0273-2289/06/129–132/680–693/$30.00

Optimization of Distilled Monoglycerides Production

LEONARDO VASCONCELOS FREGOLENTE,* CÉSAR BENEDITO BATISTELLA, RUBENS MACIEL FILHO,
AND MARIA REGINA WOLF MACIEL

Laboratory of Separation Process Development (LDPS) School of Chemical Engineering, UNICAMP CP 6066-CEP 13081-970-Campinas, SP, Brazil; E-mail: leonardo@feq.unicamp.br

Abstract

Monoglycerides (MG) are emulsifiers widely used in food and pharmaceutical industries. Current industrial processes for MG production consist of the interesterification of triglycerides with glycerol (GL), in the presence of inorganic catalysts at high temperatures (>200°C). This reaction is known as glycerolysis and produces a mixture of approx 50% of MG. This level of concentration is suitable for many applications, although, for some specific uses like margarine, shortening, icing, and cream filling, require distilled MGs, which are purified MG (min. 90%) obtained by the molecular distillation process. Therefore, in this work, a 2^3 factorial design was employed to evaluate the effects of reaction parameters in the MG content after the interesterification reaction of refined soybean oil with GL in the presence of sodium hydroxide as catalyst. After that, the MG content in the reaction product was enhanced through the molecular distillation process in order to obtain distilled MG.

Index Entries: Glycerolysis; soybean oil; distilled monoglycerides; molecular distillation; short path distillation; factorial design.

Introduction

Many different types of lipid-based emulsifier can be applied to the food, cosmetic, and pharmaceutical industries. The manufacturer must select the one that is most suitable for each particular product, considering the physicochemical properties of the final product, cost, and availability of the emulsifier and its compatibility with other ingredients *(1)*.

Monoglycerides (MG) are the predominant type of emulsifier, representing about 70% of the synthetic emulsifiers produced *(2)*. Some researchers have developed three lipase-catalyzed routes to MG: (1) hydrolysis or alcoholysis of triglyceride (TG), (2) glycerolysis of TG, and (3) esterification or transesterification of glycerol (GL), considering the mild condition

---

*Author to whom all correspondence and reprint requests should be addressed.

requirements of the lipases (low temperatures and near neutral pH). Furthermore, they can explore the lipase fatty acid selectivity and regioselectivity for the primary vs secondary positions in the GL *(3)*.

However, industrially, MG are manufactured by batch or continuous reactions at temperatures greater than 200°C, using inorganic catalysts. Products with around 50% of MG content are achieved by direct esterification reaction, when the starting material is fatty acid or, by interesterification when the starting material is TG. As the dominant part of the manufacturing cost is the price of the feed material, interesterification is preferred, because acids are more expensive than TG. Direct esterification is frequently used when MG with a specified acid distribution is required.

The MG content obtained in these chemical reactions is suitable for many applications, although for some specific uses such as cake or icing, the mouth-melt of the product is critical. The use of a commercially prepared MG emulsifier could impact texture or mouthfeel of the product *(4)*, so that distilled MG are required, which are purified MG (min. 90%), normally obtained through the molecular distillation process.

Also known as short-path distillation, molecular distillation is characterized by a short exposure of the distilled liquid to the operating temperature and high vacuum *(5,6)*. Because of these features, besides the concentration of MG, this process has been widely applied in lipid areas. Some of these applications include the recovering of carotenoids from palm oil *(7)*, recovering of tocopherol from crude deodorizer distillate of soya oil *(8)*, purification and deodorization of structured lipids *(9)*, and the preparation of purified concentrates of polyunsaturated fatty acid *(10)*.

The aim of this study is to find the best conditions for the glycerolysis of refined soybean oil in a batch reactor, carried out at relatively low temperatures (190–210°C), and also to obtain distilled MG from the reaction products using molecular distillation process.

Methods

Determination of TG, Diglycerides, MG, Free Fatty Acids, and GL

For the determination of TG, diglycerides (DG), MG, free fatty acids, and GL high-performance size exclusion chromatography (HPSEC) was used according to Schoenfelder (2003) *(11)*. The chromatographic system consists of an isocratic pump, model 515 high-performance liquid chromatography (HPLC) Pump (Waters Inc., Milford, MA), a differential refractometer detector model 2410 (Waters), and an oven for columns thermostatted at 40°C by a temperature control module (Waters). The samples were injected using a manual injector, model rheodyne 7725i with a 20-µL sample loop. Two HPSEC columns Styragel HR 1 and HR 2 (Waters), with dimensions of 7.8 × 300 mm and particule size of 5 µm packed with styrene-divinylbenzene copolymer were connected in series. The mobile phase used was HPLC-grade tetrahydrofuran from Tedia Inc. (Fairfield, OH) and the flow rate was 1 mL/min. The typical pressure at this flow rate was 470 psi. All the standards were obtained from Supelco (Supelco, Bellefonte, PA). The data processing was done by the Millenium software 2010 Chromatography Manager Software from Waters Inc.

