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OPTIMIZATION OF THE PRODUCTION OF STRUCTURED LIPID BY ENZYMATIC INTERESTERIFICATION FROM COCONUT (*COCOS NUCIFERA*) AND SESAME (*SESAMUM INDICUM*) OILS USING RESPONSE SURFACE METHODOLOGY

Subajiny Sivakanthan[1], Anura Prasantha Jayasooriya[2] and Terrence Madhujith[3]*

[1]Department of Agricultural Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka
[2]Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka
[3]Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka

ABSTRACT: *Blends of coconut (Cocos nucifera) oil (CO) and sesame (Sesamum indicum) oil (SO) were enzymatically interesrerified using aqueous lipase derived from Rhizomucor miehei and the reaction conditions, namely, temperature (45-65 °C), time (16-48 h) and mass ratio of oils (CO:SO; 70:30 - 50:50) were optimized using Response Surface Methodology (three-factor, three-level central composite design). Degree of interesterification (DI), and the ratio of monounsaturated and polyunsaturated fatty acids (MUFA:PUFA) of triacylglycerols were used as response variables. The linear effects of all factors were significant for the DI while for MUFA:PUFA, the linear effect of oil ratio and interaction effect of time and oil ratio showed significant effects. The conditions, temperature; 57.12 °C, time; 16 h and weight ratio of oil (CO:SO); 50:50 were found to be the optimum. The R^2 value for DI and MUFA:PUFA ratio were 0.80 and 0.82, respectively. Models fitted for both DI and MUFA:PUFA ratio were significant with non-significant lack of fit. Therefore, the constructed models and data provide useful information to produce structured lipid from interesterification of CO and SO in up-scaled level. The produced novel lipid containing beneficial fatty acids from both oils could be used to produce healthy fat based products.*

KEYWORDS: coconut oil, interesterification, lipase, optimization, sesame oil

INTRODUCTION

Interesterification is the exchange of fatty acids within and among triacylglycerol (TAG) moieties leading to generation of structured lipids. These specialty lipids could be designed to contain the desired fatty acid composition having a multitude of applications as for medicinal and nutritional purposes and for the food industry (Sreenivasan, 1978; Reena *et al.*, 2009). Interesterification can be performed either chemically or enzymatically. Of the two methods, enzymatic interesterification offers advantages over chemical interesterification such as mild processing conditions involved, preservation of fatty acids in *sn*-2 position when *sn*-1,3 specific lipase is used, thus preserving its natural benefits, less by-products and easy control of the process (Zhang *et al.*, 2004). Enzymatic interesterification is gaining popularity as a green technology to produce modified lipids with improved nutritional and functional benefits and without *trans* fats (Lee and Akoh, 1998). The present study employed interesterification to produce structured lipid without *trans* fats using two edible vegetable oils commonly available in Sri Lanka.

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Coconut (*Cocos nucifera*) is one of the major plantation crops cultivated in Sri Lanka over many decades while CO is the widely used edible oil in the country accounting for approximately 80% of fat intake by Sri Lankans (Amarasiri and Dissanayake, 2006). Controversy appears regarding the nutritional value of CO which is composed of 92% of saturated fatty acids of which more than 50% are medium chain fatty acids (MCFAs) such as C8:0, C10:0 and C12:0. According to the universally accepted Lipid-Heart Theory, high saturated fats lead to hypercholesterolemia and coronary heart disease. Long chain fatty acids (LCFAs) are known to be associated with the risk of increasing heart diseases. However, MCFAs which are metabolized rapidly in the liver to energy and do not participate in the biosynthesis and transport of cholesterol are known to increase serum high density lipoprotein (HDL) (Dayrit, 2003). MCFAs such as C8:0 and C10:0 follow this type of metabolism, however, there is evidence that C12:0 (lauric acid) follows the absorption pattern of both LCFAs and MCFAs, even though C12:0 is classified as MCFAs. Thus, there are concerns as lauric acid partly function like LCFA. Thus, presence of high amount of lauric acid in the diet may contribute to increase the risk of heart disease (Jandacek, 1994; Amarasiri and Dissanayake, 2006). In this backdrop, replacing some of the saturated fatty acids (SFAs) such as lauric acid and LCFAs with nutritionally important fatty acids such as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs) is beneficial.

Since antiquity, sesame (*Sesamum indicum*) oil has been well known for its nutritional and medicinal value. It is rich in unsaturated fatty acids (more than 85%) of which 39% is MUFA and 46% is PUFAs (Dubois *et al.*, 2007). Thus, incorporating these fatty acids from SO into CO by means of enzymatic interesterification will replace some lauric acid and LCFAs and contribute to superior oil.

The aim of this study was to optimize the parameters of enzymatic interesterification of CO and SO by lipase (*sn* 1, 3 specific) derived from *Rhizomucor miehei* using Response Surface Methodology (RSM). Even though studies have been carried out to enzymatically interesterifiy CO (Ibrahim *et al.*, 2008; Adhikari *et al.*, 2010; Ruan *et al*., 2014) and SO (Lopez-Hernandez *et al*., 2007) with other edible oils, studies on enzymatic interesterification of CO and SO are scanty. In Sri Lanka, no study has been carried out to date on enzymatic interesterification of edible vegetable oils. In this context, the present study will fill this gap and explore the feasibility of using enzymatic interesterification to produce a structured lipid having balanced fatty acid composition and modified physical and chemical properties from two edible oils available in the country.

