

MODIFICATION OF HYDROGENATED CANOLA OIL/PALM STEARIN/ CANOLA OIL BLENDS BY CONTINUOUS ENZYMATIC INTERESTERIFICATION

Keywords: Canola oil; palm stearin; Biobone; triglycerides; fatty acids; hydrolysis and interesterification.

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Three fat blends containing hydrogenated canola oil (HCO), palm stearin and canola oil (45/25/30%) were interesterified in a temperature and moisture controlled packed column reactor. The moisture of the fats was controlled by a pre-column packed with Biobone (a granulated chicken bone material possessing good mechanical strength). The changes in the composition of the major triglycerides (C48, C50, C52 and C54) were followed by GLC. Both the moisture content of the Biobone and the flow rate significantly affected the composition of C48, C52 and C54 triglycerides during interesterification. Other factors such as the age of the Lipozyme used, and that of the Biobone, also contributed to the variation in C48, C50 and C52 composition. An increase of more than 50% of C52 in the interesterified blends indicated that a large portion of the palmitic acid residues from the palm stearin were exchanged with an 18 carbon fatty acid present in the C54 molecules of the canola products. A flow rate of 11.4 g/h and a moisture content between 13.2% and 19.2% were most effective for interesterification.

However, the higher levels of free fatty acids (FFA), monoglycerides (MG) and diglycerides (DG) detected in the interesterified blends as compared with the control blends indicated that some degree of hydrolysis also occurred; the total amounts of FFA, MG and DG were higher by 5.9% to 13.0% in the interesterified blends.

INTRODUCTION

The reactions catalysed by lipase are of three main types and they are reversible. They include hydrolysis of esters, synthesis of esters and ester-ester interchange reactions (*i.e.* interesterification) (Lazar *et al.*, 1986). In the presence of high levels of water, lipase catalyses the hydrolysis reactions whereby ester bonds in complex chemical compounds are broken down, producing simpler molecules. After hydrolysis, the reverse reaction, *i.e.* ester synthesis or interesterification, can be made to predominate by providing a microaqueous (*i.e.* low water content) environment.

Fats are solid at room temperature and they need to be melted or dissolved in an organic solvent in order to initiate any chemical reaction. An advantage of using lipases of microbial origin for interesterification of fats is that many of them are thermostable. This characteristic allows both liquid oils and solid fats to be modified at higher temperatures in the absence of organic solvents (Muderhwa *et al.*, 1989; Chang *et al.*, 1990; Ergan *et al.*, 1990; Kalo *et al.*, 1990). Lipases secreted by *Humicola lanuginosa*, *Mucor miehei* and *Candida antarctica* can resist denaturation at temperatures as high as 40°C to 70°C (Hansen and Eigtved, 1986; Omar *et al.*, 1987; Heldt-Hansen *et al.*, 1989).

A number of factors are known to affect the equilibrium and rate of enzymatic interesterification of fats. They include the moisture content of the fat, the temperature, and the presence of impurities. The product yield, operational stability, equilibrium and rate of reaction are all affected by the amount of water present during interesterification (Macrae, 1985a, Yamane, 1987; Muderhwa *et al.*, 1988). A certain amount of moisture needs to be present in order to activate the lipase: the ester-ester interchange cannot proceed if conditions are completely anhydrous (Hoq *et al.*, 1984). On the other hand, higher quantities of water favour the hydrolysis of triglycerides to give a mixture of free fatty acids, monoglycerides and diglycerides. When free *Rhizopus arrhizus* lipase was used for glycerol ester synthesis, Touraine and Drapon (1988) found that an optimum water activity of 0.3 was required to maximize product yield. In the case of immobilized lipase, a moisture content of 10% was required for maximum activity (Wisdom *et al.*, 1985; Hansen

and Eigtved, 1986). During continuous interesterification in a packed-column reactor, water associated with the immobilized catalyst is being used up due to its slight solubility in the circulating fat and to the inevitable occurrence of a small degree of hydrolysis.

In order to maintain the activity of the enzyme, fat and oil mixture can be partially saturated with water (Macrae, 1985b). A number of adsorbents which can retain water have been used to hydrate fats during continuous interesterification. They include diatomaceous earth, silica and molecular sieves (Posorske *et al.*, 1987; Ison *et al.*, 1988). Alternatively, molecular sieves have also been used to remove water during ester synthesis in a batch system (Ergan *et al.*, 1988; Omar *et al.*, 1988); the oil was circulated through an external loop containing the adsorbent.

As already noted, some microbial lipases are known to resist denaturation at high temperatures. The optimum reaction temperature of *Humicola lanuginosa* lipase is 45°C and its full activity can be retained even at 65°C (Omar *et al.*, 1987). At higher temperatures the enzyme becomes less stable and its activity is lost after 18 hr at 65°C or 1 hr at 70°C. The half-life of immobilized *Candida antarctica* lipase is about 500 hr at 80°C but no significant deactivation has been observed even after 2000 hr of use at 60°C (Heldt-Hansen *et al.*, 1989). Immobilized *Mucor miehei* lipase has been reported to perform equally well at 60°C and 70°C in a packed column reactor (Hansen and Eigtved, 1986); the rate of interesterification was partly governed by the retention time of the fat in the reactor.