Analysis of Fatty Acid Composition

The fatty acid composition of the reaction products was determined by gas–liquid chromatography (GLC). Acylglycerols were converted into fatty acid methyl esters (FAME) according to Hartman (1973) *(12)*. The FAME mixture was analyzed by a Varian gas chromatograph model STAR 3600CX (Lexington, MA) equipped with a flame ionization detector and with a DB 23 column (30 m × 0.53 mm, J&W Scientific, Folsom, CA). Injector and detector temperatures were set at 250°C and 300°C, respectively. The carrier gas used was helium at 46 mL/min. Air and hydrogen flow rates were 334 and 34 mL/min, respectively. The program of the oven temperature was as follows: starting at 50°C for 2 min; from 50°C to 180°C at 10°C/min; 180°C was held for 5 min; from 180°C to 240°C at 5°C/min. Identification of different FAME was based on a reference standard mixture F.A.M.E. Mix C4-C24 (Supelco, PA).

Glycerolysis Reaction

In this work, to evaluate the effect of the mass ratio of GL to refined oil (GL/TG), and also the effects of the reaction temperature (T) and of the amount of catalyst (NaOH), a 2^3 factorial design with three central points was planned, as shown in Table 1. Experiments were carried out in a 250-mL glass-stoppered volumetric flask of 250 mL.

Initially, 50 g of reactants consisting of commercial refined soybean oil and GL (Labsynth, SP, Brazil) were fed in the batch reactor. Then, the mixture of reactants was heated by a stirring hot plate (Fisatom, SP, Brazil) to a defined temperature, in nitrogen atmosphere, under agitation. When the defined temperature was reached, a known amount of catalyst (NaOH, LabSynth, Brazil) was added to the reactor (reaction time = 0). In order to monitor the conversion of TG into MG and also to verify whether the reaction reached the equilibrium condition, samples were withdrawn from the reactor at different times. The mass ratio (GL/TG) of each run, as well as the amount of catalyst (amount of NaOH) and the reaction temperatures (T) studied are shown in Table 1.

Manufacturers avoid oils rich in unsaturated fatty acids, such as soybean oil, because at temperatures >200°C, they burn or polymerize causing a dark color, off-odor and burnt taste; so, usually, they use partially or fully hydrogenated fat. For these reasons, the experimental range of the reaction temperature was studied at relatively low levels, between 190°C and 210°C, and the fatty acid composition of the reaction products was accompanied. In case of significant degradation, the fatty acids composition of the reaction products may vary during the reaction time, what can

Table 1
Coded Levels and Real Levels (In Parentheses) of the Variables Studied and the Results Obtained in the Glycerolysis Reaction

Runs	NaOH (g)	T (°C)	GL/TG	% of MG[a] ($t = 90$ min)
1	–1 (0.07)	–1 (190)	–1 (0.18)	41.0
2	1 (0.13)	–1 (190)	–1 (0.18)	41.8
3	–1 (0.07)	1 (210)	–1 (0.18)	41.8
4	1 (0.13)	1 (210)	–1 (0.18)	40.7
5	–1 (0.07)	–1 (190)	1 (0.30)	48.3
6	1 (0.13)	–1 (190)	1 (0.30)	51.8
7	–1 (0.07)	1 (210)	1 (0.30)	53.8
8	1 (0.13)	1 (210)	1 (0.30)	54.5
9	0 (0.10)	0 (200)	0 (0.24)	48.6
10	0 (0.10)	0 (200)	0 (0.24)	48.6
11	0 (0.10)	0 (200)	0 (0.24)	49.6

[a] Normalized peak area.

be detected by the GLC analyses. Furthermore, if significant polymerization occurs, the molecules of high-molecular weight formed in the reaction may be detected by the HPSEC analysis, as this method of analysis identifies components by their sizes.

Usually, in the factorial design, the variables levels are denoted by coded numbers. The superior levels are represented by +1 and the inferior levels by –1. The central points, which are used for the error estimation, are denoted by 0. They are replicates in the center of the experimental range that permit the evaluation of the repeatability of the experiments. The variation between them reflects the variability of whole design *(13)*. The response analyzed was the MG concentration at the equilibrium condition.

