Response Surface Methodology Applied to Optimization of Distilled Monoglycerides Production

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ABSTRACT: This work demonstrates that response surface methodology (RSM) is a powerful tool for the optimization of the production of distilled MG. Experiments with a centrifugal molecular distillator having an evaporation area of 0.0046 m^2 were carried out using RSM to identify operating conditions that can lead to higher MG purity. The independent variables studied were the evaporator temperature (TEV) and the volumetric feed flow rate (Q). The experimental range was from 100 to 300°C for TEV and between 5 and 15 mL/min for Q. High-performance size exclusion chromatography was used to evaluate TG, DG, MG, FFA, and glycerol (GL) compositions. Results were presented as MG concentration surfaces. Starting from a material with 10.8% of TG, 37.7% of DG, 43.6% of MG, and 7.2% of GL, the maximum MG purity in the distillate stream with just one distillation step was 82.6% at a TEV equal to 250°C and Q equal to 5 mL/min. At these conditions, the MG recovery was 61%. A strategy was developed to obtain distilled MG with 96.3% purity.

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KEY WORDS: Distilled monoglycerides, molecular distillation, monoglycerides enhancement, response surface methodology, short-path distillation.

MG are widely used in food, pharmaceutical, and cosmetic industries as emulsifiers (1). They impart stability and the requisite viscosity. MG also are building blocks for syntheses of lipids, liquid crystals, and drug carriers (2).

Researchers have developed three lipase-catalyzed routes to MG—(i) hydrolysis of TG, (ii) alcoholysis of TG, and (iii) esterification or transesterification of glycerol (3)—considering the mild conditions requirements of the lipases, which are low temperatures and near neutral pH. In these routes, researchers explore the FA selectivity of the lipases and their regioselectivity for the primary vs. secondary positions in the glycerol.

However, industrially, MG production is carried out through a glycerolysis reaction at temperatures above 200°C using inorganic catalysts, since this process is cheaper than a lipase-catalyzed reaction. In this industrial reaction, the MG content in the final product is 35–50%. This level of concentration is suitable for many applications, although for some specific uses such as cake or frosting, the mouth melt of the product is critical. The use of a commercially prepared MG emulsifier could negatively affect texture or mouthfeel of the product (4), so distilled MG, which are purified MG (minimum 90%), are required for this use. Normally, MG are obtained by using the molecular distillation process (5). Also known as short-path distillation, molecular distillation is characterized by a short exposure of the distilled liquid to high operating temperature and vacuum (6,7). This process has been widely applied to lipid-containing products, including the recovery of carotenoids from palm oil (8), the recovery of tocopherol from crude deodorizer distillate of soybean oil (9), the purification and deodorization of structured lipids (10), and the preparation of purified concentrates of PUFA (11).

In this work, the molecular distillation process was applied for MG concentration, and response surface methodology (RSM) was used to obtain MG concentration surfaces in the residue and the distillate streams, both of which are product streams from the distillator. The independent variables studied were the evaporator temperature (TEV) and the feed flow rate (Q). Furthermore, a strategy was developed to purify MG using only the molecular distillation process, without the glycerol (GL) stripping process.

MATERIALS AND METHODS

Materials. The commercial MG (10.8% TG, 37.7% DG, 43.6% MG, 0.7% FFA, and 7.2% GL) that was fed into the molecular distillator was donated by Braswey S.A. (Pirapozinho, SP, Brazil). It is produced from partially hydrogenated vegetable oil.

Method of analysis. Gel permeation chromatography, also called high-performance size-exclusion chromatography (HPSEC), was used for the acylglycerols, FFA, and GL analyses (12). The chromatographic system consisted of an isocratic HPLC pump (model 515; Waters, Milford, MA), a differential refractometer detector (model 2410; Waters), and an oven for columns maintained at 40°C by a temperature control module (Waters). The samples were injected using a manual injector (model 7725i; Rheodyne; Alltech, Deerfield, IL), with a 20-μL sample loop. Two HPSEC columns, Styragel HR 1 and HR 2 (Waters), with dimensions of 7.8 × 300 mm and a particle size of 5 μm, were connected in series. These columns are packed with styrenedivinylbenzene co-polymer. The mobile phase

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used was HPLC-grade THF from Tedia Inc. (Fairfield, OH) at a flow rate of 1 mL/min. The typical pressure at this flow rate was 450 psi (3102 kPa). All the standards were obtained from Supelco, Inc. (Bellefonte, PA). Data processing was done by the Millenium software 2010 Chromatography Manager Software from Waters.

