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Optimization of Tocopherol Concentration Process From Soybean Oil Deodorized Distillate Using Response Surface Methodology

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Abstract

Soybean oil deodorized distillate is a product derived from the refining process and it is rich in high value-added products. The recovery of these unsaponifiable fractions is of great commercial interest, because of the fact that in many cases, the "valuable products" have vitamin activities such as tocopherols (vitamin E), as well as anticarcinogenic properties such as sterols. Molecular distillation has large potential to be used in order to concentrate tocopherols, as it uses very low temperatures owing to the high vacuum and short operating time for separation, and also, it does not use solvents. Then, it can be used to separate and to purify thermosensitive material such as vitamins.

In this work, the molecular distillation process was applied for tocopherol concentration, and the response surface methodology was used to optimize free fatty acids (FFA) elimination and tocopherol concentration in the residue and in the distillate streams, both of which are the products of the molecular distiller. The independent variables studied were feed flow rate (F) and evaporator temperature (T) because they are the very important process variables according to previous experience. The experimental range was 4–12 mL/min for F and 130–200°C for T. It can be noted that feed flow rate and evaporator temperature are important operating variables in the FFA elimination. For decreasing the loss of FFA, in the residue stream, the operating range should be changed, increasing the evaporator temperature and decreasing the feed flow rate; D/F ratio increases, increasing evaporator temperature and decreasing feed flow rate. High concentration of tocopherols was obtained in the residue stream at low values of feed flow rate and high evaporator temperature. These results were obtained through experimental results based on experimental design.

Index Entries: Deodorized distillate; free fatty acids; molecular distillation; response surface methodology; soya sludge; tocopherol.

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Introduction

Soybean oil deodorized distillate (SODD) is a complex mixture made up of fatty acids, sterols, tocopherols, sterol esters, hydrocarbons, breakdown products of fatty acids, aldehydes, ketones, and acylglycerol species *(1)*. It is an important source of natural tocopherols and phytosterols corresponding approx 10 and 20%, respectively. The SODD corresponds to approx 0.1 and 0.4% of crude soybean oil *(2)*. The economical value of SODD depends on tocopherols and phytosterols contents. Free fatty acids (FFA) represent 25–75% and acylglycerols about 3–56% of the SODD, depending on the raw material being refined and on the type and conditions of the refining process *(3)*.

Natural tocopherols are important antioxidants present in cereals and vegetable oils. Each one of these sources has different quantity of vitamin E. Vitamin E is the most efficient soluble, natural lipidic antioxidant and the main antioxidant membrane in mammal cells *(4)*. They avoid oxidation of vitamin A, β-carotene, and essential fatty acids *(5)*. Tocopherols prevent diseases like cancer, cardiovascular, and cataracts *(6)*. They are used in food, cosmetics, and pharmaceutical industries *(7)*. A mixture of α-, β-, γ-, and δ-isomers containing 60 wt% tocopherols is widely used as additive in many kinds of foods *(8)*. They are also used as an additive in paints based on natural oils *(9)*. Tocopherols are sensible to light, heat, alkali, and contaminant metals *(10)*.

Molecular distillation or short path distillation is characterized by a short exposure of the distilled liquid to the operating temperature and high vacuum *(11)*. This process has been applied to lipid-containing products, including monoglycerides production *(12)*, recovery of carotenoids from palm oil *(13)*, and purification of structured lipids *(14)*. Also, it can be applied to fuel product, like production of biodiesel from castor oil *(15)* and heavy petroleum characterization *(16)*. Ito et al. *(17)* studied the recovery of tocopherols and FFAs from SODD in function of the ratio (distillate flow rate [*D*]/residue flow rate [*R*]) of 0.96, and the tocopherol and FFA recovery curves had an optimum point at 73%. Martins et al *(18)* determined the best operating conditions of wiped film molecular distillator to concentrate tocopherols from SODD. Moraes et al. *(19)* studied this process experimentally and by simulation with good agreement.

To obtain maximum recovery of vitamin E, the molecular distillation process must be optimized and the response surface methodology (RSM) is an effective tool for this purpose. The main advantage of this methodology is the reduced number of experimental runs needed to provide sufficient information for statically acceptable results. According to preliminary experience, the process variables (condenser temperature and feed temperature) were not significant *(20,12)*. Then, in the feed flow rate (X_1) and evaporator temperature (X_2), process variables were selected for optimization of the unsaponifiable material from SODD through a molecular distillation process.