As GL is not totally soluble in the mixture, even in a system with good agitation, the percentage of GL varies considerably in different points of the reactor, causing sampling problem. To avoid this problem, the percentage of MG (in the equilibrium condition) reported in Table 1 was obtained by normalizing the peak areas of TG, DG, and MG. Furthermore, this procedure permits the comparison among runs, with different initial GL concentration.

Molecular Distillation

Molecular distillation, also known as short path distillation, is a separation process characterized by a short exposure of its distilled liquid to the operating temperature, high vacuum in the distillation space (distance between the evaporator and condenser), and a small distance between these elements. The separation principle is the vacuum (enabling the molecules to evaporate from the evaporator to the condenser) and, in case of a centrifugal distillator, the centrifuge force (promoting a thin film on the evaporator

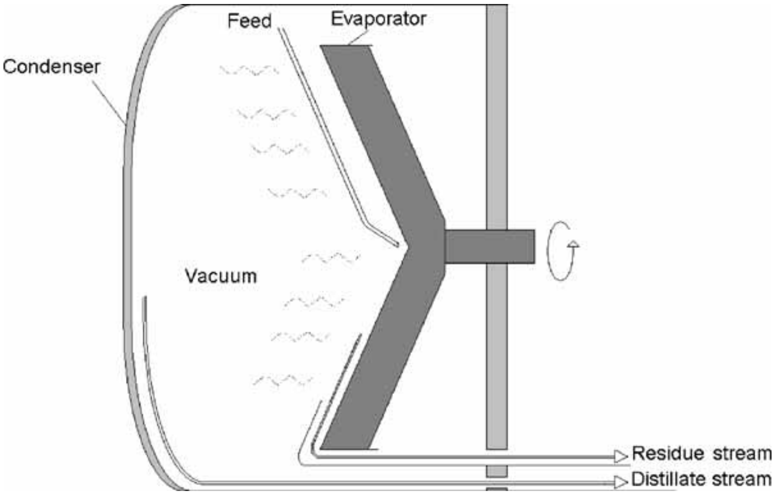

Fig. 1. Simplified scheme of a centrifugal molecular distillator.

surface). Two product streams are generated: Distillate (rich in the molecules that escape from the evaporator and reach the condenser) and Residue (rich in the heavier molecules that remain in the evaporator).

In this work, the equipment used was a centrifugal distillator from Myers Vacuum Inc. (Kittanning, PA), with an evaporator area of 0.0046 m^2. The system was operated at a pressure of 1.6×10^{-4} bar. The starting material was fed at 60°C in the center of the evaporator (as indicated in Fig. 1), with a defined flow rate (Q) and evaporator temperatures (TEV). The condenser temperature is fixed at 55°C.

Results and Discussion

Glycerolysis Optimization

As can be seen in Fig. 2, after 1 h, the system reached the equilibrium condition, for all the runs. It can be noted that the higher reaction yields were obtained in the runs with GL/TG = 0.30 (runs 5–8). Analyzing the three central points (runs 9–11), the good repeatability of the experiments can be seen, as their results are very close.

Figure 3A shows the time-course of the glycerolysis for run 2, representing the runs carried out with GL/TG = 0.18 and Fig. 3B shows the time-course of the glycerolysis for run 7, representing the runs carried out with GL/TG = 0.30.

It can be seen that, in the experiments carried out with the mass ratio GL/TG = 0.30, the difference between the MG and DG concentrations at the equilibrium condition is approx 20%, whereas in the experiments carried out with GL/TG = 0.18, these concentration values are very close. Furthermore, it can be noted that the TG equilibrium concentration in the

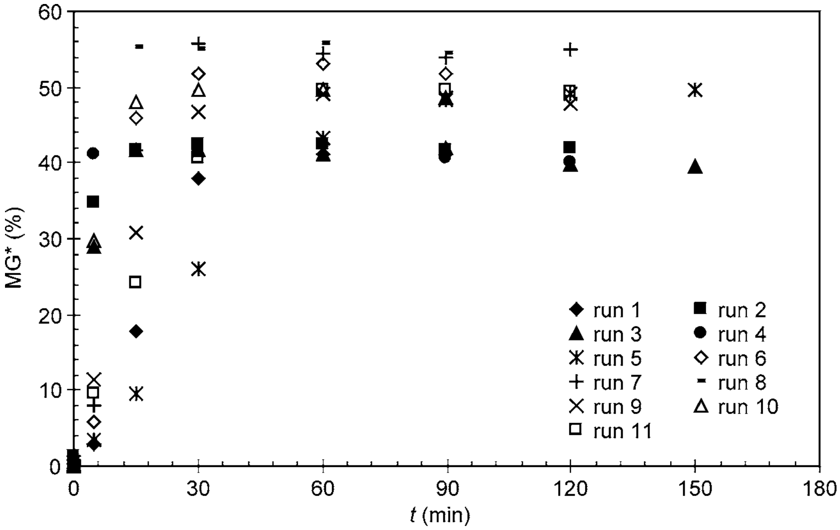

Fig. 2. MG concentration during the glycerolysis reaction. *Normalized peak areas of TG, DG, and MG.