The activity of lipases can be affected by the impurities present in fats and oils. While Ca²⁺, Mg²⁺ and Co²⁺ are favourable for the activity of *Candida deformans* lipase, Cu²⁺, Zn²⁺ and Fe²⁺ act as strong inhibitors; Ni²⁺ and Mn²⁺ slightly reduce the activity of the enzyme (Muderhwa *et al.*, 1985). In the case of *Humicola lanuginosa* lipase, alkali metal ions such as Li⁺, K⁺ or Ca²⁺ were found to enhance its activity, while Co²⁺, Ni²⁺, Cu²⁺, Sn²⁺ and Hg²⁺ had inhibitory effects (Omar *et al.*, 1987). A slight inhibitory effect was also observed when Zn²⁺, Mg²⁺, sulphhydryl reagents, ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulphate (SDS) were present. Wisdom *et al.* (1987) reported that the activity of immobilized *Rhizopus arrhizus* lipase was reduced by the

impurities present in a low grade fat used. A similar observation was made by Posorske *et al.* (1987) when Lipozyme (an immobilized *Mucor miehei* lipase preparation) was used for interesterification of soya bean oil which may have contained oxidation products as impurities. Also, the initial rate of transesterification of lauric acid with triolein was found to be lower in the presence of 0.5% phospholipid (Wang and Gordon, 1991). The ability of various phospholipids to inactivate Lipozyme was in the increasing order: phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol. Therefore, as with chemical interesterification, the use of refined oil is recommended for enzymatic interesterification.

The aim of this study was to establish operating conditions for continuous interesterification of fats in a bench-top reactor, and to evaluate Biobone (a food-grade and porous granulated by-product of the chicken deboning industry possessing good mechanical strength) containing various amounts of water for hydrating fats during interesterification.

MATERIAL AND METHODS

Refined, bleached and deodorized canola oil was purchased from a local supermarket and hydrogenated under various selectivity conditions. Fractionated palm stearin (PS) was obtained from Lam Soon Bhd., Petaling Jaya, Malaysia.

HYDROGENATION OF CANOLA OIL

Canola oil was hydrogenated under non-selective conditions (140°C, 310.1 KPa (45 psi)), selective conditions (200°C, 51.7 KPa (7.5 psi)) and intermediate (AOCS) conditions (175°C, 103.4 KPa (15 psi)). One litre of oil was hydrogenated in a 2 L hydrogenation reactor (Parr Instrument Co., Moline, IL) using an agitation rate of 600 rpm. The reaction was catalysed with 0.4% (w/w) Nysosel 222 nickel catalyst (Harshaw/Filtrol Partnership, Cleveland, OH) which contains approximately 22% nickel by weight. The concentration of nickel in the oil was 0.08 percent. The details of the hydrogenation procedure have been described in the AOCS (1981) procedure Tz 1a-78. The hydrogenated fats were filtered twice through a Whatman No. 4 and

a No. 2 filter paper successively under vacuum. Iodine value was then determined according to method Cd 1-25 of the AOCS (1981).

Formulation of fat blends

Three fat blends (A, B and C) containing hydrogenated canola oil, palm stearin and canola oil in the ratio 45/25/30 (w/w) were formulated. They contained canola oil hydrogenated under non-selective conditions (Iodine Value = 59.9), intermediate conditions (I.V. = 56.2) and selective conditions (I.V. = 58.7), respectively.

Enzymatic interesterification

Intesterification was carried out at 60°C in a temperature and moisture controlled bench-top reactor consisting of two 1.0 x 20 cm water-jacketed glass columns (Pharmacia LKB, Uppsala, Sweden) arranged in series. The pre-column (for moisture control) was packed with 6.0 g of Biobone (Bioprotein Inc., Hamilton, Ontario) of 10 mesh size, and the enzyme column was packed with 5.0 g of Lipozyme (NOVO Industri, Bagsvaerd, Denmark). An Econo-Column variable speed peristaltic pump (Bio-Rad, Richmond, California) was used to circulate the fat through the reactor. The pump head was fitted with a 30 cm Tygon R-3603 tubing (1.6 mm I.D., 0.8 mm wall thickness) which was changed after every 18 hr of operation to minimize variation in flow rates. The fat was pumped against gravity from the pre-column to the enzyme column without recycling.

Samples of interesterified fats were collected every 15 min by a fraction collector and their triglyceride composition were determined by gas liquid chromatography (GLC). The free fatty acid (FFA), monoglyceride (MG) and diglyceride (DG) contents were also determined.

Free fatty acid, partial glyceride and triglyceride analysis

Triglyceride composition was determined by gas liquid chromatography (GLC) (Litchfield, 1972; d'Alonzo *et al.*, 1981). The free fatty acid, mono- and di-glyceride contents were determined by GLC following derivatization into the trimethylsilyl derivatives (Goh and Timms, 1985). The details of the conditions used in our laboratory have been previously published (Cho *et al.*, 1993).