Molecular distillator. The model used in this work was a centrifugal distillator system from Myers Vacuum Inc. (Kittanning, PA), with an evaporation surface area of 0.0046 m^2. The feed temperature was 60°C; the condenser temperature was 30°C in the first factorial design and 60°C in the second. The typical pressure of the system was 16 Pa, and the evaporator rotation velocity was 1350 rpm.

RSM. The aim of this work was to optimize conditions for the MG concentration and then to develop a strategy to obtain distilled MG. Therefore, RSM, a set of mathematical and statistical methods developed for modeling phenomena, was applied to find combinations of a number of experimental factors that will lead to optimal responses (13).

In RSM, simple coded models such as linear and quadratic expressions usually 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).

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2 \quad [1]$$

By analyzing Equation 1, which is a quadratic model with two variables, one can see that it contains 6 parameters, so the number of combinations of the independent variable levels must be higher than 6, since it is not possible to predict values when the number of equation parameters is higher than the number of independent variable levels. Thus, factorial designs consisting of 2^2 trials plus a star configuration (4 axial points) with 3 central points were 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 from the central points and n is the number of independent variables (14). This kind of factorial design, also known as central composite design, is suitable for the fit of Equation 1, because, for two independent variables, it contains 9 different combinations of the independent variable levels. The 3 central points are important since they represent a set of experimental conditions at which 3 replicates are carried out. The variation between them represents the deviation of all experiments (15). Furthermore, they provide additional DF for error estimation.

All data were treated with the aid of STATISTICA 7 from StatSoft Inc. (Tulsa, OK). The quality of the fitted models was evaluated by ANOVA, based on the F-test (16) 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 (17).

RESULTS AND DISCUSSION

Factorial designs. In the molecular distillation process, two product streams are generated: distillate (rich in the molecules that escape from the evaporator) and residue (rich in the heavier molecules). The process initially was carried out following a factorial design to characterize the system behavior and to verify whether the experimental value range should be adjusted to surround the optimal region, which means the maximum of MG concentration in the distillate stream (MGD). The independent variables studied were TEV and Q, since they are very important process variables in the molecular distillation process.

Experimental values were chosen according to previous experience. Values of feed flow rate lower than 4 (mL/min) may not be high enough to form a uniform thin film on the evaporator surface. A uniform thin film promotes efficient mass and energy transfers (18). For feed flow rate, values higher than 15 (mL/min), the system operated with low effectiveness because the residence time of the molecules on the evaporator is too low. Therefore, the feed flow rate was varied from 5 to 15 (mL/min). The TEV was varied from 100 to 250°C. At 100°C, the first significant GL drops on the condenser wall can be observed. The experimental conditions and the results for the first factorial design are shown in Table 1.

The fitted coded models for the MGD and MGR are shown in Equations 2 and 3, respectively, where all the coefficients of Equation 1 are considered.

$$\begin{aligned} \text{MGD} = {} & 56.18 + 22.43 \times \text{TEV} - 7.90 \times \text{TEV}^2 \\ & - 11.06 \times \text{Q} - 6.81 \times \text{Q}^2 - 4.60 \times \text{TEV} \times \text{Q} \end{aligned} \quad [2]$$

$$\begin{aligned} \text{MGR} = {} & 42.62 - 1.82 \times \text{TEV} - 1.85 \times \text{TEV}^2 \\ & + 0.64 \times \text{Q} - 0.11 \times \text{Q}^2 + 0.82 \times \text{TEV} \times \text{Q} \end{aligned} \quad [3]$$

By analyzing the ANOVA, shown in Table 2, one may conclude that the models fit the experimental data quite well, since the calculated F values (lack of fit/pure error) are lower than the critical F value ($F_{0.95;3;2} = 19.16$) at 95% confidence (i.e., at this confidence level, there is no evidence of lack of fit for the models). Furthermore, the results show that the model for the MGD accounted for a high percentage of the explained variance (97.36%), and the calculated F value (regression/residual) is more than seven times higher than the critical F value at 95% confidence ($F_{0.95,5,5} = 5.05$), indicating that the regression is statistically significant.

As can be seen in Figure 1, obtained from Equation 2, the MG concentration depends more on TEV than on Q; however, both operating variables are important. An increase in TEV and a decrease in Q led to an increase in the MGD. Therefore, to maximize MGD, TEV must be kept at the highest tested levels and Q must be kept at the lowest levels. The maximum of MGD seems to be near TEV = 250°C and Q = 5 mL/min.