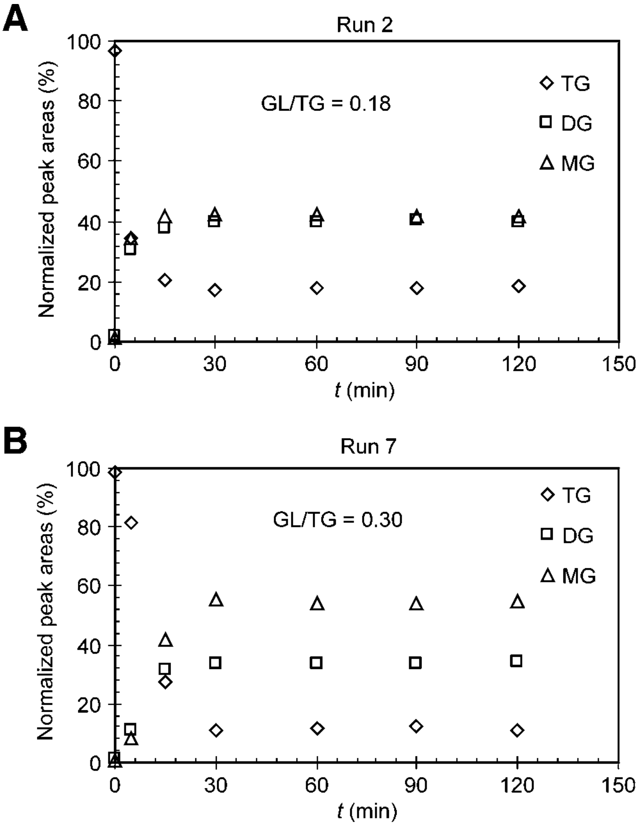

Fig. 3. Comparison among the runs with GL/TG = 0.18 **(A)** and runs with GL/TG = 0.30 **(B)**.

Table 2
Main Effects of GL/TG, T, Amount of NaOH, and the Binary Interaction Effect Involving These Factors

	Effect (%)	Standard error	t (2)	p-value
(1)NaOH	0.96	0.204	2.388	0.1395
(2)T[a]	1.97	0.204	4.838	0.0401
(3)GL/TG[a]	10.77	0.204	26.393	0.0014
1 by 2	–1.20	0.204	–2.878	0.1025
1 by 3	1.17	0.204	2.756	0.1103
2 by 3[a]	2.13	0.204	5.205	0.0350

[a]Significant effects at 95% of confidence.

runs with GL/TG = 0.18 is approximately twice the TG equilibrium concentration when GL/TG = 0.30. This confirms that, at the experimental conditions studied, the higher yields were obtained with the GL/TG = 0.30.

The main effects of the three variables studied and the binary interaction effect involving these factors are shown in Table 2. It can be seen that, at 95% of confidence, GL/TG, T, and the interaction between T and GL/TG presented significant effects (p-value < 0.05). As shown before, the effect of the GL/TG is very relevant in the experimental condition studied, presenting an effect of 10.77%, what means that, on an average, the percentage of MG increases 10.77% when the level of the GL/TG is increased from –1 to +1.

The effect of T is positive and also significant, but much lower than the GL/TG effect. This may be owing to the narrow T experimental range studied. The interaction effect between T and GL/TG is significant at 95% of confidence, but also lower than the GL/TG effect. As expected, the main effect of the amount of catalyst (NaOH) is not significant at this level of confidence, as the catalyst concentration does not displace the equilibrium condition toward any side (product or reagent sides).

Because all the variables studied showed positive main effects, new experiments were carried out in order to explore a new experimental range, confirming the results obtained in the experimental design. Thus, higher levels of the mass ratio GL/TG was studied (Fig. 4) as its effect was significant at 95% of confidence level (the highest effect among the studied variables). The temperature was kept at 210°C (the highest studied level in the experimental design). Although its effect is positive and significant at 95% of confidence level, in the experimental range studied, temperatures >210°C were not studied because it may cause considerable polymerization reactions. The amount of catalyst (NaOH) added in the system did not affect significantly the equilibrium condition, therefore it was fixed in 0.13 g for 50 g of reactants.