MATERIALS AND METHODS

Materials

Solvents, chemicals, lipase derived from *R. miehei* (≥30,000U/g) and Tween® 40 (polyoxyethylenesorbitan monopalmitate) **and** authentic fatty acid standards for Gas Liquid chromatography (GLC) (SUPELCO 37 Component FAME Mix) and Thin Layer Chromatography (TLC) (1-oleoyl-*rac*-glycerol, 1, 2-dipalmitoyl-*sn*-glycerol, 1,2-dipalmitoyl-*rac*-glycerol glyceryltrilaurate, glyceryltrioleate and glyceryltripalmitate) were purchased from Sigma Aldrich, USA. TLC plates (TLC silica gel 60 F_{254}, 20x20cm) were purchased from Merck (Darmstadt, Germany). Gases used for GLC: helium (purity 99.99%) and hydrogen were purchased from Ceylon Oxygen (Pvt) Ltd, Sri Lanka. All chemicals, solvents and gases

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used in the study were of analytical grade or chromatographic grade with the highest purity available. Regular CO (copra oil) was purchased from a local oil mill located in Kegalle, Sri Lanka and SO was purchased from a local oil mill located in Jaffna, Sri Lanka. Oil samples were stored in tightly closed glass containers covered with aluminium foil after flushing with nitrogen gas at 4 °C.

METHODS

Enzymatic Interesterification

The reaction parameters used for the RSM: temperature (°C) (X_1), time duration of reaction (h) (X_2) and weight ratio of oils (w/w) (X_3) and their levels used are shown in Table 1. CO and SO were weighed at particular ratio (50:50, 60:40 or 70:30) keeping the total weight of substrate 30 g into a clean, dry Erlenmeyer flask and 0.5% (w/w) of Tween® 40 was added. The flask was covered with an aluminium foil, stoppered and stirred for 10 min at 150 rpm using a magnetic stirrer. Lipase derived from *R. miehei* diluted in phosphate buffer (0.2 M, pH 8) was added, stoppered and reacted immediately in a shaking water bath (Yamato BW 100) at different temperatures (45, 55 or 65 °C) and 100 rpm and samples were drawn at the particular time intervals (16, 32 or 48h). Samples were added into glass vials (3 mL) and enzyme was inactivated by adding acetic acid (0.25%). Then the samples were sealed using Parafilm and stored at 2-8 °C for further analysis.

Table 1. The levels of independent variables used for RSM

Independent variable (X_i)	Levels		
	-1	0	+1
Temperature (°C) (X_1)	45	55	65
Time (h) (X_2)	16	32	48
Oil ratio* (X_3)	0.5	0.6	0.7

*The values 0.5, 0.6 and 0.7 are used to denote the weight ratios of oils (CO:SO) such as 50:50, 60:40 and 70:30 respectively.

Statistical Design

Reaction parameters were optimized using RSM. MINITAB 17 statistical software was used to design the experiments using RSM. A three-factor and three-level CCD (face-centred cube design) with 20 individual design points was used. Responses or dependent variables (Y) studied were DI (%) and MUFA:PUFA ratio of the TAG fraction of the interesterified oils. Table 2 shows the experimental design with coded and actual values of independent variables such as temperature, time and oil ratio. Triplicate experiments were carried out for each run.

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Table 2. Experimental design for DI and MUFA:PUFA ratio of interesterified oil with coded and actual values of independent variables such as temperature (X_1) (°C), time (X_2) (h) and oil ratio (X_3) according to CCD (face-centred cube design)

Run	Independent variables			Responses	
	X_1	X_2	X_3	DI	MUFA:PUFA
1	55(0)	32(0)	0.6(0)	15.65±0.04	1.40±0.03
2	65(+1)	16(-1)	0.7(+1)	17.17±0.49	1.43±0.05
3	55(0)	16(-1)	0.6(0)	18.51±1.13	1.34±0.02
4	55(0)	32(0)	0.6(0)	16.82±2.86	1.32±0.00
5	65(+1)	16(-1)	0.5(-1)	21.94±0.72	1.47±0.00
6	45(-1)	32(0)	0.6(0)	18.44±2.74	1.25±0.02
7	65(+1)	48(+1)	0.5(-1)	16.60±0.56	1.31±0.00
8	55(0)	48(+1)	0.6(0)	16.65±0.36	1.29±0.03
9	65(+1)	48(+1)	0.7(+1)	9.12±1.63	1.66±0.07
10	55(0)	32(0)	0.6(0)	16.02±0.65	1.39±0.06
11	45(-1)	16(-1)	0.7(+1)	26.31±1.78	1.37±0.05
12	55(0)	32(0)	0.6(0)	13.68±2.39	1.34±0.01
13	55(0)	32(0)	0.6(0)	13.56±2.53	1.32±0.01
14	45(-1)	48(+1)	0.5(-1)	23.26±1.68	1.24±0.01
15	55(0)	32(0)	0.5(-1)	25.31±1.05	1.27±0.01
16	55(0)	32(0)	0.6(0)	17.23±3.09	1.25±0.01
17	45(-1)	16(-1)	0.5(-1)	23.27±1.68	1.36±0.01
18	45(-1)	48(+1)	0.7(+1)	13.64±0.54	1.51±0.03
19	65(+1)	32(0)	0.6(0)	17.65±0.67	1.26±0.01
20	55(0)	32(0)	0.7(+1)	17.81±3.11	1.43±0.02

