# Optimization of Enzymatic Interesterification of Coconut (*Cocos nucifera*) and Sesame (*Sesamum indicum*) Oils using *Thermomyces lanuginosus* Lipase by Response Surface Methodology

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**ABSTRACT.** *Blends ofcoconut (Cocosnucifera) oil (CO) and sesame (Sesamumindicum) oil (SO) were enzymatically interesrerified using aqueous lipase derived from Thermomyceslanuginosusand the reaction conditions were optimized using Response Surface Methodology (RSM). A three-factor, three-level central composite design (face-centred cube design) was employed to optimize the reaction parameters, namely temperature (45-65 °C), time (16-48 h) and mass ratio of oils (CO:SO; 70:30 - 50:50). Lipase, diluted in phosphate buffer (pH 8) was used at 0.2% (v/w) of the substrate. Degree of interesterification (DI), and the ratio of monounsaturated and polyunsaturated fatty acids (MUFA:PUFA) of triacylglycerols (TAGs)were used as response variables. Triacylglycerol (TAG) fractions of the samples were separated using Thin Layer Chromatography (TLC) and the fatty acid composition of TAGs was determined using Gas-Liquid Chromatography (GLC). Thelinear and squared effects of temperature and time were significant ($p<0.05$) for DI whilethe reaction conditions did not exhibit a significant ($p<0.05$) effect on MUFA:PUFA ratio. The optimum conditions for enzymatic interesterificationwere45 °C (temperature), 40.24 h (time) and 70:30 (weight ratio of CO:SO).Under these optimized conditions, the DI was 28.98% and MUFA:PUFA was 1.50±0.06. According to the response surface regression analysis, the $R^2$ value for DI versus reaction parameters was 91.85% and MUFA:PUFA ratio versus reaction parameters was 61.82%. Therefore it can be concluded that enzymatic interesterification can effectively be applied to develop nutritionally and functionally superior modified oilknown as structured lipids using coconut and sesame oils.*

***Keywords:*** *Coconut oil, interesterification, lipase, optimization, sesame oil*

## INTRODUCTION

Structured lipids are thetriacylglycerols (TAG), re-structured or modified to change the fatty acid composition and/or their positional distribution in the glycerol molecule by chemical or enzymaticinteresterification (Timm-Heinrich*et al*., 2003). Structured lipids havea multitude of applications as speciality lipids for medicinal and nutritional purposes (Sreenivasan, 1978; Reena*et al.,* 2009).Interesterificationis the exchange of fatty acid molecules within and among TAG moieties which can be performed chemically as well as enzymatically (Fig. 1.).Of the two methods of interesterification, enzymatic interesterificationoffers advantages

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over chemical interesterification. The use of mild processing conditions such as low temperature, preservation of fatty acids in *sn*-2 position due to the specificity of lipases used, preservation of natural benefits of the oils, less by-product generation and ease of controlling the interesterificaiton process are some of the important benefits of enzymatic interesterification over chemical interesterification (Zhang *et al.,* 2004).

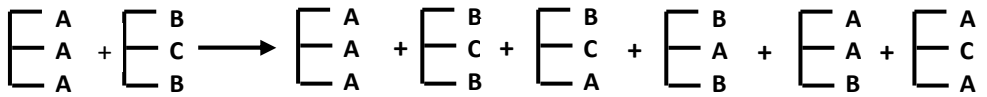



**Fig. 1. Different triacylglycerols (TAG)that can be generated through enzymatic interesterification of two TAGs using *Sn*-1,3-specific lipase.**

Coconut (*Cocosnucifera*) is one of the major plantation crops cultivated in Sri Lanka over many decades whilecoconut oil(CO) is the mostly used edible oil in the country. In 2015, the total area under the coconut cultivation stood approximately at 455,000 hawhile CO production was about 52,790 MT (Central Bank of Sri Lanka, 2016).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) (C8-C12).According to the universally accepted Lipid-Heart Theory, high saturated fats lead to hypercholesterolemia and coronary heart disease. Particularly long chain saturated fatty acids (LCSFAs) are known to be associated with the risk of increasing cardio vascular diseases.However, MCFAs such as C8:0 and C10:0 which are rapidly metabolized in the liver to energy and not participatory in the biosynthesis and transport of cholesterol are known to increase serum high density lipoprotein cholesterol (HDL) (Dayrit, 2003). However,the contribution of lauric acid (C12:0) which has originally been classified as an MCFA remains controversial. Despite the fact that lauric acid is classified as MCFA, there iscompelling evidence that lauric acidfollows the absorption pattern of bothLCSFAs and MCFAs. Thus the presence of high quantity of lauric acid in the diet may contribute to increase the risk of heart disease (Jandacek, 1994;Amarasiri and Dissanayake, 2006; Dubois, 2007). In this backdrop, replacing some of the SFAs such as lauric acid and LCSFAswith nutritionally important fatty acids such as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs) is beneficial.