Figure 4 shows the MG concentration (normalized peak areas of TG, DG, and MG) in the equilibrium condition as a function of the GL/TG ratio. It can be noted that, in this new experimental range explored

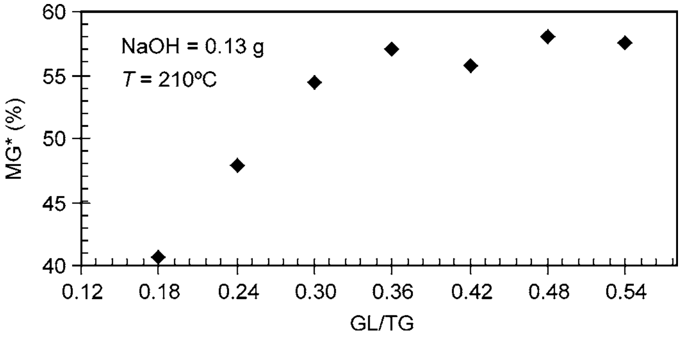

Fig. 4. MG concentration in the equilibrium condition as a function of the GL/TG ratio. *Normalized peak areas of TG, DG, and MG.

Table 3
Fatty Acids Composition of Acylglycerols

t	Fatty acid composition (%)[a]								
(min)	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
0	12.1	3.3	21.8	54.2	5.4	0.4	0.2	0.5	0.2
30	12.4	3.8	22.3	53.2	5.2	0.4	0.2	0.5	0.2
60	12.6	3.4	22.5	53.2	5.1	0.4	0.2	0.5	0.2
90	12.8	3.7	22.2	52.4	5.1	0.4	0.2	0.5	0.2

[a] 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, α-linolenic acid; 20:0, arachidic acid; 20:1, eicosenoic acid; 22:0, behenic acid; 24:0, lignoceric acid. GL/TG = 0.48, T = 210°C, NaOH = 0.13 g, mass of reagents = 50 g.

(GL/TG values >0.30), there is a small increase in the equilibrium MG concentration. However, it seems that for GL/TG values greater than 0.36, the equilibrium MG concentration remains the same. The maximum MG concentration obtained (as normalized peak area) in the studied experimental range is around 57%.

Table 3 contains experimental data on fatty acid composition of acylglycerols at different times of reaction. One can see that, at the experimental conditions studied, the fatty acid composition changes lightly with time, indicating just a moderate occurrence of polymerization. The concentrations of linoleic acid and α-linolenic acid, both polyunsaturated, decrease because they are more susceptible to degradation. This partial degradation causes a change in the color of the oil, which becomes darker as well as a change in the odor. Despite the moderate degradation of the polyunsaturated, HPSEC analysis did not detect polymers formed.

Molecular Distillation Optimization

Fregolente et al. (2005) *(14)* have shown a study to find optimized operating conditions of the molecular distillation in order to enrich the distillate

Table 4
Properties of the Commercial MG Used as Starting Material

Product name	Source	Acid value (mg KOH/g)	Iodine value (cg I 2/g)	MG content (%)	Physical form
BRAWAX MGS C	Vegetable	1.3	58.3	43.6	Waxy solid

Table 5
Central Composite Design Carried Out to Optimize the Molecular Distillation Process

TEV (°C)	Q (mL/min)	MGD (%)	MGR (%)
–1(215)	–1(6.5)	78.1	37.5
+1(285)	–1(6.5)	70.9	25.7
–1(215)	+1(13.5)	56.3	41.8
+1(285)	+1(13.5)	77.7	36.1
–1.41(200)	0(10)	56.9	41.5
1.41(300)	0(10)	65.2	29.3
0(250)	–1.41(5)	74.8	26.8
0(250)	1.41(15)	68.8	41.0
0(250)	0(10)	75.2	37.4
0(250)	0(10)	77.5	36.6

stream in MG, starting from a commercial MG. Its properties are shown in Table 4. The composition of this commercial MG is similar to the composition of the products obtained in the glycerolysis products (40–50%).

The variables studied were the evaporator temperature (TEV) and the feed flow rate (Q), because they are very important process variables in the molecular distillation process. The experimental range of these variables was chosen according to previous experience. Values of feed flow rate <4 mL/min may not be high enough to form a uniform thin film on the evaporator surface. This uniform thin film promotes efficient mass and energy transfers *(15)*. For feed flow rate values greater than 15 mL/min, it was noted that the system operated with low effectiveness, as the residence time of the molecules on the evaporator is too low. Therefore, the feed flow rate varied from 5 to 15 mL/min. The variation range of TEV was from 200 to 300°C (Table 5).