Separation of Lipid Fractions by Thin Layer Chromatography (TLC)

TAG fraction of the interesterified oil samples as well as their respective blends were separated using TLC. Sample (1 mL) was dissolved in 4 mL of hexane and spotted on a TLC plate. Solvent mixture of hexane:diethylether:glacial acetic acid (70:30:1) was used as the mobile phase. Separated components were identified by spraying boric acid solution (10% boric acid in 20% ethanol). The spots were identified by comparing the R_f value of authentic standards (1-oleoyl-*rac*-glycerol, 1, 2-dipalmitoyl-*sn*-glycerol, 1,2-dipalmitoyl-*rac*-glycerol glyceryltrilaurate, glyceryltrioleate and glyceryltripalmitate). The TAG spot was carefully scraped off along with silica and transferred into a screw capped tube containing 0.6 mL of hexane and centrifuged at 1500 rpm for 10 min. Then hexane layer was transferred into another tube. The extraction process was repeated once more and the hexane containing TAGs was combined and evaporated to concentrate fatty acids by flushing with nitrogen and used for analysis of fatty acid composition by GLC.

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Determination of Triacylglycerol (TAG) Composition

Fatty acid composition of the separated TAGs were determined by GLC. Fatty acid methyl esters (FAMEs) were prepared according to Christie (1992) and analyzed by injecting 1 µL into GLC (Shimadzu, 14-B, Japan), equipped with a Flame Ionization Detector (FID) and a fused silica capillary column (100 m, 0.25 mm id and 0.20 µm film thickness. The split ratio was set at 80:1. Helium was used as carrier gas at flow rate of 20 mL/min. Injector and detector temperatures were maintained at 260 °C. The initial column temperature was maintained at 140°C for 5 min and increased to 220°C at the rate of 4°C/min, then maintained at that temperature for 10 min. Fatty acids were identified by comparison of their retention time with authentic standards (SUPELCO 37 Component FAME Mix). The amount of each fatty acid in the sample was expressed as % of the sum of all fatty acids in the sample.

Degree of Interesterification (DI)

DI was determined using the equation explained by Nunes *et al.* (2011) with slight modifications. Fatty acids with major increment and fatty acids with major decrement were considered to determine the DI. The DI is defined as follows;

$$DI\ (\%) = \frac{\Sigma(FA_{IT} - FA_{I0})}{\Sigma\ (FA_{D0})} \times 100$$

Where, FA_I is the % area of fatty acids which increased during the reaction, FA_D is the % area of fatty acids, which decreased during the reaction, subscripts T and 0 represent the area % of fatty acids at a given reaction time and at the beginning of the reaction, respectively.

Determination of Mufa: Pufa Ratio of TAGs

Based on the fatty acid composition of TAGs as determined by GLC, the MUFA:PUFA ratio was calculated.

Scaling up and Determination of Proportion of Lipid Classes of Interesterified Oil

Interesterification reaction was carried out in scaled up level using the optimized parameters determined based on the analysis of RSM design. The total amount of substrate used for the scaled up reaction was 1 kg. The proportion of lipid classes such as TAG, diacylglycerol (DAG), monoacylglycerols (MAG) and free fatty acids of oil interesterified under optimized conditions were determined. Lipid classes such as TAG, DAG, MAG and free fatty acids were separated using TLC as explained above and identified using authentic standards. Each spot was marked and scraped off separately and placed in glass vials. A known quantity of internal standard (methyl heptadecanoate; 1 mg/mL) was added to each tube and fatty acids were extracted into hexane and analyzed for the fatty acid composition using GLC. The peak areas of fatty acids and internal standard recorded on the gas chromatograms were used for estimation of relative proportions of different lipid classes.

RESULTS AND DISCUSSION

Lipase can catalyze the hydrolysis reaction in aqueous mixtures but the substrates are generally insoluble in water. For industrial applications, interesterification reactions are best carried out either in organic media or in non-solvent systems in which the water content can be controlled

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(Maruyama, Nakajima, Ichikawa, Nabetania, Furusaki, and Seki, 2000). The present study was carried out in solvent-free system. Therefore, surfactant (Tween®40) was used in this study to make emulsions in which lipase can react effectively.