Since antiquity, sesame (*Sesamumindicum*) oil (SO) is well known for its nutritional and medicinal value. It is rich in unsaturated fatty acids (>85%) of which 39% is MUFAand 46% is PUFAs (Dubois *et al.,* 2007). Thus, incorporating these fatty acids from SO into CO by means of enzymatic interesterificationreplacescertainLCSFAs including lauric acid and brings about a nutritionally superior oil. SO helps maintain HDL and lowerlow density lipoprotein (LDL).However, despite the benefits associated with, SO remains as an underutilized oil in Sri Lanka as many dislike the direct consumption of this important edible oil due to itsflavour. Therefore, this study focused on enzymatic interesterification of these two major edible oils available in Sri Lanka.

The enzymatic interesterification reaction depends on reaction parameters such as temperature, time, pH, substrate composition, surface active agents etc. (Willis and Maragoni, 2002). In order to obtain the optimum output from the interesterification reaction, the parameters should be optimized. Response Surface Methodology (RSM) is a statistical technique used in engineering and science to optimize parameters affecting yield or any other expected outcome. The main advantage of RSM is the reduced number of experimental runs

required to provide sufficient information for statistically acceptable results (Shieh *et al*., 1995). The present study used this statistical design to optimize the reaction parameters of the interesterification of CO and SO. The conditions optimized using RSM could be used in up-scaled level to produce modified lipid using CO and SO through enzymatic interesterification.

Studies on enzymatic interesterification of CO and SO are scanty while this is the first time an interesterification study was reported in Sri Lanka.This study aimed at optimizing the parameters of enzymatic interesterification of two edible oils available in Sri Lanka such as CO and SO by lipase (*sn* 1, 3 specific) from *T.lanuginosus*using RSM. The reaction conditions optimized for interesterification in the laboratory level could be used in up-scaled level to produce modified lipid with nutritionally beneficial and balanced fatty acid composition. These structured lipid could potentially be used in preparation of*zero-trans*margarine, shorteningsand fat spreads.

## MATERIALS AND METHODS

### Materials

Solvents, chemicals, lipase derived from *T.lanuginosus* (≥100,000U/g) and Tween® 40 (Polyoxyethylenesorbitanmonopalmitate) 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%) and hydrogen were purchased from Ceylon Oxygen (Pvt) Ltd, Sri Lanka. All chemicals, solvents and gases used in the study were of analytical grade or chromatographic grade with the highest purity available.Regular coconut oil(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.

### Lipase catalysed interesterification

The reaction parameters used for the RSM: temperature (°C) ($X_1$), time duration of reaction (h) ($X_2$), weight ratio of oils (w/w) ($X_3$) and their levels used are shown in Table 1. Coconut oil and sesame oils were weighed at particular weight 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 *T. lanuginosus* 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 the samples were drawn at the particular time intervals (16, 32 or 48h). Samples were added into glass vials and enzyme was inactivated by adding acetic acid (0.25% v/v). Then the samples were sealed using Parafilm$^{TM}$and stored at 2-8 °C for further analysis.

**Table 1. The range and levels of independent variables used for RSM**

| Independent variable ($X_i$) | Range and 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): 50:50, 60:40 and 70:30, respectively.

**Statistical design**

Reaction parameters were optimized using RSM model. MINITAB 17 statistical software was used to design the experiments using RSM. A three-factor, three-level central composite design (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. Triplicate experiments were carried out for each run.