Nowadays, in several areas of process development, RSM are being applied. Chemical processes have many variables, which need to be optimized in relation to cost, yield, and purity taking into consideration the interactions among them. Maciel Filho et al. *(21)* evaluated atmospheric and vaccum petroleum residues using a two-level factorial design. The error repetition was estimated by three runs on the central point of the experimental design. Fregolente et al. *(22)* studied the optimization of distilled monoglycerides production through molecular distillation. The 2^3 factorial design was used to evaluate the effects of reaction parameters and the central composite design to optimize the molecular distillation process. The objective of this work is to understand the relationships between the independent (X_1 and X_2) and dependent variables (Distillate flow rate/Feed flow rate [D/F]) ratio, Y_1; FFAs content in the distillate stream (FFAD), Y_2; and total tocopherols content in the residue stream (TocoR), Y_3.

Materials and Methods

Materials

SODD was provided by Bunge Ltda, (São Paulo, Brazil). All samples were stored in the refrigerator at 4°C until analysis. All solvents and reactants for the analysis were of analytical grade. A tocopherol kit consisting of α-, β-, γ-, and δ-tocopherol (purity ≥95%) was purchased from Calbiochem (San Diego, CA) and used as reference standards for tocopherols analysis.

Molecular Distillator

Molecular distillation or short path distillation is a separation process characterized by short exposure of the feed inside the equipment, and so to the operating temperature, by high vacuum and by a small distance between evaporator and condenser (in the order of mean free path of the molecular involved) *(13)*. In this study, the centrifugal molecular distillator from Myers Vacuum Inc. (Kittaning, PA), with an evaporator area of 0.0046 m^2 was used. Figure 1 shows a scheme of this equipment. Two product streams are generated: distillate (rich in the volatile compounds that escape from the evaporator and reach the condenser, in this case FFA) and residue (rich in the heaviest compounds that remain in the evaporator surface, in this case tocopherols). The process conditions were maintained at 13.3 Pa, the feed temperature at 50°C, condenser temperature at 50°C, at *g*-force value equal to 5.5*g*. The molecular distillation experiments were conducted according to the following procedure: a sample of SODD was homogenized before feeding the equipment. For each molecular distillation run, samples of both streams (distillate [D] and residue [R]) were collected and submitted to FFAs and tocopherols analysis.

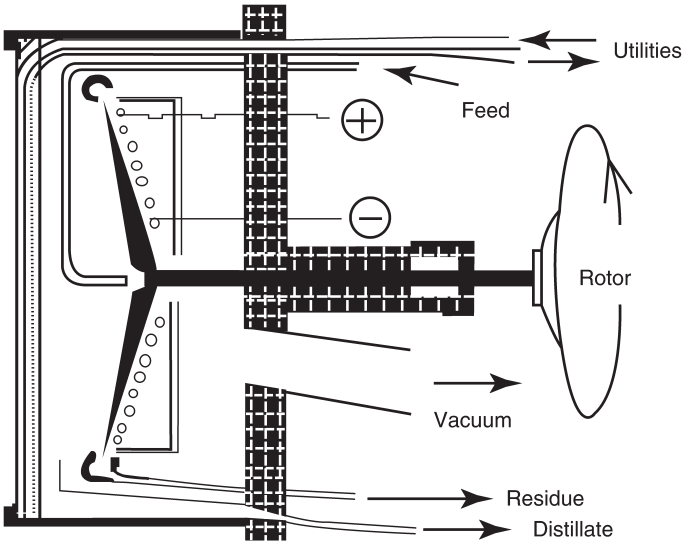

Fig. 1. Centrifugal molecular distillator scheme.

Response Surface Methodology

A central composite design was used to study the dependent variables which are called responses, which are D/F (split ratio), Y_1; FFAD, Y_2; and TocoR, Y_3. The independent variables were X_1 and X_2 representing feed flow rate and evaporator temperature, respectively. The experiments were carried out according to a 2^2 complete factorial design plus three central points and four axial points, called star points. The distance of the star points from the center point is given by $\alpha = (2^n)^{1/4}$, where n is the number of independent variables, for two factors $\alpha = 1.41$ *(23)*. Experimental values were chosen according to previous experience. Values of feed flow rate lower than 4 mL/min might be high enough to form a uniform thin film on the evaporator surface. An efficient mass and energy transfers were promoted by a uniform, thin film. For feed flow rate greater than 15 mL/min, the system operated with low effectiveness, because of the low residence time. The first significant FFA drops on the condenser wall sets the lower evaporator temperature level *(12)*. The settings for the independent variables were as follows (low/high values): evaporator temperature (°C), 140/190, and the feed flow rate (mL/min), 5.1/10.9.