To find the optimized operating conditions, response surface methodology (RSM) was applied, that is a set of mathematical and statistical methods developed for modeling phenomena and finding combinations of a number of experimental factors that will lead to optimum responses *(16)*. Usually, in the RSM, simple coded models such as linear and quadratic are fitted. In this work, independent and dependent variables were fitted to a second-degree polynomial equation (Eq. 1), where y is the estimated response (MG concentration in the distillate stream [MGD], or MG concentration in the residue stream [MGR]), b_0 is a constant, b_{ij} are the coefficients for each term and x_i are the independent factors in coded values (x_1 corresponds to the coded value for TEV and x_2 corresponds to the coded value for Q).

$$y = b_0 + b_1\, x_1 + b_2\, x_2 + b_{11}\, x_1^{\,2} + b_{22}\, x_2^{\,2} + b_{12}\, x_1 x_2 \tag{1}$$

Analyzing Eq. 1, which is a quadratic model with two variables, it can be seen that it contains six parameters, so that the number of combinations of the independent variable levels must be >6, as it is not possible to predict values when the number of equation parameters is higher than the number of independent variable levels. Thus, a factorial design consisting of 2^2 trials plus a star configuration (four axial points) with three central points was carried out. The distance of the axial points from the central point is calculated from the equation, $\alpha = (2^n)^{1/4}$, where α is the distance of the axial points and n is the number of independent variables *(17)*. This kind of factorial design, also known as central composite design, is suitable for the fit of Eq. 1, because for two independent variables, it contains nine different combinations of the independent variable levels.

The quality of the fitted models was evaluated by the analysis of variance (ANOVA), based on F-test *(18)* and on the percentage of explained variance, which provides a measurement of how much of the variability in the observed response values could be explained by the experimental factors and their interactions *(19)*.

The fitted coded models for the MGD and MGR are shown in Eqs. 2 and 3, respectively, in which, all the coefficients of Eq. 1 were considered.

$$\begin{aligned}\text{MGD} = 75.27 + 3.25 \times \text{TEV} - 6.04 \times \text{TEV}^2 - 2.94 \times Q \\ - 0.66 \times Q^2 + 7.13 \times \text{TEV} \times Q\end{aligned} \tag{2}$$

$$\begin{aligned}\text{MGR} = 36.88 - 4.37 \times \text{TEV} - 0.59 \times \text{TEV}^2 + 4.35 \times Q \\ - 1.32 \times Q^2 + 1.51 \times \text{TEV} \times Q\end{aligned} \tag{3}$$

Through the ANOVA, shown in Table 6, it can be concluded that there is no evidence of lack of fit for the fitted models, since the calculated F-value (lack of fit/pure error) are lower than the critical F-value ($F_{0.95,3,2} = 19.16$) at 95% confidence, for both models. Furthermore, the results show that the model for the MG concentration in the residue (MGR), Eq. 3, is predictive in the experimental conditions studied, because the percent of explained variance is high (98.53%) and the calculated F-value (regression/residual) is more than 13 times higher than the critical F-value at 95% of confidence ($F_{0.95,5,5} =$ 5.05). As a practical rule, the regression can be considered useful to predict values when the calculated F-value (regression/residual) is more than 10 times higher than the critical F-value *(20)*.

As can be seen in Fig. 5, obtained from Eq. 3, at low feed flow rate, the MGD increases up to a maximum of 80% approx, and starts decreasing at TEV higher that 250°C owing to the significant amount of DG that leave the distillator in the distillate stream at these conditions. The feed flow rate (Q) is important, because at high Q, the residence time of the molecules on

Table 6
Analysis of Variance for the Fitted Models[a]

Source of variation	Model	Sum of square	Degrees of freedom	Mean square	*F*-ratio
Regression	Eq. 2 (MGD)	570.002	5	114.000	10.57[b]
	Eq. 3 (MGR)	323.592	5	64.718	66.93[b]
Residual	Eq. 2 (MGD)	53.919	5	10.783	–
	Eq. 3 (MGR)	4.833	5	0.967	–
Lack of fit	Eq. 2 (MGD)	44.262	3	14.754	3.06[c]
	Eq. 3 (MGR)	4.373	3	1.458	6.34[c]
Pure error	Eq. 2 (MGD)	9.657	2	4.829	–
	Eq. 3 (MGR)	0.460	2	0.230	–
Total	Eq. 2 (MGD)	623.921	10		
	Eq. 3 (MGR)	328.425	10		

[a] MGD, percent of explained variance, 91.36; percent of explicable variance, 98.45; MGR, percent of explained variance, 98.53; percent of explicable variance, 99.86

[b] *F*-ratio (regression/residual)

[c] *F*-ratio (lack of fit/pure error).