**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 [free fatty acid, monoacylglycerol (MAG), diacylglycerol (DAG) and TAG]. The TAG spots were carefully scraped off along with silicaand transferred into a screw capped tube containing 0.6 mL of hexaneand centrifuged at 1500 rpm for 10 min. Then hexane layer was transferred into another tube and the extraction process was repeated once more. Subsequently,the hexane containing TAGs was combined and evaporated to concentrate fatty acids by flushing with nitrogen and used for determination of fatty acid profile using GLC.

**Determination of the composition of triacylglycerol (TAG)**

Fatty acid composition of the separated TAGs was 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) attached with Chromatopac data processor (Model-CR6A, Shimadzu, Japan). The split ratio was set at 80:1, injector and detector temperatures were maintained at 260°C. Helium was used as carrier gas at flow rate of 20 mL/min. 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 comparing their retention times with those of authentic standards. The amount of each fatty acid in the sample was expressed as percentage of the sum of all fatty acids in the sample.

**Determination of Degree ofInteresterification (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;

$$DI(\%) = (\Sigma(FA_I - FA_0)/(\Sigma(FA_D)) * 100$$

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 0represent 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:TAG, diacylglycerol (DAG), monoacylglycerols(MAG) and free fatty acids of oil interesterified under optimized conditions were determined.Lipid classes:TAG, DAG, MAG and free fatty acids were separated using TLC as explained above and identified using comparing with authentic standards. Each spot was marked and scraped off separately and placed inglass vials. A known quantity of internal standard (methylheptadecanoate, 1 mg/mL) was added to each tube and fatty acids were extracted into hexane and analysed for the fatty acid composition using GLC. The area under the peak corresponding to each fatty acid and internal standard was recorded on the gas chromatogramswhich were used for estimation of relative proportions of different lipid classes.

## RESULTS AND DISCUSSION

**Optimization of interesterification by Response Surface Methodology (RSM)**

The DI and MUFA:PUFA ratio of TAGs of interesterified oils are shown in Table 2. The DI ranged from 13.40 to 33.23% with the highest value reported in the oil blend containing CO:SO at 70:30 ratio interesterified at 45 °C for 16 h while the lowest DI was reported in the oil blend containing CO:SO at 70:30 ratio interesterified at 65 °C for 16 h. The values of MUFA:PUFA ratio ranged from 1.18 to 1.70 with the highest (1.70) reported in the oil blend containing CO:SO at 70:30 ratio interesterified at 55 °C for 32 h while the lowest (1.18) reported in the oil blend containing CO:SO at 50:50 ratio interesterified at 65 °C for 48 h.

Estimated effects, standard error coefficients, t-values and p-values for DI and MUFA:PUFA ratio of TAG of interesterified oils according to RSM are shown in Table 3. Linear effect of

temperature and time, squared effect of temperature and time and interaction effect of temperature and time and temperature and oil ratio exhibited significant effect on DI. All effects were not significant for MUFA:PUFA ratio.

**Table 2. Experimental design for DI and MUFA:PUFA ratio of interesterifiedoils and physical blends with coded and actual values of independent variables: temperature, time and oil ratio according to face-centred cube design**

| Run Order | Independent variables | | | Responses | |
|---|---|---|---|---|---|
| | Temperature (°C) | Time (h) | Oil ratio | DI (%) | MUFA:PUFA |
| 1 | 55(0) | 32(0) | 0.6(0) | 19.28 | 1.56 |
| 2 | 45(-1) | 48(+1) | 0.5(-1) | 15.73 | 1.48 |
| 3 | 65(+1) | 48(+1) | 0.5(-1) | 21.42 | 1.26 |
| 4 | 65(+1) | 16(-1) | 0.7(+1) | 13.40 | 1.43 |
| 5 | 55(0) | 48(+1) | 0.6(0) | 14.95 | 1.46 |
| 6 | 55(0) | 16(-1) | 0.6(0) | 18.57 | 1.35 |
| 7 | 55(0) | 32(0) | 0.6(0) | 17.19 | 1.31 |
| 8 | 45(-1) | 32(0) | 0.6(0) | 27.09 | 1.31 |
| 9 | 55(0) | 32(0) | 0.6(0) | 19.77 | 1.33 |
| 10 | 45(-1) | 16(-1) | 0.7(+1) | 33.23 | 1.18 |
| 11 | 55(0) | 32(0) | 0.7(+1) | 18.34 | 1.70 |
| 12 | 65(+1) | 32(0) | 0.6(0) | 21.96 | 1.30 |
| 13 | 55(0) | 32(0) | 0.6(0) | 19.68 | 1.40 |
| 14 | 45(-1) | 16(-1) | 0.5(-1) | 21.98 | 1.49 |
| 15 | 55(0) | 32(0) | 0.6(0) | 17.82 | 1.40 |
| 16 | 65(+1) | 16(-1) | 0.5(-1) | 22.49 | 1.35 |
| 17 | 55(0) | 32(0) | 0.6(0) | 19.12 | 1.44 |
| 18 | 55(0) | 32(0) | 0.5(-1) | 18.58 | 1.30 |
| 19 | 45(-1) | 48(+1) | 0.7(+1) | 21.24 | 1.66 |
| 20 | 65(+1) | 48(+1) | 0.7(+1) | 19.49 | 1.41 |