Table 2 shows the DI and MUFA:PUFA ratio of oils interesterified according to CCD. The DI varied from 9.12±1.63 to 26.31±1.78%. The values of MUFA:PUFA ratio ranged from 1.24±0.01 to 1.66±0.07. Based on the RSM analysis, optimum reaction parameters selected to maximize both responses such as DI and MUFA:PUFA ratio were temperature; 57.12 °C, time; 16 h and weight ratio of oil (CO:SO) 50:50. According to the analysis, under these optimized conditions, the expected DI and MUFA:PUFA ratio were 22.60±2.19% and 1.43±0.05, respectively at 95% confidence interval.

Estimated effects, standard error coefficients, t-values and p-values for DI and MUFA:PUFA ratio of TAG of interesterified oil are shown in Table 3. All three factors exhibited significant ($p<0.05$) linear effect on DI, while, linear effect of oil ratio and interaction effect of time and oil ratio had significant effect on MUFA:PUFA ratio.

Table 3. Estimated effects, standard error coefficients, t-values and p-values for DI and MUFA:PUFA ratio of TAG of interesterified oil according to CCD (face-centred cube design)

Response variable	Independent and variable interactions	Estimated effects	SE Coefficient	t-value	p-value
DI	X_1	-4.487	0.825	-2.72	0.002*
	X_2	-5.589	0.825	-3.39	0.007*
	X_3	-5.265	0.825	-3.19	0.010*
	X_1^2	-0.19	1.57	-0.06	0.953
	X_2^2	-1.13	1.57	-0.36	0.727
	X_3^2	6.84	1.57	2.17	0.055
	$X_1 X_2$	-0.179	0.922	-0.10	0.924
	$X_1 X_3$	-1.419	0.922	-0.77	0.460
	$X_2 X_3$	-3.842	0.922	-2.08	0.064
MUFA:PUFA	X_1	0.0795	0.0191	2.09	0.064
	X_2	0.0080	0.0191	0.21	0.838
	X_3	0.1520	0.0191	3.99	0.003*
	X_1^2	-0.0355	0.0363	-0.49	0.636
	X_2^2	0.0741	0.0363	1.02	0.332
	X_3^2	0.1508	0.0363	2.07	0.065
	$X_1 X_2$	0.0140	0.0213	0.33	0.750
	$X_1 X_3$	0.0072	0.0213	0.17	0.869
	$X_2 X_3$	0.1657	0.0213	3.89	0.003*

*$p<0.05$, X_1=temperature, X_2= time, X_3= oil ratio

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Regression analysis was performed in order to fit the response variables as a function of independent variables. The regression equations for DI and MUFA: PUFA ratio as a function of temperature (X_1), time (X_2) and oil ratio (X_3) are shown in the Equations 1 and 2, respectively.

$$DI = 120.8 + 0.32\ X_1 + 0.718\ X_2 - 359\ X_3 - 0.0009\ X_1^2 - 0.00221\ X_2^2 - 342\ X_3^2 - 0.00056\ X_1 X_2 - 0.709\ X_1 X_3 - 1.201\ X_2 X_3 \quad \text{.....1}$$

$$MUFA:PUFA = 4.15 + 0.0199\ X_1 + 0.0425\ X_2 - 10.14\ X_3 - 0.000178\ X_1^2 - 0.000145\ X_2^2 + 7.54\ X_3^2 - 0.000044\ X_1 X_2 + 0.0036\ X_1 X_3 + 0.0518\ X_2 X_3 \quad \text{.....2}$$

These two models were validated by analysis of variances (ANOVA) which is shown in Table 4. The model fitted for both DI and MUFA:PUFA ratio were significant at 95% confidence level with non-significant lack of fit. The R^2 value for DI was 80.35% and MUFA:PUFA ratio was 82.46%. This indicates that these models can explain more than 80% of the variability for DI and more than 82 % of the variability for MUFA:PUFA ratio.

Table 4. Analysis of Variance (ANOVA) of the fitted models for DI and MUFA:PUFA ratio of TAG of interesterified oil according to CCD (face-centred cube design)

Response variable	Factor	Degrees of freedom	Adjusted sum of square	Adjusted mean square	F-value	p-value
DI	Model	9	278.185	30.9095	4.54	0.013*
	X_1	1	50.334	50.3343	7.40	0.022*
	X_2	1	78.082	78.0820	11.48	0.007*
	X_3	1	69.307	69.3071	10.19	0.010*
	X_1^2	1	0.025	0.0245	0.00	0.953
	X_2^2	1	0.877	0.8773	0.13	0.727
	X_3^2	1	32.130	32.1299	4.72	0.055
	$X_1 X_2$	1	0.064	0.0643	0.01	0.924
	$X_1 X_3$	1	4.025	4.0247	0.59	0.460
	$X_2 X_3$	1	29.517	29.5165	4.34	0.064
	Error	10	68.029	6.8029		
	Lack-of-fit	5	55.901	11.1801	4.61	0.059
	Pure error	5	12.129	2.4257		
MUFA:PUFA	Model	9	0.1707	0.0189	5.23	0.008
	X_1	1	0.0157	0.0157	4.35	0.064
	X_2	1	0.0001	0.0001	0.04	0.838
	X_3	1	0.0577	0.0577	15.91	0.003
	X_1^2	1	0.0008	0.0008	0.24	0.636
	X_2^2	1	0.0037	0.0037	1.04	0.332
	X_3^2	1	0.0156	0.0156	4.31	0.065
	$X_1 X_2$	1	0.0003	0.0003	0.11	0.750
	$X_1 X_3$	1	0.0001	0.0001	0.03	0.869
	$X_2 X_3$	1	0.0548	0.0548	15.12	0.003
	Error	10	0.0363	0.0036		
	Lack-of-fit	5	0.0200	0.0040	1.23	0.413
	Pure error	5	0.0162	0.0032		