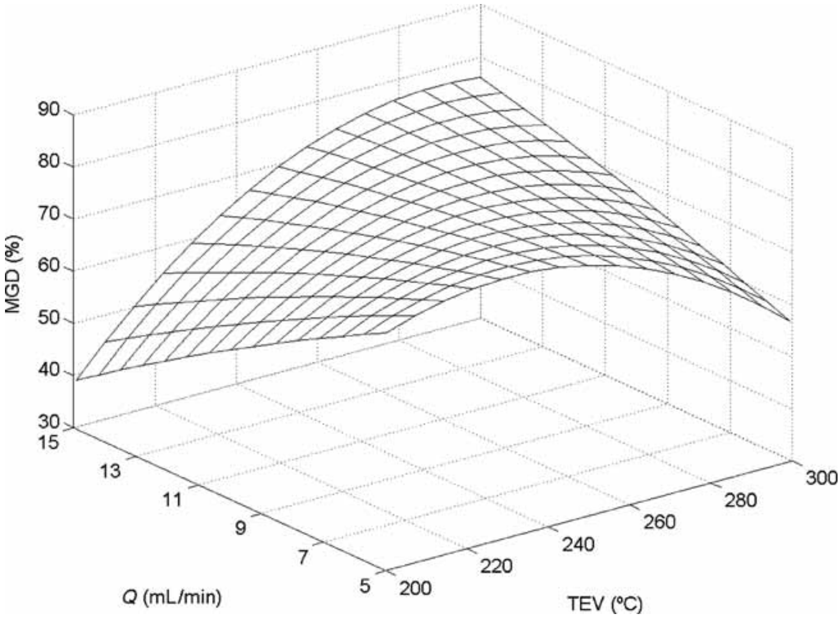

Fig. 5. Response surface for the MG concentration in the distillate stream.

the evaporator surface is low, so that the DG evaporation may not be high enough to dilute the MGD.

Distillation of the Glycerolysis Reaction Product

To obtain enough material for the distillation of the glycerolysis product in a optimized operating condition, the 50 g experiments was scaled up to 500 g with the following conditions: GL/TG = 0.48, T = 210°C and

Table 7
Composition of the Two Phases Separated, After the Glycerolysis Reaction in the 500 g System and the Compositions of the Streams Obtained in the Molecular Distillations

Material analyzed	TG (%)	DG (%)	MG (%)	FFA (%)	GL (%)
Superior phase (acylglycerols)	8.22	30.99	50.13	0.85	9.81
Inferior phase (GL)		0.34	1.84	0.9	96.91
Distillate stream of the first distillation		3.11	80.81	0.91	15.17
Residue stream of the second distillation		3.78	90.88	0.81	4.53

NaOH = 1.3 g. When the system reached the equilibrium condition, the reaction mixture was transferred to a separatory funnel in order to separate the insoluble GL. The composition of the two phases formed is shown in Table 7.

It is clear that the MG losses were small, as the MG concentration in the inferior phase is low (1.84%). Furthermore, it can be seen that the MG concentration level reached is 50.13, a good value when it is compared with reactions carried out at higher temperatures.

The superior phase was submitted to a first distillation at TEV = 250°C and $Q = 5$ mL/min, because according to Fig. 5, this is a suitable condition for the concentration of MG in the distillate stream. The MG concentration obtained in the distillate stream was 80.81 as shown in Table 7, with a MG recovery of 56%. It can be noted that the GL concentration in the distillate is high (15.17%). Therefore, a second distillation was carried out at TEV = 170°C and $Q = 5$ mL/min in order to remove the GL from the distillate stream. The MG concentration obtained in the residue stream of the second distillation is 90.88%, which can already be considered a commercial distilled monoglyceride (min. 90%).

Besides the MG content, the color of the product, as well as odor and taste, are essential properties to be considered. Therefore, it can be seen that the change in the color of the reaction products during the glycerolysis does not compromise the quality of the distilled MG obtained, as it is practically colorless. Furthermore, one can note that the odor was effectively removed by the molecular distillation process, as verified by Kuhrt et al. (1950) *(21)*.

Concluding Remarks

This work represents an important contribution for the manufacturers of MG, as it shows an optimization study of the MG production from soybean oil and also a study of the usual process for MG concentration.