From Equation 2, the dependent variable has negative values at high Q and low TEV, which has no physical meaning. This happens owing to the low MGD at this condition (high GL concentration in the distillate stream). However, this does not compromise the conclusions from the surface analysis.

TABLE 1
Coded Levels, Real Levels (in parentheses), and Concentration of MG in the Distillate and Residue Streams for the First (A) and Second Factorial Design[a] (B)

	(A) First factorial design				(B) Second factorial design			
Run	TEV (°C)	Q (mL/min)	MGD (%)	MGR (%)	TEV (°C)	Q (mL/min)	MGD (%)	MGR (%)
1	−1 (122)	−1 (6.5)	19.6	42.2	−1 (215)	−1 (6.5)	78.1	37.5
2	+1 (228)	−1 (6.5)	79.6	38.0	+1 (285)	−1 (6.5)	70.9	25.7
3	−1 (122)	+1 (13.5)	9.1	42.5	−1 (215)	+1 (13.5)	56.3	41.8
4	+1 (228)	+1 (13.5)	50.8	41.5	+1 (285)	+1 (13.5)	77.7	36.1
5	−1.41 (100)	0 (10)	14.7	41.8	−1.41 (200)	0 (10)	56.9	41.5
6	1.41 (250)	0 (10)	69.2	35.3	1.41 (300)	0 (10)	65.2	29.3
7	0 (175)	−1.41 (5)	61.7	41.6	0 (250)	−1.41 (5)	74.8	26.8
8	0 (175)	1.41 (15)	26.9	42.5	0 (250)	1.41 (15)	68.8	41.0
9	0 (175)	0 (10)	55.1	41.9	0 (250)	0 (10)	75.2	37.4
10	0 (175)	0 (10)	60.9	42.9	0 (250)	0 (10)	77.5	36.6
11	0 (175)	0 (10)	51.8	43.0	0 (250)	0 (10)	73.1	36.6

[a]TEV, evaporator temperature; Q, volumetric feed flow rate; MGD, MG concentration in the distillate stream; MGR, MG concentration in the residue stream.

The surface shown in Figure 2 was obtained from Equation 3. This figure shows a small increase in MG content in the residue when the temperature is increased from 100 to approximately 160°C. In explanation, at these conditions, the evaporation rate of GL (molecule of lowest vapor pressure in the starting material) is higher than the evaporation rate for MG, so a considerable amount of GL leaves the distillator in the distillate stream, which does not occur with the MG molecules. Figure 2 confirms that TEV is more important than Q since, at a given TEV, the MGR varies just slightly.

TABLE 2
ANOVA for the Fitted Models[a]

Source of variation	Model	Sum of squares	DF	Mean square	*F*-ratio
Regression	Equation 2	5549.264	5	1109.853	36.84[b]
	Equation 3	52.620	5	10.524	11.37[b]
	Equation 4	570.002	5	114.000	10.57[b]
	Equation 5	323.592	5	64.718	66.93[b]
Residual	Equation 2	150.615	5	30.123	—
	Equation 3	4.629	5	0.926	—
	Equation 4	53.919	5	10.783	—
	Equation 5	4.833	5	0.967	—
Lack of fit	Equation 2	105.507	3	35.205	1.56[c]
	Equation 3	3.925	3	1.308	3.71[c]
	Equation 4	44.262	3	14.754	3.06[c]
	Equation 5	4.373	3	1.458	6.34[c]
Pure error	Equation 2	45.108	2	22.554	—
	Equation 3	0.704	2	0.352	—
	Equation 4	9.657	2	4.829	—
	Equation 5	0.460	2	0.230	—
Total	Equation 2	5699.879	10		
	Equation 3	57.248	10		
	Equation 4	623.921	10		
	Equation 5	328.425	10		

[a]Equation 2, percent of explained variance, 97.36; percent of explicable variance, 99.21. Equation 3, percent of explained variance, 91.92; percent of explicable variance, 98.77. Equation 4, percent of explained variance, 91.36; percent of explicable variance, 98.45. Equation 5, 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).