Based on the RSM analysis, optimum reaction parameters selected to maximize both responses: DI and MUFA:PUFA ratio were temperature (45 °C); time (40.24 h), and weight ratio of oil-CO:SO, (70:30).

**Table 3. Estimated effects, standard error coefficients, t-values and p-values for DI and MUFA:PUFA ratio of TAG of oilsinteresterifiedaccording to central composite design**

| Response variable | Independent variable and interactions | Estimated effects | SE Coefficient | t-value | p-value |
|---|---|---|---|---|---|
| DI | $X_1$ | -4.100 | 0.535 | -3.83 | 0.003* |
| | $X_2$ | -3.370 | 0.535 | -3.15 | 0.010* |
| | $X_3$ | 1.099 | 0.535 | 1.03 | 0.329 |
| | $X_1^2$ | 10.70 | 1.02 | 5.24 | 0.000* |
| | $X_2^2$ | -4.82 | 1.02 | -2.36 | 0.040* |
| | $X_3^2$ | -1.43 | 1.02 | -0.70 | 0.499 |
| | $X_1 X_2$ | 5.815 | 0.598 | 4.86 | 0.001* |
| | $X_1 X_3$ | -6.944 | 0.598 | -5.80 | 0.000* |

| | | | | | |
|---|---|---|---|---|---|
| | $X_2 X_3$ | 0.354 | 0.598 | 0.30 | 0.774 |
| MUFA:PUFA | $X_1$ | -0.0765 | 0.0348 | -1.10 | 0.298 |
| | $X_2$ | 0.0956 | 0.0348 | 1.37 | 0.200 |
| | $X_3$ | 0.1003 | 0.0348 | 1.44 | 0.180 |
| | $X_1^2$ | -0.1960 | 0.0664 | -1.48 | 0.171 |
| | $X_2^2$ | -0.0021 | 0.0664 | -0.02 | 0.988 |
| | $X_3^2$ | 0.1993 | 0.0664 | 1.50 | 0.164 |
| | $X_1 X_2$ | -0.1437 | 0.0389 | -1.854 | 0.095 |
| | $X_1 X_3$ | 0.0863 | 0.0389 | 1.11 | 0.294 |
| | $X_2 X_3$ | 0.1385 | 0.0389 | 1.78 | 0.106 |

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

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.

$$DI = 76.3 - 4.59 X_1 - 0.568 X_2 + 279 X_3 + 0.0535 X_1^2 - 0.00942 X_2^2 - 72 X_3^2 + 0.01817 X_1 X_2 - 3.472 X_1 X_3 - 0.111 X2 X_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$MUFA:PUFA = 3.30 + 0.0924 X_1 + 0.0020 X_2 - 15.21 X_3 - 0.000980 X_1^2 - 0.000004 X_2^2 + 9.97 X_3^2 - 0.000449 X_1 X_2 + 0.0431 X_1 X_3 + 0.0433 X_2 X_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