Method for establishing moisture calibration curve for Biobone

About 250 g of Biobone was dried in an air oven at 110° C for 12 hours to constant weight. Portions of 10 g were weighed out in polystyrene screw-capped containers and 10%, 20%, 30% or 40% (w/w) distilled water was added. The containers were sealed and the contents were allowed to equilibrate for 24 hr at room temperature. The moisture content of the Biobone was then determined as described below.

Conditioning of Biobone for pre-column use

Biobone was dried for 24 hr at 110° C and weighed out in three air-tight glass containers. Three levels of distilled water (8%, 16% or 24% (w/w)) were added and the mixtures were allowed to equilibrate for 24 hr at room temperature. The preparations were then stored at 5° C and re-equilibrated to room temperature before use. The moisture contents of the Biobone preparations were determined at the beginning and at the end of the processing period (18 days) as described below.

Determination of moisture in Biobone

Two grams of Biobone were weighed in aluminium dishes which had been previously dried to constant weight at 110° C and cooled in a desiccator. The Biobone was then dried for 12 hr in an oven at the same temperature and then cooled in a desiccator before weighing. Moisture content was then calculated from the difference between the initial and final weight of the sample.

Measurement of residence time

Sudan III (tetrazobenzene- β -naphthol) was dissolved in canola oil and used as a tracer to estimate the mean residence time of fats in both the Biobone and Lipozyme columns. The time taken for the red colour at the two ends of the columns to match in intensity was measured. The time taken for the red colour to clear when pure canola oil was circulated through the columns was also recorded.

The experiment was repeated several times and the mean residence time of the fat in both columns was calculated. The residence time at various flow rates is shown in *Table 1*.

Experimental design

The effects of 3 flow rates and 3 levels of water content on the triglyceride composition, free fatty acid, monoglyceride and diglyceride contents of 3 fat blends were investigated in a 3³ factorial design. Twenty-seven treatment combinations were arranged in an incomplete block design. It was adapted from plan 6.7 (Cochran and Cox, 1957) to accommodate three blocking factors (age of Biobone, age of Lipozyme and block as described below).

The treatment combinations used for the 2 replicates of the experiment are shown in *Table 2*. The three factors (blends, levels of moisture and flow rates) were randomized and the experiment was replicated. A total of 54 treatment combinations were run in 18 consecutive days; the reactor was run continuously for 3 hr with each fat blend and a total of 3 blends were interesterified daily (total = 9 hr). The effects of age of Biobone (pre-column) and age of Lipozyme (enzyme column) on the composition of the blends were also evaluated to discern any variation due to depletion of water (in the pre-column) and decrease in Lipozyme activity (in the enzyme column) with time. Another factor (simply called 'block') which evaluated the effect of changing the Lipozyme column after every three days was also included in the design. During the processing period, the pre-column and the enzyme columns were repacked after every 9 hr (daily) and 27 hr (3 days) of operation, respectively.

Analysis of data

The C48, C50, C52, C54 triglyceride, free fatty acid, monoglyceride and diglyceride contents of the fat blends were analysed. The ratios of the composition of the various components in the interesterified blends to their control blends (straight blends with no interesterification involved) were calculated. These ratios were then analysed according to the following linear model using the *glm* procedure (SAS Institute Inc., 1985a).

$$Y_{ijklmno} = \mu + R_i + K_{ij} + L_{ik} + I_{il} + B_m + M_n + F_o + (MF)_{no} + (MB)_{nm} + (FB)_{om} + \epsilon_{ijklmno}$$

TABLE 1. MEAN RESIDENCE TIME OF FATS IN LIPOZYME AND BIOBONE COLUMNS AT VARIOUS FLOW RATES

Flow rate (g/hr)	Mean residence time ^a (min)	
	Biobone column	Lipozyme column
11.4	40.5 ± 10.5	45.0 ± 7.8
20.5	19.8 ± 7.4	18.9 ± 5.6
28.3	13.3 ± 6.5	14.5 ± 6.8

^a Mean and standard deviation of 4 determinations.