*$p<0.05$, X_1=temperature, X_2= time, X_3= oil ratio

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According to Figure 1 (a), DI can be maximized by using low temperature for low duration. Figure 1 (b) illustrates that higher DI could be obtained at low temperature with oil ratio of CO:SO, 50:50. According to Figure 1 (c), DI can be maximized by reducing the time and using oil ratio to have high proportion of CO or equal proportion of CO and SO. MUFA:PUFA ratio can be maximized by using high temperature and high time duration [Figure 2 (a)] or using high temperature and using oil ratio to have high proportion of CO [Figure 2 (b)] or by combination of using high time duration and using oil ratio to have high proportion of CO [Figure 2 (c)].

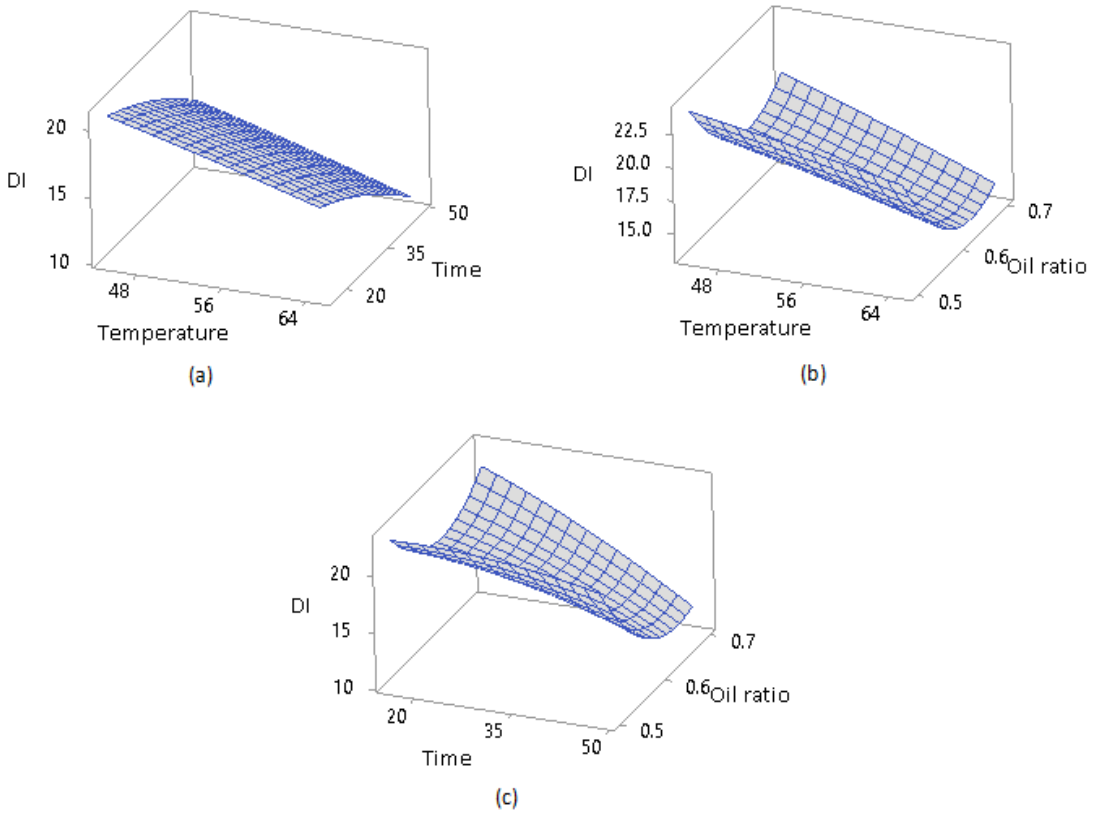

Figure 1. (a)Three dimensional surface plot of DI versus time and temperature; (b): Surface plot of DI versus oil ratio and temperature; (c): Surface plot of DI versus oil ratio and time.