These two models were validated by analysis of variance (ANOVA) which is shown in Table 4. The model fitted for DI was significant at 95% confidence level with non-significant lack of fit, however, the model fitted for MUFA:PUFA ratio was not significant at 95% confidence level without significant lack of fit. The $R^2$ value produced by regression analysis for DI versus reaction parameters (time, temperature and weight ratio of oils) was 91.85% and MUFA:PUFA ratio versus reaction parameters was 61.82%. This indicates that these models can explain more than 92% of the variability of DI and more than 62% 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 interesterifiedoils according to the central composite design**

| Response variable | Factor | Degrees of freedom | Adjusted sum of square | Adjusted mean square | F-value | p-value |
|---|---|---|---|---|---|---|
| DI | Model | 9 | 322.451 | 35.8279 | 12.52 | 0.000* |
| | $X_1$ | 1 | 42.025 | 42.0247 | 14.68 | 0.003* |
| | $X_2$ | 1 | 28.387 | 28.3865 | 9.92 | 0.010* |
| | $X_3$ | 1 | 3.020 | 3.0196 | 1.05 | 0.329 |
| | $X_1^2$ | 1 | 78.704 | 78.7040 | 27.49 | 0.000* |
| | $X_2^2$ | 1 | 16.002 | 16.0022 | 5.59 | 0.040* |
| | $X_3^2$ | 1 | 1.409 | 1.4087 | 0.49 | 0.499 |
| | $X_1 X_2$ | 1 | 67.630 | 67.6296 | 23.63 | 0.001* |
| | $X_1 X_3$ | 1 | 96.430 | 96.4297 | 33.69 | 0.000* |
| | $X_2 X_3$ | 1 | 0.250 | 0.2503 | 0.09 | 0.774 |
| | Error | 10 | 28.625 | 2.8625 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Lack-of-fit | 5 | 23.030 | 4.6059 | 4.12 | 0.073 |
| | Pure error | 5 | 5.596 | 1.1191 | | |
| MUFA:PUFA | Model | 9 | 0.1962 | 0.0218 | 1.80 | 0.187 |
| | $X_1$ | 1 | 0.0146 | 0.0146 | 1.21 | 0.298 |
| | $X_2$ | 1 | 0.0228 | 0.0228 | 1.88 | 0.200 |
| | $X_3$ | 1 | 0.0251 | 0.0251 | 2.08 | 0.180 |
| | $X_1^2$ | 1 | 0.0264 | 0.0264 | 2.18 | 0.171 |
| | $X_2^2$ | 1 | 0.000003 | 0.000003 | 0.00 | 0.988 |
| | $X_3^2$ | 1 | 0.0273 | 0.0273 | 2.25 | 0.164 |
| | $X_1 X_2$ | 1 | 0.0412 | 0.0412 | 3.41 | 0.095 |
| | $X_1 X_3$ | 1 | 0.0148 | 0.0148 | 1.23 | 0.294 |
| | $X_2 X_3$ | 1 | 0.0383 | 0.0383 | 3.17 | 0.106 |
| | Error | 10 | 0.1211 | 0.01211 | | |
| | Lack-of-fit | 5 | 0.0826 | 0.01652 | 2.14 | 0.212 |
| | Pure error | 5 | 0.0385 | 0.0077 | | |

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

**Effect of parameters and response surface plotting**

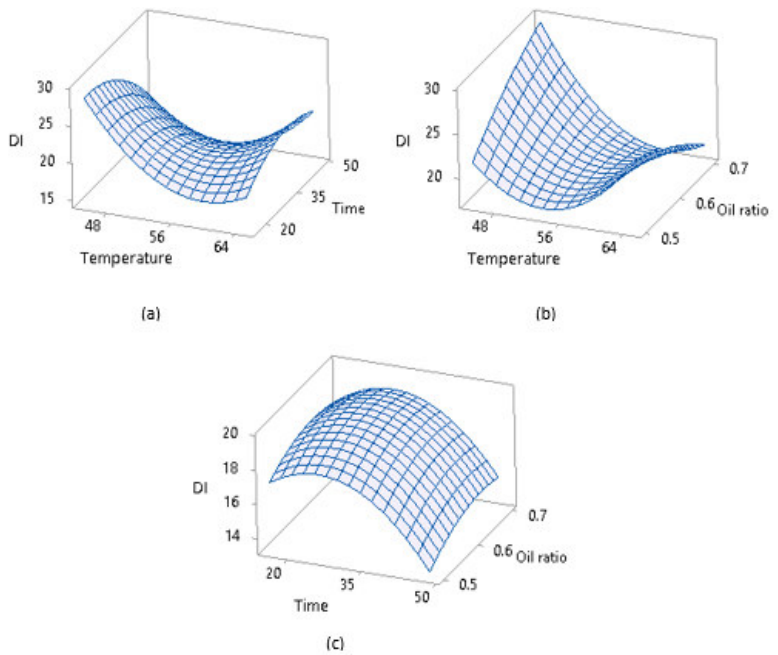



**Fig. 2. Surface plotsillustrating the effect of time and temperature (a) oil ratio and temperature(b)and oil ratio and time (c) on the degree of interesterification (DI).**

