for 4 h using palm oil and methyl stearate, at a mole ratio of 1:4, as substrates and 18 wt% of enzyme with initial a_w = 0.11. The chemical and physical properties of cocoa butter equivalent such as free fatty acid, iodine number, sponification value and melting point similar to typical cocoa butter. However, yield of cocoa butter equivalent under optimized conditions will be further investigated.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support from The Thailand Research Fund.

REFERENCES

[1] D. Undurraga, A. Markouits and S. Erazo, "Cocoa butter equivalent through enzymic interesterification of palm oil midfraction," *Process Biochem,* vol. 36, pp. 933–939, Octo. 2001.
[2] M. Lipp, C. Simoneau, F. Ulberth, E. Anklam, C. Crews, P. Prereton, W. Greyt, W. Schwack and C. Wiedmaier, "Composition of genuine cocoa butter and cocoa butter equivalents," *J. Food Compos. Anal.,* vol. 14, pp. 399-408, Mar. 2001.
[3] K. J. Liu, H. M. Chang and K. M. Liu, "Enzymatic synthesis of cocoa butter analog through interesterification of lard and tristearin in supercritical carbon dioxide by lipase," *Food Chem*., vol. 100, pp. 1303-1311, Dec. 2007.
[4] V. K. S. Shukla, *Confectionery fats.* Blackie Academic and Professional, Glasgow, 1996, pp. 66-94.
[5] F. Padley, F. Gunstone, J. Harwood, "Occurrence and characteristics of oils and fats," Chapman and Hall, London, 1986, pp. 49-170.
[6] H. X. Wang, H. Wu, C. T. Ho and X. C. Weng, "Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters," *Food Chemistry.,* vol. 97, pp. 661-665, Apr. 2006.
[7] M. K. Chang, G. Abraham and V. T. John, "Production of cocoa butter-like fat from interesterification of vegetable oils," *J. Am. Oil Chem. Soc.,* vol. 67, pp. 832-834, Aug. 1990.
[8] A. R. Macrae, "Lipase-catalyzed interesterification of oils and fats," *J. Am. Oil Chem. Soc.,* vol. 60, pp. 291-294, Feb. 1983.
[9] R. Sridhar, G. Lakshminarayana and T. N. B. Kaimal, "Modification of selected Indian vegetable fats into cocoa butter substitutes by lipase-catalyzed ester interchange," *J. Am. Oil Chem. Soc.,* vol. 68, pp. 726-730, June. 1991.
[10] R. A. Wisdom, P. Dunnil and M. D. Lilly, "Enzymatic interesterification of fats : laboratory and pilot-scale studies with immobilized lipase from Rhizopus arrhizus," *Biotechnol. Bioeng.,* vol. 29, pp. 1081-1085, Dec. 1986.
[11] P. Villeneuve, A. Skarbek, M. Pina, J. Graille and T. A. Foglia, "Catalytic behavior of Carica papaya latex in transesterification reactions," *Biotech. Tech.,* vol. 11, pp. 637-639, May. 1997.
[12] J. M. Graille, D. M. Pina and J. M. Muderwha, "Making value-added products from palm oil by 1-3 regioselectivity enzymatic interesterification," *Elaeis.,* vol. 41, pp. 10-13, Jan. 1991.
[13] T. A. Foglia and P. Villeneuve, "Carica papaya latex lipase-catalyzed synthesis of structured triacylglycerols," *J. Am. Oil. Chem. Soc.,* vol. 74, pp. 1447-1450, Nov. 1997.
[14] D. Y. Kwon and J. S. Rhee, "A simple and rapid colorimetric method for determination of free fatty acids for lipase assay," *J. Am. Oil. Chem. Soc.,* vol. 63, pp. 89-92, Jan. 1986.
[15] R. Giordani, A. Moulin and R. Verger, "Tributyroylglycerol hydrolase activity in Carica papaya and other lattices," *Phytochemistry,* vol. 30, pp. 1069-1072, May. 1991.
[16] L. W. Khumalo, L. Majoko, J. S. Read and I. Ncube, "Characterisation of some underutilized vegetable oils and their evaluation as starting materials for lipase-catalysed production of cocoa butter equivalents," *Ind. Crops Products*, vol. 16., pp. 237-244., Aug. 2002.
[17] S. Bloomer, P. Adlercreatz and B. Mattiasson, "Triglyceride interesterification by lipase. 1. Cocoa butter equivalents from a fraction of palm oil," *J. Am. Oil Chem. Soc.*, vol. 67, pp. 519-524, Aug. 1990.
[18] L. Mojovic, S. Siler-Marinkovic, G. Kukic and G. Vunjak-Novakovic, "Rhizopus arrhizus lipase-catalyzed interesterification of the mid fraction of palm oil to a cocoa butter equivalent fat," *Enzyme Microb. Technol.*, vol. 15, pp. 438-443, May. 1993.
[19] H-X. Wang, H. Wu, C-T. Ho and X-C. Weng, "Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters," *Food Chemistry,* vol. 97, pp. 661-665, Apr. 2006.
[20] H. L. Goderis, "Lipase-catalyzed ester exchange reaction in organic media with controlled humidity," *Biotechnol. Bioeng.*, vol. 130, pp. 256-265, Apr. 1990.

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