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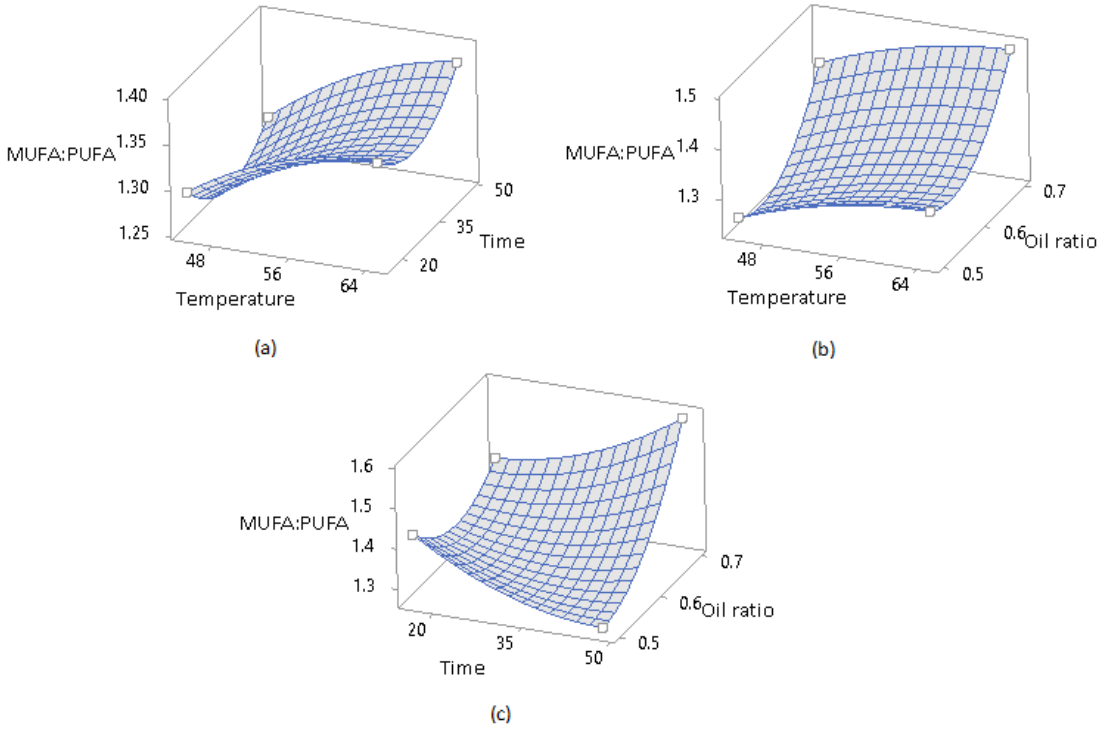

Figure 2. (a)Surface plot of MUFA:PUFA versus time and temperature; (b): Surface plot of MUFA:PUFA versus oil ratio and temperature; (c): Surface plot of MUFA:PUFA versus oil ratio and time.

The yield of enzymatic reactions depends on reaction parameters such as temperature, time, pH, substrate composition, surface active agents etc. (Willis and Maragoni, 2002). In interesterification, the optimum conditions required for the reaction depend on the expected outcome. In the present study, conditions were selected to maximize the incorporation of fatty acids from SO into CO and thus increase MUFA:PUFA ratio of the TAG. In addition, the optimum conditions may differ depending on the activity of enzyme, micro-aqueous environment of the reaction medium, fatty acid composition of the substrate etc. Therefore, hardly the conditions obtained from this study can be compared with the optimum conditions obtained from the other studies. To the best of our knowledge, no studies have been carried out on optimization of enzymatic interesterification of CO and SO. A study has been carried out by Reena and Lokesh (2007) to study the hypolipidimic effect of structured lipid prepared by interesterification of blended oil comprising CO and SO using lipase from *R. miehei* for 72 h at 37°C using animal models. However the study did not include optimization of the reaction parameters. However, in the present study, the robustness of the RSM as evaluated by the regression coefficients (R^2) for both responses (DI and MUFA:PUFA ratio) indicate that the developed models can explain the effect of variables (reaction parameters).

The fatty acid composition and MUFA:PUFA, SFA:MUFA:PUFA and MCFA:LCFA ratios of TAGs of CO, SO and oil interesterified under optimized conditions and its blend are shown in Table 5. During interesterification of CO and SO using *R. miehei* lipase, major changes in the amount of fatty acids occurred in lauric and oleic acids compared to other fatty acids. There was no significant ($p>0.05$) difference between the MUFA:PUFA ratio of interesterified oil

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and physical blend, the interesterified oil had balanced proportion of SFA:MUFA:PUFA (1.9:1.5:1) compared to that of blend (2.4:1.4:1).

Table 5. The fatty acid composition and fatty acid ratios of TAGs of CO, SO and oil interesterified using R. miehei lipase under optimized conditions and its blend

Fatty acid/ fatty acid ratio	SO	CO	Blend	IE
Caprylic acid	ND	2.67±0.27^{a}	1.22±0.04^{b}	2.00±0.15^{c}
Capric acid	ND	3.60±0.08^{a}	1.70±0.01^{c}	2.49±0.16^{b}
Lauric acid	ND	52.15±0.52^{a}	26.38±1.34^{b}	21.11±0.15^{c}
Myristic acid	ND	21.20±0.64^{a}	10.42±0.40^{b}	7.86±0.64^{c}
Palmitic acid	7.82±0.24^{b}	8.80±0.64^{ab}	8.06±0.14^{b}	9.35±0.32^{a}
Stearic acid	3.30±0.14^{a}	0.84±0.05^{c}	2.40±0.14^{b}	0.59±0.04^{c}
Oleic acid	48.88±0.95^{a}	8.47±0.74^{d}	28.76±1.47^{c}	33.69±0.72^{b}
Linoleic acid	39.61±0.56^{a}	2.29±0.33^{d}	21.07±0.29^{c}	22.93±0.28^{b}
Linolenic acid	0.41±0.02	ND	ND	ND
MUFA:PUFA	1.22±0.04^{b}	3.72±0.22^{a}	1.36±0.05^{b}	1.47±0.05^{b}
SFA:MUFA:PUFA	1:4.3:3.6	39:3.7:1	2.4:1.4:1	1.9:1.5:1
MCFA:LCFA	-	1.40±0.01^{a}	0.34±0.003^{c}	0.41±0.03^{b}