Each variable to be optimized was coded at five levels, –1.41, –1.0, +1, and +1.41. This gives a range of 130–200°C and 4–12mL/min, to these variables, respectively, including the star points. Three replicate runs at the center (0, 0) of the design were performed to allow the estimation of the pure error. All experiments were carried out in a randomized order to minimize the effect of unexplained variability in the observed responses owing to

Table 1
Independent Variables and Their Levels for Central Composite Design in Optimization of Molecular Distillation Process of SODD

		Coded variable levels				
Independent variables	Symbol	–1.41 (–α)	–1	0	+1	+1.41 (+α)
Feed flow rate (mL/min)	X_1	4.0	5.1	8.0	10.9	12.0
Evaporator temperature (°C)	X_2	130	140	165	190	200

extraneous factors. The RSM was chosen to study the optimization of two selected factors, feed flow rate (*F*) and evaporator temperature (*T*). Table 1 shows the independent variables and the coded levels.

FFA Analysis

FFA content was determined according to the method AOCS Ca 5a-40 *(24)*. This method uses titration with a standard alkali, NaOH. The FFA content is expressed as percentage of oleic acid ($C_{18:1}$). The expression is:

$$\text{FFA as oleic acid (\%)} = \frac{\text{Alkali volume (mL)} \cdot \text{Alkali normality} \cdot 28.2}{\text{Sample weight (g)}} \quad (1)$$

Tocopherol Analysis

The method AOCS Ce 8–89 *(25)* was used to determine the α-, β-, γ-, and δ-tocopherol contents. A known amount of the sample was dissolved in hexane (approx 1 mg/mL) and 20 μL of the solution was injected into a high-performance liquid chromatography modular equipment made up of Waters delta 600 high-performance liquid chromatography pump (Mildford, MA), equipped with a fluorescence detector (Waters model 2475 multifluorescence). The flow rate of the mobile phase (hexane : isopropanol, 99 : 01 [v/v]) was set at 1.0 mL/min. The separation was conducted in a microporasil column 125 Å, with particle size of 10 μm and 3.9 × 300 mm^2 of dimension (Waters, Ireland). Figure 2 shows the tocopherols chromatogram. The tocopherols detected in the chromatograms were identified comparing the retention time of the compounds with the retention time of the standard solutions. Quantification of each type of tocopherols was done using calibration curves. The data processing was carried out through the Millennium software (Waters, Mildford, MA).

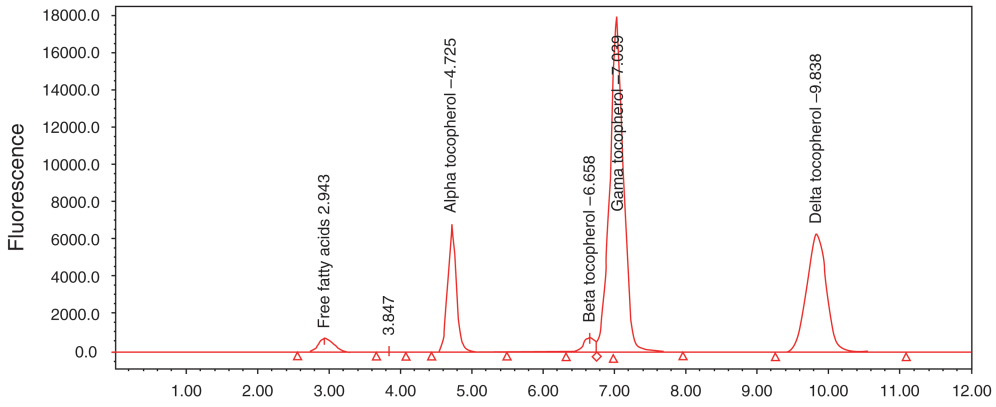

Fig. 2. Chromatogram of SODD for tocopherols analysis.

Table 2
Raw Material Characteristics

Analysis	SODD
FFA (wt% as oleic acid)	53.04 ± 1.10
α-Tocopherol	1.41 ± 0.25
β-Tocopherol	0.12 ± 0.05
γ-Tocopherol	1.95 ± 0.15
δ-Tocopherol	0.58 ± 0.09
Tocopherol total	4.06 ± 0.54

Results and Discussion

The raw SODD was analyzed in relation to the FFA and tocopherols contents. Table 2 shows the characteristics of the SODD. The SODD used is brownish and semisolid at room temperature.