Figure 2 (a) clearly depicts that DI can be maximized by using low temperature for low duration. Figure 2 (b) illustrates that higher DI is obtainable at low temperature with oil ratio of CO:SO, 70:30. According to Fig. 2 (c), the DI can be maximized by reducing the time and oil ratio to have high proportion of CO.

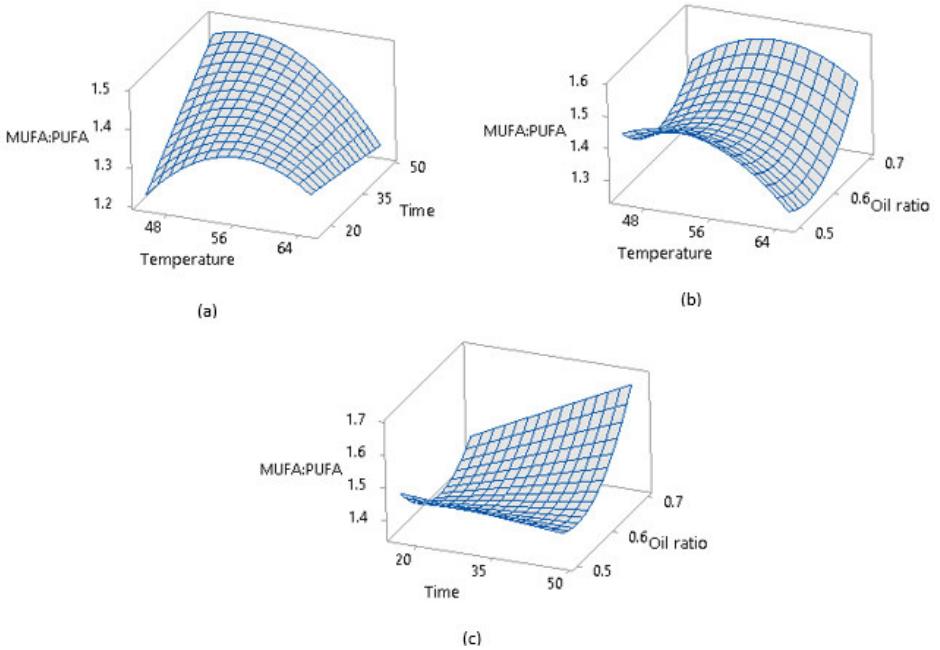



**Fig. 3. Surface plots illustrating the effect of time and temperature (a) oil ratio and temperature (b) and oil ratio and time (c) onthe MUFA:PUFA ratio**

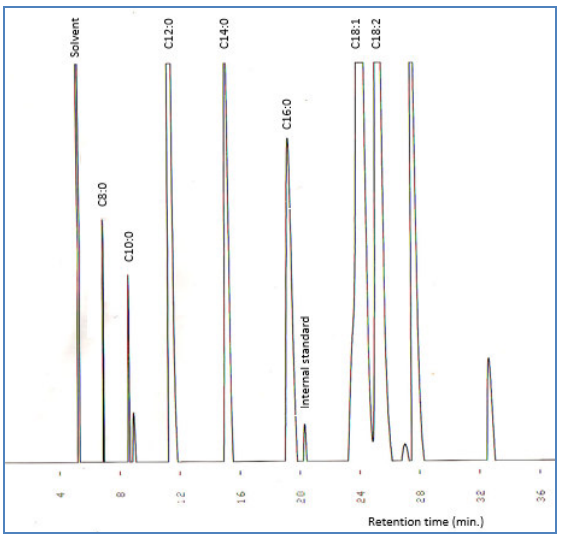
MUFA:PUFA ratio can be maximized by using low temperature and high time duration [Fig. 3 (a)] or using medium temperature and using oil ratio to have high proportion of CO [Fig. 3 (b)] or by combination of using high time duration and using oil ratio to have high proportion of CO [Fig. 3 (c)].