Values with different superscripts in the same raw imply significant differences ($p < 0.05$).

Abbreviations: IE; interesterified oil

Even though lauric acid is classified under the group of MCFAs, during metabolism, it behaves like long chain saturated fatty acids (Jandacek, 1994). Thus, reducing the amount of lauric acid to some extent may be beneficial to reduce the risk of heart diseases, even though lauric acid exerts some beneficial effect as MCFA. Therefore in this study, reduction in the amount of lauric acid in the interesterified TAG could be considered a positive effect. The oxidative stability of the oil depends on the ratio of MUFA:PUFA rather than the total amounts of MUFA and PUFA. In the present study, even though MUFA:PUFA ratio of SO, blend and interesterified oil did not differ significantly ($p>0.05$), total amount of MUFA and PUFA increased significantly ($p<0.05$). The aim of the study was to maximize the incorporation of MUFA and PUFA from SO into TAGs of CO considering their nutritional and health benefits. Considering SFA:MUFA:PUFA ratio, interesterified oil had balanced fatty acid composition compared to original oils and blend.

SO is mainly composed of unsaturated fatty acids (>90 %), mainly oleic and linoleic acids. Oleic acid (C18:1) is the MUFA (39%) and linoleic acid (C18:2) is the PUFA (45%) (Dubois et al., 2007). MUFA is well known for its nutritional and functional benefits and it is less prone to oxidative deterioration compared to PUFAs. Even though linoleic acid is an essential fatty acid, it can easily be oxidized thus may impart a negative effect on the oxidative stability of the interesterified oils. Even though, SO is highly stable against oxidation owing to the presence of natural antioxidants such as tocopherol and other minor components, inferior oxidative stability of structured lipids with respect to original oils have been reported, attributed mainly to the loss of endogenous antioxidants (Martin *et al.*, 2010; Wirkowska *et al.*, 2012). Due to these reasons, in this study it was decided to maximize the amount of MUFA while reducing the amount of PUFA. Even though saturated fatty acids are linked with causation of

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coronary heart diseases, MCFAs (C8 and C10) which is present in CO are easily metabolized in the body and does not contribute to adipogenesis. Therefore these MCFAs from CO are considered beneficial for health. The oil interesterified under optimum conditions showed significantly ($p<0.05$) higher proportion of MCFA:LCFA than pure coconut oil.

The lipase used in the present study is *sn*-1 and 3 specific, hence, they can act only on *sn*-1 and 3 positions. Since most saturated fatty acids are found in external positions (*sn*-1 and 3) (Pham and Gregorio, 2008), they can be interesterified by the lipase used in the study. Even though most unsaturated fatty acids are found in *sn*-2 position, SO has relatively high amounts of trilinoleic and trioleic TAGs. Therefore the oleic and linoleic acids are also interesterified using the lipases used in the study. Hence, it could be possible to incorporate the fatty acids from SO TAGs in to TAGs of CO and *vice-versa*.

The interesterification reaction using *R. miehei* lipase was carried out under the optimized conditions in up-scaled level (total weight of the substrate was 1 kg) to confirm the results obtained by RSM. The DI and MUFA:PUFA ratio of interesterified oil produced under these optimum conditions in scaled up level were 24.62±1.91% and 1.47±0.05, respectively. These values are comparable to the expected values produced by RSM analysis (22.60±2.19% and 1.43±0.05, respectively). During interesterification reaction TAG molecules are hydrolysed and fatty acids are rearranged in glycerol molecule. The proportions of different classes were TAG; 69.52%, DAG; 5.67%, MAG; 10.69% and free fatty acids; 14.12%. When compared to the original oils which contained more than 90% of TAG, interesterified oil contained high amount of DAG, MAG and free fatty acids which are formed during interesterification reaction as by-products. These by-products need to be removed by post-processing operations in order to improve the oxidative stability of the interesterified oil as these partial acylglycerols and free fatty acids increase the autoxidation of the interesterified oil and impart objectionable odors.

CONCLUSION

The reaction parameters for the interesterification of CO and SO using lipase derived from *R. miehei* were optimized using RSM. The R^2 values of regression analysis shown that the models used can explain the variability for both responses measured. From the present study, it could be concluded that the obvious reduction in total SFA and simultaneous increase in desirable MUFA and PUFA could be achieved successfully through enzymatic interesterification of CO and CO blend using lipases derived from *R. miehei*. The outcome of this study provides valuable information for the formulation of more healthy fat and oil out of locally available oils namely CO and SO. Furthermore, the structured lipids generated out of these oils can potentially be used to manufacture margarines, shortenings and fat spreads. Thus, there is a promising possibility for the production of nutritionally and functionally superior lipids using locally available raw materials through exploring interesterification process as forefront lipid modification technology in the country.