After 60 min, the glycerolysis of the soybean oil reached the equilibrium condition for all the runs. Among the three variables studied (T, GL/TG, and NaOH), the GL/TG is the most relevant, in the experimental range studied. Its main effect is 10.77%. The maximum of MG concentration obtained in the glycerolysis reaction was around 50%, when the independent variables *T* and NaOH were fixed in the their superior levels studied and the GL/TG ratio was greater than 0.36.

For effective separation of the reaction products, careful attention must be given to feed flow rates (*Q*) and evaporator temperature (TEV). According to the results obtained in this work, with only one distillation step, the max. MGD was 80.81%, at evaporator temperature = 250°C and feed flow rate = 5 mL/min. From this stream, a second distillation step was carried out in order to remove GL of the distillate stream (evaporator temperature = 170°C and feed flow rate = 5 mL/min), obtaining a colorless product with 90.88% of MG, which has the desired characteristics of Distilled MG.

Acknowledgment

The authors are grateful to the financial support of CAPES, CNPq, and FAPESP.

References

1 Akoh, C. C. and Min, D. B. (1998), *Food Lipids: Chemistry, Nutrition, and Biotechnology*, Marcel Dekker, New York.
2 Ferreira-Dias, S., Correia, A. C., Baptista, F. O., and Fonseca, M. M. R. (2001), *J. Mol. Catal B: Enzymatic*. **11,** 669.
3 Bornscheuer, U. T. and Kazlauskas, R. J. (1999), Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations, Wiley-VCH, Weinheim.
4 Bailey, A. E. (1996), *Bailey's Industrial Oil & Fat Products*, 5th ed., Edited by Y. H. Hui, John Wiley, New York, pp. 569–601.
5 Mikov, M., Lutisan, J., and Cvengros, J. (1997), *Sep. Sci. Technol.* **32,** 3051–3066.
6 Batistella, C. B., Maciel, M. R. W., and Maciel Filho, R. (2000), *Comput. Chem. Eng.* **24,** 1309–1315.
7 Batistella, C. B., Moraes, E. B., Maciel Filho, R., and Maciel, M. R. W. (2002), *Appl. Biochem. Biotechnol.* **98,** 1149–1159.
8 Moraes, E. B., Batistella, C. B., Alvarez, M. E. T., Maciel Filho, R., and Maciel, M. R. W. (2004), *Appl. Biochem. Biotechnol.* **113,** 689–711.
9 Xu, X., Jacobsen, C., Nielsen, N. S., Heinrich, M. T., and Zhou , D. (2002), *Eur. J. Lip. Sci. Technol.* **104,** 745–755.
10 Breivik, H., Haraldsson, G. G., and Kristinsson , B. (1997), *J. Am. Oil Chem. Soc.* **74,** 1425–1429.
11 Schoenfelder, W. (2003), *Eur. J. Lip. Sci. Technol.* **105,** 45–48.
12 Hartman, L. and Lago, R. C. A. (1973), *Lab. Practice* **22,** 475–476.
13 Carvalho, C. M. L., Serralheiro, M. L. M., Cabral, J. M. S., and Aires-Barros, M. R. (1997), *Enzyme Microb. Technol.* **21,** 117–123.
14 Fregolente, L. V., Batistella, C. B., Maciel Filho, R., and Maciel, M. R. W. (2005), *J. Am. Oil Chem. Soc.* **82,** 673–678.
15 Batistella, C. B. and Maciel, M. R. W. (1998), *Comput. Chem. Eng.* **22,** S53–S60 .
16 Ferreira-Dias, S., Correia, A. C., Baptista, F. O., and Fonseca, M. M. R. (2001), *J. Mol. Catal B: Enzymatic*. **11,** 669–711.
17 Khuri, A. I. and Cornell, J. A. (1987), *Response Surface Design and Analyses,* Marcel Dekker, New York.

18 Box, G. E. P., Hunter, W. G., and Hunter, J. S. (1978), *Statistics for Experimenters,* Wiley, New York, pp. 510–539.
19 Burkert, J. F. M., Maugeri, F., and Rodrigues, M. I. (2004), *Bioresour. Technol.* **91,** 77–84.
20 Barros Neto, B., Scarminio, I. S., and Bruns, R. E. (2003), *Como Fazer Experimentos*, 2nd ed., Editora da UNICAMP, São Paulo, Brazil, pp. 251–266 (in Portuguese).
21 Kuhrt, N. H., Welch, E. A., and Kovarik, F. J. (1950), *J. Am. Oil Chem. Soc.* **27,** 310–313.