The yield of enzymatic reactions depends on reaction parameters such as temperature, time, pH, substrate composition and surface active agents (Willis and Maragoni, 2002). In interesterification, the optimum conditions for the reaction depend on the expected outcome. In the present study, the operating conditions were selected in order to maximize the incorporation of fatty acids from SO into CO which will in turn increase the 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, among others. Therefore, the conditions optimized in this study are not directly comparable with the optimum conditions determined inother studies. To our knowledge, no studies have been carried out so far to optimize the reaction parameters of enzymatic interesterification of CO and SO. A study has been carried out by Reena and Lokesh (2007) to study the hypolipidemic effect of structured lipid prepared by interesterification of blended oil comprising of 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. Moreover, when lipases derived from other sources such as*T. lanuginosus* were used for

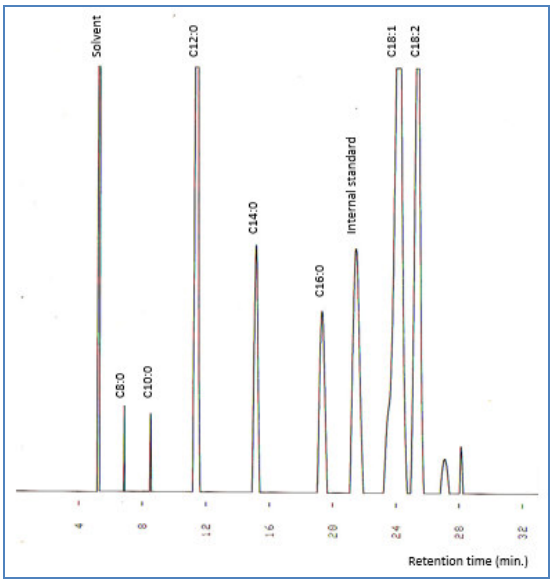
enzymatic interesterification, the conditions have been different. As an example,a study by Lee *et al.* (2010) carried out to synthesize human milk fat substitute from tripalmitin-rich fraction and ethyl oleate using *T. lanuginosus*lipase through interesterification indicated that 50°C and 3h werethe optimum reaction temperature and time duration, respectively.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) indicated that the developed models can explain the effect of variables (reaction parameters).MUFA:PUFA ratio can be maximized by using low temperature and high time duration [Fig. 3 (a)] or using medium temperature and using oil ratio to have high proportion of CO [Fig. 3 (b)] or by combination of using high time duration and using oil ratio to have high proportion of CO [Fig. 3 (c)].

**Changes in the composition of fatty acids in TAG**

The fatty acid compositions of TAGs of CO, SO and interesterified oil under optimized conditions and its blend are given in the Table 5. Compared to the blend, caprylic, palmitic and oleic acids have been increased during the reaction while lauric, stearic and linoleic acids have been reduced. However, major changes occurred in the amount oflauric and oleic acids compared to the other fatty acids (Table 5). Figure 3 illustrates two representative gas liquid chromatograms indicating the peaks corresponding to major fatty acids of CO and SO blend at 70:30 ratio and the interesterified oil.




(a)




(b)

**Fig. 3. Gas chromatograms of TAGs of oil blend (CO:SO; 70:30) (a) and oil interesterified under the conditions optimized by RSM (b).**

Even though lauric acid is classified under the group of MCFAs, during metabolism, it behaves like LCSFAs (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 an oil depends on the ratio of MUFA:PUFA rather than the absolute quantities of MUFA and PUFA. In the present study, even though MUFA:PUFA ratio of SO, blend and interesterified oil did not differ significantly, total amount of MUFA and PUFA increased significantly. 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 associated with them. Considering SFA:MUFA:PUFA ratio, interesterified oil had balanced fatty acid composition compared to original oils and blend.