TABLE 2. MOISTURE, FLOW RATE AND BLEND TREATMENT COMBINATIONS FOR ENZYMATIC INTERESTERIFICATION OF FATS

	Age of Lipozyme (days)	Age of Biobone (hr)		
		0	3	6
Replicate 1				
Block 1	0	M ₁ F ₁ B _A	M ₁ F ₂ B _C	M ₁ F ₃ B _B
	1	M ₂ F ₁ B _C	M ₂ F ₂ B _B	M ₂ F ₃ B _B
	2	M ₃ F ₁ B _B	M ₃ F ₂ B _A	M ₃ F ₃ B _C
Block 2	0	M ₃ F ₃ B _A	M ₃ F ₁ B _C	M ₃ F ₂ B _B
	1	M ₁ F ₃ B _C	M ₁ F ₁ B _B	M ₁ F ₂ B _A
	2	M ₂ F ₃ B ₃	M ₂ F ₁ B _A	M ₂ F ₂ B _C
Block 3	0	M ₂ F ₂ B _A	M ₂ F ₃ B _C	M ₂ F ₁ B _B
	1	M ₃ F ₂ B _C	M ₃ F ₃ B _B	M ₃ F ₁ B _A
	2	M ₁ F ₂ B _B	M ₁ F ₃ B _A	M ₁ F ₁ B _C
Replicate 2				
Block 1	0	M ₁ F ₁ B _A	M ₁ F ₂ B _C	M ₁ F ₃ B _B
	1	M ₂ F ₁ B _C	M ₂ F ₂ B _B	M ₂ F ₃ B _A
	2	M ₃ F ₁ B _C	M ₃ F ₂ B _A	M ₃ F ₃ B _C
Block 2	0	M ₃ F ₃ B _A	M ₃ F ₁ B _C	M ₃ F ₂ B _B
	1	M ₁ F ₃ B _C	M ₁ F ₁ B _B	M ₁ F ₂ B _A
	2	M ₂ F ₃ B _B	M ₂ F ₁ B _A	M ₂ F ₂ B _C
Block 3	0	M ₂ F ₂ B _A	M ₂ F ₃ B _C	M ₂ F ₁ B _B
	1	M ₃ F ₂ B _C	M ₃ F ₃ B _B	M ₃ F ₁ B _A
	2	M ₁ F ₂ B _B	M ₁ F ₃ B _A	M ₁ F ₁ B _C

Subscripts 1, 2 and 3 associated with M represent moisture content levels of 7.4%, 13.3% and 19.2%, respectively.
 Subscripts 1, 2 and 3 associated with F represent flow rates of 11.4, 20.5 and 28.3 g/hr, respectively.
 Subscripts A, B and C associated with B represent the three fat blends used.

where $i = 1, 2; j, k, l, m, n$ and $o = 1, 2, 3$
 $Y =$ observed ratio
 $\mu =$ mean effect
 $R_i =$ effect of i th replicate
 $K_{ij} =$ effect of j th block within the i th replicate
 $L_{ik} =$ effect of k th age of Lipozyme within i th replicate
 $I_{il} =$ effect of l th age of Biobone within i th replicate
 $B_m =$ effect of m th blend
 $M_n =$ effect of n th level of moisture
 $F_o =$ effect of o th flow rate
 $MF_{no} =$ interaction effect of n th level of moisture with o th flow rate
 $MB_{nm} =$ interaction effect of n th level of moisture with m th blend
 $FB_{om} =$ interaction effect of o th flow rate with m th blend.
 $\epsilon =$ random residual effect assumed to be $N(0, \sigma^2)$

The analysis of variance table for the design used is shown in *Table 3*. Response surface plots for C48, C50, C52 and C54 triglyceride ratios with respect to moisture content levels and flow rates were generated by the *g3d* procedure (SAS Institute Inc., 1985b) for each blend using the following regression model:

$$z_{ij} = \beta_0 + \beta_1 m_i + \beta_2 f_j + \beta_3 m_i^2 + \beta_4 f_j^2 + \epsilon_{ij}$$

where z is the observed ratio response, $m =$ moisture, $f =$ flow rate, $\epsilon =$ residual effect assumed to be $N(0, \sigma^2)$, and all the β s are constant coefficients.

The above model does not include the blocking factors, blend or replicate effect. The estimated surface will not be affected because moisture and flow rate are orthogonal to these factors, but the estimate of the error variance, σ^2 , will be affected. Therefore, the average standard error of the fitted values was calculated by the following equation (Box *et al.*, 1978):

$$\text{S.E. (predicted Y)} = \sqrt{(p\sigma^2/n)}$$

where $p =$ number of parameters fitted (4), $n =$ number of observations (54) and $\sigma^2 =$ pooled error mean square obtained by fitting the following model:

$$z_{ij} = R_i + K_{ij} + L_{jk} + I_{il} + B_m + \beta_0 + \beta_1 m_i + \beta_2 f_j + \beta_3 m_i^2 + \beta_4 f_j^2 + \epsilon_{ij}$$

RESULTS AND DISCUSSION

During modification of the fat blends, conditions favouring the equilibrium of the reaction

towards interesterification were attempted but the production of some FFA, MG and DG was inevitable because of some hydrolysis which occurred simultaneously. In order to follow the progress of both interesterification and hydrolysis reactions, the results of compositional analysis of the fat blends have been expressed as C48, C50, C52, C54, FFA, MG and DG ratio. This allows their changes in composition to be compared under various moisture and flow rate conditions. The term 'ratio' in the following discussion is defined as follows:

$$\text{Component ratio} = \frac{\text{composition of component in interesterified blend (\%)}}{\text{composition of same component in control blend (\%)}}$$

The control blends contained the same fat and oil components as the interesterified blends but they were not interesterified.