REFERENCES

Adhikari, P., Shin, J., Lee, J.H., Hu, J.N., Zhu, X.M., Akoh C.C. and Lee, K.T. (2010). Production of *trans*-free margarine stock by enzymatic interesterification of rice bran

ISSN 2056-5798(Print), ISSN 2056-5801(online)

oil, palm stearin and coconut oil. *Journal of the Science of Food and Agriculture*, 90:703–711.

Amarasiri, W.A.D.L. and Dissanayake, A.S. (2006). Coconut fats. *Ceylon Medical Journal*, 51:47-51.

Christie, W.W. (1992). *Gas chromatography and lipids. A practical guide*. Ayr, UK: The oily Press.

Dayrit, C.S. (2003). Coconut oil: atherogenic or not? (What therefore causes atherosclerosis?). *Philippine Journal of Cardiology*, 31, 97-104.

Dubois, V., Breton, S, Linder, M., Fanni, J. and Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, 109:710–732.

Ibrahim, N.A.B., Guo, Z. and Xu, X. (2008). Enzymatic interesterification of palm stearin and coconut oil by a dual lipase system. *Journal of the American Oil Chemists' Society*, 85:37-45.

Jandacek, R.J. (1994). Structured Lipids: An Overview and Comments on Performance Enhancement Potential. In B.M. Marriott (ed.), *An evaluation of potential performance-enhancing food components for operational rations.* Washington, DC: The national Academic Press. pp. 351-380

Lee, K.T and Akoh, C.C. (1998). Characterization of enzymatically synthesized structured lipids containing eicosapentaenoic, docosahexaenoic, and caprylic acids. *Journal of the American Oil Chemists' Society*, 75:495-499.

Lopez-Hernandez, A., Otero, C., Hernandez-Martin, E., Garcia, H.S. and Hill, C.G. (2007). Interesterification in batch and continuous flow processes of sesame oil and fully hydrogenated fat catalyzed by immobilized lipase. *European Journal of Lipid Science and Technology*, 109: 1147-1159.

Martin, D., Reglero, G. and Senorans, F.J. (2010). Oxidative stability of structured lipids. *European Food Research and Technology*, 231:635–653.

Maruyama, T., Nakajima, M., Ichikawa, S., Nabetania, H., Furusaki, S. and Seki, M. (2000). Oil-Water Interfacial Activation of Lipase for Interesterification of Triglyceride and Fatty Acid. *Journal of the American Oil Chemists' Society*, 77:1121-1126.

Nunes, G.F.M., de Paula, A.V., de Castro, H.F. and dos Santos, J.C. (2011). Compositional and textural properties of milk fat-soybean oil blends following enzymatic interesterification. *Food Chemistry*, 125:133–138.

Pham, L.J. and Gregorio, M.A. (2008). The Triacylglycerol Structure of Coconut Oil Determined by Chromatography Combined with Stereospecific Analysis. *Philippine Agricultural Scientist*, 91: 343-347.

Reena, M.B. and Lokesh, B.R. (2007). Hypolipidemic effect of oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with rice bran oil or sesame oil. *Journal of Agricultural and Food Chemistry*, 55:10461-10469.

Reena, M.B., Reddy, S.R.Y. and Lokesh, B.R. (2009). Changes in triacylglycerol molecular species and thermal properties of blended and interesterified mixtures of coconut oil or palm oil with rice bran oil or sesame oil. *European Journal of Lipid Science and Technology*, 111:346–357.

Ruan, X., Zhu, X., Xiong, H., Wang, S., Bai, C. and Zhao, Q. (2014). Characterisation of zero-trans margarine fats produced from camellia seed oil, palm stearin and coconut oil using enzymatic interesterification strategy. *International Journal of Food Science and Technology*, 49:91–97.

Sreenivasan, B. (1978). Interesterification of fats. *Journal of the American Oil Chemists' Society*, 55: 796-805.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Willis, W.M. and Maragoni, A.G. (2002). Enzymatic Interesterification. In C.C.,Akoh and Min, D.B. (Eds.). *Food Lipids: Chemistry, Nutrition, and Biotechnology*. New York: Marcel Dekker, Inc. pp. 839-876.

Wirkowska, M., Bryś, J., Górska, A., Ostrowska-Ligęza, E. and Koczoń, P. (2012). Oxidative properties of interesterified mixtures of milk fat, rapeseed oil and n-3 fatty acids. *Chemical Technology*, 61:11-13.

Zhang, H., Smith, P. and Adler-Nissen, J. (2004). Effects of degree of enzymatic interesterification on the physical properties of margarine fats: Solid fat content, crystal behavior, crystal morphology, and crystal network". *Journal of Agricultural and Food Chemistry*, 52:4423–4431.

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