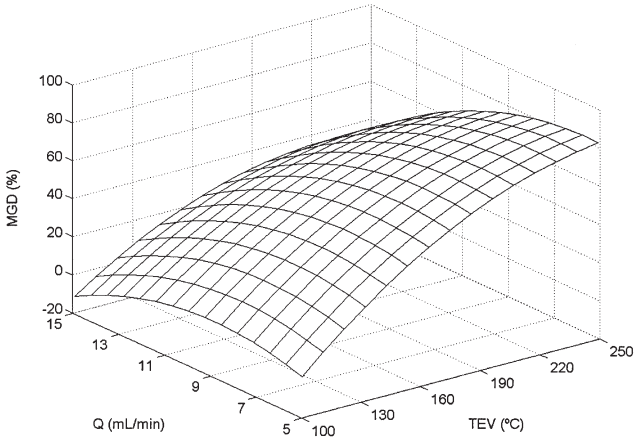

FIG. 1. Response surface for the MG concentration in the distillate stream for the first factorial design. TEV, evaporator temperature; Q, volumetric feed flow rate; MGD, MG concentration in the distillate stream.

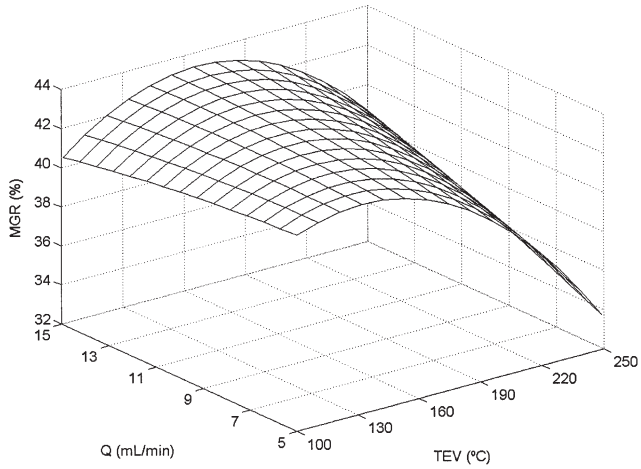

FIG. 2. Response surface for the MG concentration in the residue stream for the first factorial design. For abbreviations see Figure 1.

At high temperatures and low feed flow rate, MGR decreases drastically (Fig. 2) since a significant amount of MG leaves the distillator in the distillate stream. This observation agrees with Figure 1.

Thus, based on the first factorial design, a second factorial design was carried out to confirm the tendency presented in Figure 1 and to find the optimal region for the MGD. In the second factorial design, the level of Q was kept the same while the TEV experimental range was increased. The new TEV experimental range was from 200 to 300°C, as shown in Table 1.

Table 3 contains experimental data on the ratio of D to F at different operating conditions, where F is the mass of the starting material fed into the distillator and D is the mass of the distillate stream obtained in the distillation. It can be noted that, as expected, TEV and Q are also important operating variables in the yield of the process. D/F increases with increasing TEV and decreases with increasing Q owing to the diminution of the residence time of the molecules on the evaporation surface.

The coded models for the MGD and MGR in the new factorial design are shown in Equations 4 and 5, respectively, where, again, all the coefficients of Equation 1 were considered.

$$\text{MGD} = 75.27 + 3.25 \times \text{TEV} - 6.04 \times \text{TEV}^2 - 2.94 \times \text{Q} - 0.66 \times \text{Q}^2 + 7.13 \times \text{TEV} \times \text{Q} \quad [4]$$

$$\text{MGR} = 36.88 - 4.37 \times \text{TEV} - 0.59 \times \text{TEV}^2 + 4.35 \times \text{Q} - 1.32 \times \text{Q}^2 + 1.51 \times \text{TEV} \times \text{Q} \quad [5]$$

Through the ANOVA, shown in Table 2, one can see that there is no evidence of lack of fit for the fitted models in the second factorial design, since the calculated F values (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), Equation 5, is predictive in the experimental conditions studied, since the percentage 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 (19).

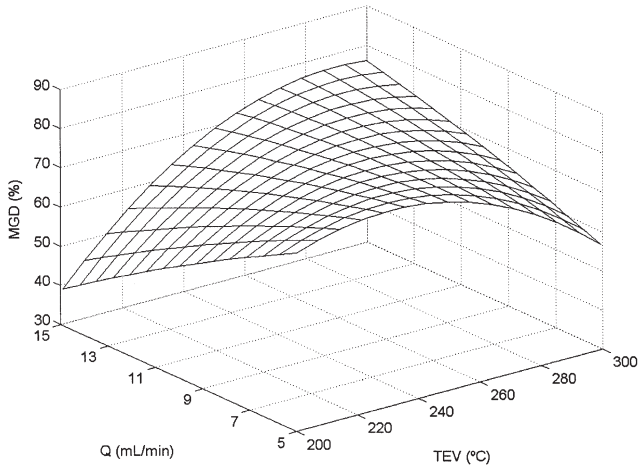

FIG. 3. Response surface for the MG concentration in the distillate stream for the second factorial design. For abbreviations see Figure 1.