Since the three fat blends used (A, B and C) contained mainly fatty acids with 16 and 18 carbon chains, the following discussion will concentrate on the composition of C48, C50, C52 and C54 triglycerides. A number of factors which contributed to the variation in triglyceride composition during interesterification has been identified. The main effects of moisture and flow rate significantly ($P \leq 0.01$) contributed to the variation in the ratio of composition of the various triglycerides during interesterification, one exception being the effect of moisture on the C50 composition (*Table 4*). The same table shows that, at a lesser level of significance, the age of Lipozyme and Biobone also contributed to the variation in the ratio of composition of some triglycerides (C48, C50 and C52). Repacking the enzyme column after every three days of processing (called 'block') also significantly contributed to the change in the ratio of composition of all of the four triglycerides. Some significant differences in C48 ($P \leq 0.01$) and C54 ($P \leq 0.1$) composition were also observed between replicates 1 and 2. These results suggest that significant changes occurred in both the Lipozyme and the Biobone which contributed to the changes in the triglyceride composition besides the interesterification reaction itself. As there was no evidence of two factor interactions for C48 to C54 triglycerides, the main effect means of moisture, flow rate and blend have been reported in *Table 5*.

TABLE 3. PLAN FOR THE ANALYSIS OF VARIANCE OF FREE FATTY ACID, MONOGLYCERIDE AND DIGLYCERIDE CONTENTS, AND TRIGLYCERIDE COMPOSITION OF CONTROL AND INTERESTERIFIED FAT BLENDS

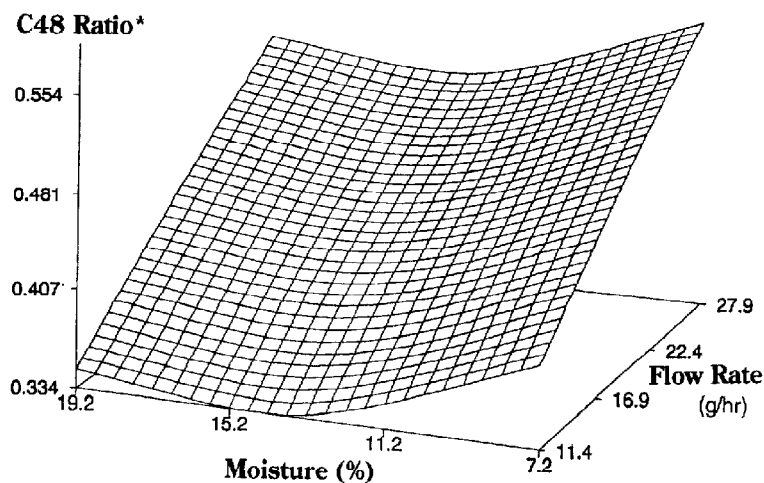
Sources of variation	Degree of freedom
Replication	1
Block within replicate	4
Age of Lipozyme within replicate	4
Age of Biobone within replicate	4
Moisture	2
Flow rate	2
Blend	2
Moisture × Flow rate	4
Moisture × Blend	4
Flow rate × Blend	4
Error	14
Total	53

TABLE 4. ANALYSIS OF VARIANCE OF THE TRIGLYCERIDE, FREE FATTY ACID AND PARTIAL GLYCERIDE RATIOS

Sources of variation	Mean Square						
	C48r ^a	C50r	C52r	C54r	FFAr	MGr	DGr
Replication	0.0748 ***	0.0017	0.0041	0.0007 *	12.4032	2.1440 ***	14.0964
Block (rep)	0.0945 ***	0.0317 ***	0.0450 ***	0.0026 ***	4.3066	0.2673	3.9040
Age of Lipozyme (rep)	0.0104 **	0.0037 **	0.0321 ***	0.0005	73.3725 ***	0.2855	42.7978
Age of Biobone (rep)	0.0073 **	0.0023 *	0.0260 **	0.0002	47.8151 ***	0.0359	27.254 ***
Moisture	0.0166 ***	0.0018	0.0809 ***	0.0015 ***	19.4217	0.1196	18.6744 *
Flow rate	0.1238 ***	0.0147 ***	0.4141 ***	0.0079 ***	3.7986	0.1588	0.5146
Blend	0.0021	0.0028 *	0.0078	0.0030 ***	85.7688 ***	0.3262	3580.3802 ***
Moisture × Flow rate	0.0026	0.0019	0.0059	0.0003	22.4356	0.2979	9.0560
Moisture × Blend	0.0006	0.0002	0.0048	0.0002	8.6806	0.1635	3.6079
Flow rate × Blend	0.0050	0.0005	0.0091	0.0001	16.0733	0.3440	5.2882
Error	0.0025	0.0009	0.0074	0.0003	11.3455	0.2331	6.4490

^ar - ratio of composition of interesterified to control blend
 FFA - Free fatty acid; MG - monoglyceride; DG - diglyceride
 * P ≤ 0.10, ** P ≤ 0.05, *** P ≤ 0.01