**Table 5. The fatty acid composition and MUFA:PUFA, SFA:MUFA:PUFA and MCFA:LCFA ratios of TAGs of SO, CO and the oil interesterified under optimized conditions and its blend**

| Fatty acid | SO | CO | Blend | IE |
|---|---|---|---|---|
| Caprylic (C8:0) | ND | $2.67\pm0.27^{a}$ | $1.72\pm0.06^{b}$ | $2.27\pm0.10^{a}$ |
| Capric (C10:0) | ND | $3.60\pm0.08^{a}$ | $2.52\pm0.28^{b}$ | $2.54\pm0.16^{b}$ |
| Lauric (C12:0) | ND | $52.15\pm0.52^{a}$ | $36.65\pm0.28^{b}$ | $24.33\pm0.51^{c}$ |
| Myristic (C14:0) | ND | $21.20\pm0.64^{a}$ | $15.30\pm0.92^{b}$ | $12.52\pm1.04^{b}$ |
| Palmitic (C16:0) | $7.82\pm0.24^{b}$ | $8.80\pm0.64^{b}$ | $8.30\pm0.08^{b}$ | $10.32\pm0.45^{a}$ |
| Stearic (C18:0) | $3.30\pm0.14^{a}$ | $0.84\pm0.05^{c}$ | $1.57\pm0.16^{b}$ | $0.42\pm0.07^{d}$ |
| Oleic (C18:1) | $48.88\pm0.95^{a}$ | $8.47\pm0.74^{d}$ | $20.55\pm0.13^{c}$ | $28.5\pm0.99^{b}$ |

| | | | | |
|---|---|---|---|---|
| Linoleic (C20:2) | 39.61±0.56[a] | 2.29±0.33[d] | 13.40±0.55[c] | 19.1±1.41[b] |
| Linolenic (C20:3) | 0.41±0.02 | ND | ND | ND |
| MUFA:PUFA | 1.2:1 | 3.7:1 | 1.5:1 | 1.5:1 |
| SFA:MUFA:PUFA | 1:4.3:3.6 | 39:3.7:1 | 4.9:1.5:1 | 2.7:1.5:1 |
| MCFA:LCFA | - | 1.40:1 | 1:1.4 | 1:2.4 |

Values (Means± SD) with different letters in the same raw imply significant differences ($p < 0.05$).

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 negativeeffect 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 tocopherols, tocotrienols and other phenolic constituents, inferior oxidative stability of structured lipids containing SO has been reported. This is most likely attributable to the loss of endogenous antioxidants during the interesterification process (Martin *et al.,* 2010; Wirkowska *et al.,* 2012). Due to these reasons, in the present 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 ofcoronary heart diseases, MCFAs (C8 and C10) which are 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. Hence, maximum incorporation of these two MCFAs into the interesterified portion wasone of the main objectives of this study.

In this study,*sn*-1,3 specific lipase was used for interesterification. The*sn*-1,3 specific lipase re-esterify the fatty acids esterified at*sn*-1 and 3 keeping the fatty acid at *sn*-2 position intact. Therefore, preservation of fatty acid at *sn*-2 position would result in more natural fat compared to chemical interesterification which results in random exchange of fatty acids in all three positions of the glycerol backbone(Willis and Maragoni, 2002).

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 into TAGs of CO and *vice-versa*.

Lipase can catalyze the hydrolysis reaction in aqueous mixtures, however, 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 (Maruyama *et al.,* 2000). The present study was carried out in solvent-free system. Therefore, surfactant (Tween-40) was used in this study to emulsify the substrates enabling the lipase to react effectively.

**Scalingup of interesterification and analysis of lipid classes of ineresterified oil**

In order to confirm the results obtained by RSM, the interesterification reaction was carried out under the optimized conditions in up-scaled level. The DI and MUFA:PUFA ratio of interesterified oil produced under these optimum conditions in scaled up level were 28.98% and 1.5±0.06, respectively. These values are comparable to the expected values obtained through RSM analysis (expected DI and MUFA:PUFA ratio were 26.75% and 1.55,respectively). During interesterification reactions, the TAG molecules are hydrolysed and fatty acids are rearranged into different positions. During this process, free fatty acids and partial acylglycerols such as MAG and DAG can be formed. Therefore, the proportions of these different classes were determined. The values were TAG; 69.18%, DAG; 11.38%, MAG; 1.03% and free fatty acids; 18.42%.

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 can possibly be formed during interesterification reactionas 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 odours.

## CONCLUSIONS

The reaction parameters for the interesterification of CO and SO using lipase derived from *T.lanuginosus*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 *T. lanuginosus*. The outcome of this study provides valuable information for the formulation of more healthy fat and oil out of locally available oils namely coconut and sesame oils. 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.

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