TABLE 3
Experimental Data of the Ratio D/F at Different Operating Conditions[a]

	100 D/F (%)		
TEV (°C)	Q = 5 mL/min	Q = 10 mL/min	Q = 15 mL/min
200	13.8	6.5	4.5
220	21.5	10.3	7.3
240	35.3	16.7	7.4
260	46.7	19.4	14.0
280	58.3	31.2	16.6
300	78.5	35.8	17.1

[a]D, mass of distillate stream obtained in the distillation; F, mass of starting material fed into the distillator; for other abbreviations see Table 1.

TABLE 4
Component Concentrations and the Individual MG Recovery for Each Stream[a]

Stream	TG (%)	DG (%)	MG (%)	FFA (%)	GL (%)	MG recovery
Starting material	10.8	37.7	43.6	0.7	7.2	
1D	ND	2.6	82.6	1.5	13.4	0.609
1R	17.2	50.0	31.4	0.9	0.6	0.391
2D	ND	ND	36.8	4.9	58.2	0.096
2R	ND	5.3	90.2	1.0	3.6	0.904
3D	ND	ND	85.8	3.8	10.2	0.087
3R	ND	6.5	91.5	1.4	0.6	0.913
4D	ND	2.1	96.3	ND	1.6	0.436
4R	ND	12.1	87.1	0.3	0.6	0.564

[a]GL, glycerol; ND, not detected; D, distillate; R, residue.

As can be seen in Figure 3, obtained from Equation 4, at low values of Q, the MGD starts decreasing at temperatures above 250°C, owing to the significant amount of DG that leaves the distillator in the distillate stream under these conditions. Q is important because, at high values of Q, the residence time of the molecules on the evaporator surface is short and DG evaporation may not be high enough to dilute the MG in the distillate stream. Figure 3 shows that the maximum MG content obtained in the studied conditions, with just one distillation, is approximately 80%.

Strategy for the distilled MG production. A strategy consisting of four distillation steps was developed to obtain distilled MG. In this strategy, the first step was based on the results given by the response surfaces. The other three steps were carried out as a refinement, to obtain more highly concentrated MG. Actually, the best operating conditions for these three steps cannot be obtained from the factorial design, since the feed concentrations of the components in these distillations are different from the starting material used in the factorial designs. However, the MG concentration surfaces give very important information about the experimental range of TEV and Q that must be explored.

This strategy is presented in Figure 4, where the distillate streams are represented by the symbol D and the residue streams are represented by the symbol R. The numbers 1, 2, 3, and 4 refer to the number of distillations carried out. The first distillation was carried out at TEV = 250°C and Q = 5mL/min, since, as shown in Figure 3, maximal MG content in the distillate was obtained around this condition. Two subsequent distillations were carried out to decrease the GL concentration. A last distillation at TEV = 250°C and Q = 5 mL/min was made to obtain a final product containing 96.3% MGD.

Table 4 contains experimental data on the component concentrations, such as the individual MG recovery for each stream. In this table, the MG concentrations obtained in the distillate and residue streams of the first distillation (streams 1D and 1R) are 82.6 and 31.4%, respectively. Comparing these values with the values predicted by the fitted models in the first and second factorial designs confirmed the good agreement between the experimental observations and the mathematical models. In the first factorial design, the predicted values for the MGD and MGR are 83.3 and 33.6%. In the second factorial design, the predicted values are 78.1 and 28.1%, respectively. In

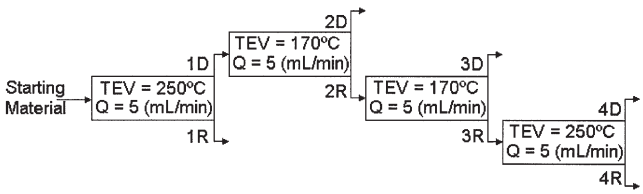

FIG. 4. Scheme of the strategy adopted for the production of distilled MG. D, distillate; R, residue; for other abbreviations see Figure 1.

analyzing the MG recovery, it can be concluded that a product with a MG content higher than 90% (stream 2R) can be reached with a total MG recovery of 55.0%.

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