BLEND A



*Ratio of Composition of Interesterified to Control Blend

Average standard error of fitted values = 0.021

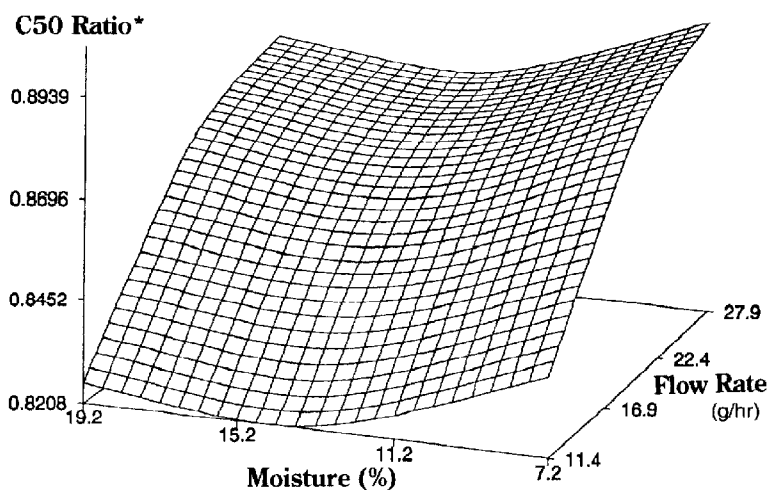
$$z = 0.4005 - 0.0286 m + 0.0144 f + 0.0009 m^2 - 0.0001 f^2$$

Blend A contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.

The canola oil was hydrogenated under non-selective conditions (140° C, 310.1 KPa (45 psi)).

Figure 1. Effect of moisture and flow rate on C48 triglyceride ratio of blend A.

BLEND A



*Ratio of Composition of Interesterified to Control Blend

Average standard error of fitted values = 0.014

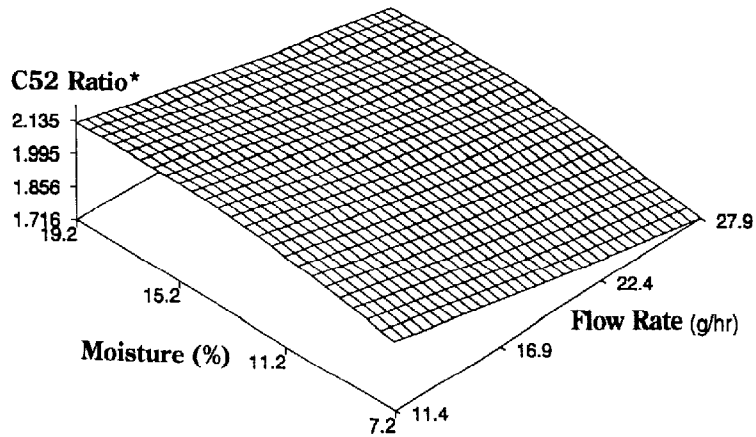
$$z = 0.7964 - 0.0094 m + 0.0104 f + 0.0003 m^2 - 0.0002 f^2$$

Blend A contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.

The canola oil was hydrogenated under non-selective conditions (140° C, 310.KPa (45 psi)).

Figure 2. Effect of moisture and flow rate on C50 triglyceride ratio of blend A.

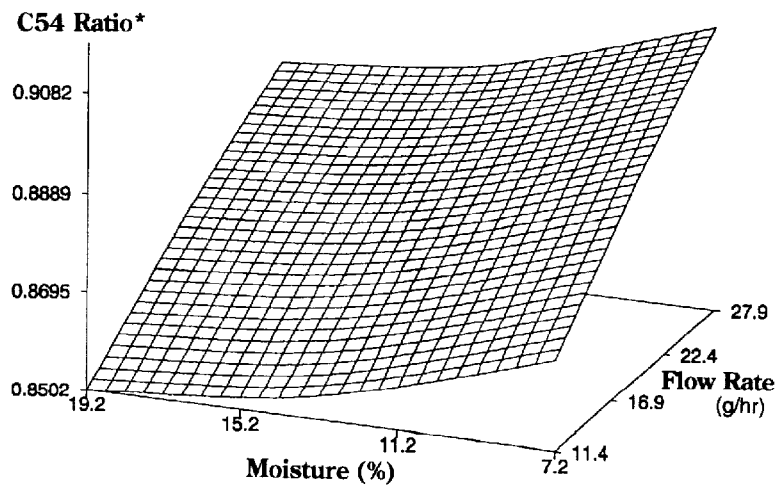
BLEND A



Ratio of Composition of Interesterified to Control Blend
 Average standard error of fitted values = 0.026
 $z = 1.9785 + 0.0513 m - 0.0241 f - 0.0016 m^2 + 0.0002 f^2$
 Blend A contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
 The canola oil was hydrogenated under non-selective conditions (140° C, 310.1 KPa (45 psi)).

Figure 3. Effect of moisture and flow rate on C52 triglyceride ratio of blend A.

BLEND A



*Ratio of Composition of Interesterified to Control Blend
 Average standard error of fitted values = 0.006
 $z = 0.8565 - 0.0045 m + 0.0036 f + 0.0001 m^2 - 0.0003 f^2$
 Blend A contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
 The canola oil was hydrogenated under non-selective conditions (140° C, 310.1 KPa (45 psi)).

Figure 4. Effect of moisture and flow rate on C54 triglyceride ratio of blend A.

The combination effects of moisture and flow rate are more clearly depicted in *Figures 1 to 4*; similar trends were observed for blends B and C. The trend depicted in the 3-dimensional plots indicates that the greatest change in ratio of triglycerides (C48, C50, C52 and C54) occurred at a flow rate of 11.4 g/hr when a moisture content between 13.2% and 19.2% was present in the Biobone. The effect of moisture and operation time on the C52 triglyceride composition of blend A at a flow rate of 11.4 g/hr is shown in *Figure 5*. The greatest change in C52 composition was observed after about 30 minutes and the composition started to stabilize after about 1 hour of processing. The graph also indicates that the greatest change in C52 composition occurred at a moisture content of 19.2%; this confirms the conclusion drawn earlier.

The processing parameters which significantly contributed to an increase in FFA and DG contents were moisture content of Biobone, age of Lipozyme and age of Biobone (*Table 4*). The monoglyceride (MG) content was not affected by these factors. However, there was a significant difference ($P \leq 0.01$) in the monoglyceride content between replicates 1 and 2. There was also evidence that the blends used contributed significantly ($P \leq 0.01$) to the variation in the FFA content ($P \leq 0.01$) and DG content ($P \leq 0.01$) of the interesterified blends.

The standard errors in *Table 5* indicate that the composition of FFA, MG and DG between most of the means (moisture, flow rate and blend) were significant, but no significant interactions between any two factors were observed. Due to inconsistency in the treatment means, no trend could be established for FFA and MG content with respect to moisture, flow rate or blend. However, the lowest amount of DG was observed when the reactor was operated at a flow rate of 11.4 g/hr with a moisture content of 7.3 percent. In contrast, this flow rate condition produced the greatest change in triglyceride composition as discussed earlier.

The largest amounts of FFA, MG and DG were produced during the first three hours of Biobone and the first day of Lipozyme utilization. The content of these three components was lower after 6 hours and day 2, respectively. However, a longer processing period is required before further conclusions can be drawn.

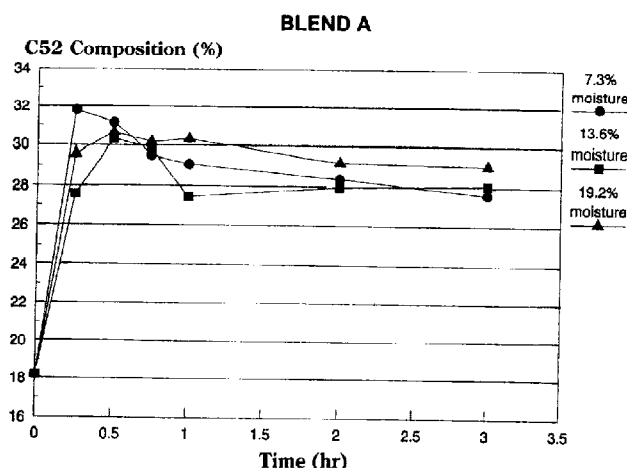
The variations in FFA, MG and DG reported in *Table 5* were most probably due to the frequent changes in both moisture contents and flow rates during the 18 days of the processing period. The operation of the reactor under fixed moisture and flow rate conditions is expected to produce lesser variations in the results.

The ability of Biobone to retain water was expressed as water retention capacity (WRC) which was defined as follows:

$$\text{WRC} = \frac{\text{amount of water retained (\%)}^a}{\text{amount of water added (\%)}^a} \times 100$$

^aThe amount of water added and retained as a percentage of dry weight of Biobone was determined following 24 hr of equilibrium at room temperature (20°C).

The amount of water retained by the Biobone increased with increasing amount of water added (*Table 6*). These data indicate that the material was not fully saturated and it could still hold more water than the levels (8%, 16% and 24%) used in the present study. If the Biobone were saturated, no additional water would be retained. As a result, the



Blend A contained 45%, 25% and 30% of hydrogenated canola oil (I.V. = 59.9), palm stearin and canola oil, respectively. The canola oil was hydrogenated under non-selective conditions (140°C, 310.1 KPa (45 psi)).

Figure 5. Effect of moisture and operation time on the composition of C52 triglyceride of blend A at a flow rate of 11.4 g/hr.

TABLE 5 . MEANS OF TRIGLYCERIDE, FREE FATTY ACID AND PARTIAL GLYCERIDE RATIOS FOR MAIN EFFECTS OF MOISTURE, FLOW RATE AND BLEND

Sources of variation	Mean						
	C48 ratio	C50 ratio	C52 ratio	C54 ratio	FFA ratio	MG ratio	DG ratio
Moisture							
(%)							
7.3	0.48	0.87	1.85	0.89	3.14	1.23	5.95
13.6	0.42	0.85	1.97	0.88	5.19	1.21	7.60
19.2	0.44	0.85	1.97	0.87	3.87	1.36	7.81
S.E. ^a	0.02	0.01	0.03	0.01	1.12	0.16	0.86
Flow rate							
(g/hr)							
11.4	0.36	0.83	2.09	0.86	4.46	1.37	6.92
20.5	0.46	0.87	1.91	0.88	3.56	1.22	7.24
28.3	0.52	0.88	1.79	0.89	4.17	1.20	7.19
S.E. ^b	0.02	0.01	0.03	0.01	1.12	0.16	0.86
Fat blend							
A	0.45	0.88	1.91	0.89	2.80	1.22	2.83
B	0.44	0.85	1.94	0.87	6.59	1.42	11.73
C	0.46	0.86	1.95	0.87	2.81	1.16	6.79
S.E. ^c	0.02	0.01	0.03	0.01	1.12	0.16	0.86

^{a,b,c} Standard error of difference between any two means.
 FFA – free fatty acid; MG – monoglyceride; DG – diglyceride.

TABLE 6. WATER RETENTION CAPACITY OF BIOBONE AT VARIOUS LEVELS OF MOISTURE

Day 1			Day 18	
Water added (%)	Water retained (%)	WRC ^a (%)	Water retained (%)	WRC (%)
8.0	7.2	90.0	7.4	92.5
16.0	13.6	85.0	13.3	83.1
24.0	19.2	80.8	19.2	80.0

$$^a \text{ Water retention capacity} = \frac{\text{amount of water retained (\%)}}{\text{amount of water added (\%)}} \times 100$$

where the amount of water added and retained were expressed as a percentage of the dry weight of Biobone.

TABLE 7. EFFECT OF ENZYMATIC INTERESTERIFICATION ON THE TRIGLYCERIDE COMPOSITION, FREE FATTY ACID AND PARTIAL GLYCERIDE CONTENT OF FAT BLENDS

Blend	Composition by carbon number (%)							Total ^a
	48	50	52	54	FFA	MG	DG	
A (CN)	7.9	14.5	18.2	50.6	0.7	1.1	2.4	4.3
A (I)	2.8	12.8	32.9	43.9	2.0	1.3	6.8	10.2
B (CN)	8.2	14.9	18.6	49.9	0.7	1.1	0.8	2.7
B (I)	3.8	13.5	30.9	44.2	4.6	1.4	9.4	15.7
C (CN)	7.1	14.5	18.4	53.3	0.7	1.1	1.0	2.8
C (I)	3.0	12.9	32.3	45.0	2.1	1.3	6.6	9.9

^aTotal of free fatty acids, monoglycerides and diglycerides.

The control blends were not interesterified.

All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.

Blends A, B and C contained hydrogenated canola oil (I.V. = 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140°C, 310.1 KPa (45 psi)), intermediate AOCS conditions (175°C, 103.4 KPa (15 psi)) and selective conditions (200°C, 51.7 KPa (7.5 psi)), respectively.

WRC would tend to zero. The ability of the Biobone to maintain its moisture during the 18 days of interesterification is also shown in *Table 6*. The stock containers of Biobone were stored at 10°C and 6-gram portions were removed to repack the pre-column during the experimental period (18 days). Very little or no change in the moisture content was observed when the data at the beginning and at the end of the 18-day storage period were compared.

All the samples collected for each blend during processing were pooled and their triglyceride composition determined. A decrease of more than 50% in C48 triglyceride content indicates that a large portion of the trisaturate has been eliminated during interesterification (*Table 7*). The palmitic acid residue was exchanged with an 18 carbon fatty acid in C54 triglycerides to yield C52 triglycerides. A less drastic decrease in C50 triglyceride composition could be due to the replacement of either one palmitic acid residue in C48 or the replacement of a second 18 carbon fatty acid in C54 triglycerides. The decrease in C54 triglyceride content with simultaneous formation of C52 triglycerides produced a more heterogeneous triglyceride composition in the blends.

The higher levels of FFA, MG and DG in the pooled interesterified fat samples as compared with the control blends indicated that some degree of hydrolysis inevitably occurred during processing (*Table 7*). The FFA, MG and DG contents of the interesterified blends were 2.0% to 4.6%, 1.3% to 1.4%, and 6.6% to 9.4%, respectively. Using the same enzyme for batch interesterification, Posorske *et al.* (1988) found that the levels of FFA, MG and DG were 7%, 1% and 12%, respectively. But after a second use they were lowered to 2%, 0% and 6%, respectively. The results of the present study, therefore, indicate that an operation time of more than 3 hr is required to stabilize the reactor in order to further minimize the production of these hydrolytic products.

This study has shown that the incomplete block design evaluated was useful in discriminating processing parameters and identifying factors which affect both interesterification and hydrolysis reactions in a bench-top packed-column reactor. Biobone was found to be suitable for hydrating fats during continuous enzymatic interesterification. By operating the reactor for extended hours, it is speculated that the production of FFA, MG and DG would be lower than the levels reported in this study.

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