Experimental Design

The response were analyzed using Statistica 7.0 software (StatSoft. Inc.). A quadratic polynomial regression model was assumed for predicting Y_1, Y_2, and Y_3. The model proposed for each response of Y was:

$$Y = A + B_1X_1 + B_2X_2 + C_{11}X_{1^2} + C_{22}X_{2^2} + C_{12}X_1X_2 \quad (2)$$

where A is a constant, B_i is a first order model coefficient, C_{ij} is a second order model coefficient, X_1 and X_2 are independent variables. The model fitting was evaluated by the coefficient of determination (R^2), and by the analysis of variance (ANOVA). Table 3 shows the coded levels and the responses obtained through the molecular distillation process (central composite design). The fitted coded models for the D/F ratio, FFA content

Table 3
Coded Levels and the Results Obtained in the Molecular Distillation Process (central composite design)

Run	X_1	X_2	Y_1	Y_2	Y_3
1	−1	−1	12.70	93.61	4.07
2	+1	−1	2.60	93.13	4.27
3	−1	+1	63.30	101.49	9.91
4	+1	+1	9.20	94.71	5.74
5	−1.41	0	48.50	97.60	7.14
6	+1.41	0	10.50	95.57	3.99
7	0	−1.41	3.50	93.14	4.22
8	0	+1.41	32.50	104.00	10.19
9	0	0	26.70	98.13	4.63
10	0	0	25.50	98.89	5.23
11	0	0	24.70	99.33	5.10

X_1, feed flow rate; X_2, evaporator temperature; Y_1, D/F split ratio; Y_2, FFAs content in the distillate stream (FFAD); and Y_3, tocopherols content in the residue stream (TocoR).

in the distillate stream, and tocopherol content in the residue stream were shown in Eqs. 3–5. All the coefficients of Eq. 2 were considered:

$$Y_1 = 0.256 + 0.123X_1 - 0.142X_1^2 - 0.147X_2 + 0.015X_2^2 - 0.01122X_1X_2 \quad (3)$$

$$Y_2 = 98.78 + 3.10X_1 - 0.57X_1^2 - 1.27X_2 - 1.76X_2^2 - 1.58X_1X_2 \quad (4)$$

$$Y_3 = 4.99 + 1.97X_1 + 1.01X_1^2 - 1.05X_2 + 0.19X_2^2 - 1.09X_1X_2 \quad (5)$$

The model validation was determined by the ANOVA in relation to the responses Y_1, Y_2, and Y_3. Table 4 shows the ANOVA data. It can be concluded that there is no evidence of lack of Fit for the fitted models, because the calculated F values $\left(\frac{\text{Mean square lack of fit}}{\text{Mean square pure error}}\right)$ are lower than the critical F value ($F_{0.95,8,2} = 19.37$) at 95% confidence, for the three models. The results show the model for tocopherols content in the residue (Y_3). Equation 5 is predictive in the experimental conditions studied, because the percent of explained variable is high (98.7%) and the calculated F value $\left(\frac{\text{Mean square regression}}{\text{Mean square residual}}\right)$ is more than 65 times higher than the critical F value at 95% of confidence ($F_{0.95,2,8} = 4.46$). As a practical rule, regression can be considered useful to predict values, when the F value $\left(\frac{\text{Mean square regression}}{\text{Mean square residual}}\right)$ is more than ten times higher than the critical F value *(26)*. An excellent repeatability of results was obtained, the pure error is low in comparison

Table 4
ANOVA for the Fitted Models

Source of variation	Model	Sum of square	Degree of freedom	Mean square	*F*-ratio
Regression	Eq. 3	0.35	2	0.18	57.97[a]
	Eq. 4	113.56	2	56.78	7.13[a]
	Eq. 5	50.52	2	25.26	65.70[a]
Residual	Eq. 3	0.006	8	0.0007	–
	Eq. 4	14.28	8	1.78	–
	Eq. 5	0.69	8	0.09	–
Lack of fit	Eq. 3	0.0053	8	0.0007	–
	Eq. 4	13.55	8	1.69	–
	Eq. 5	0.49	8	0.06	–
Pure error	Eq. 3	0.0002	2	0.0001	0.28[b]
	Eq. 4	0.74	2	0.37	0.24[b]
	Eq. 5	0.20	2	0.10	0.03[b]
Total	Eq. 3	0.363	10	0.036	–
	Eq. 4	127.84	10	12.78	–
	Eq. 5	51.21	10	5.12	–

Equation 3, explained variance (98.5%), explicable variance (96.95%); Eq. 4, explained variance (88.8%), explicable variance (99.42%); and Eq. 5, explained variance (98.7%), explicable variance (99.61%).

[a]*F*-ratio (regression/residual).

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

with the experimental values. As can be seen in Fig. 3, obtained from Eq. 2, the D/F ratio increases, increasing the evaporator temperature and decreasing the feed flow rate. In the maximum conditions, almost all the feed was distilled. The maximum is got at higher temperature and lower feed flow rate levels.

Figure 4 shows the response surface for FFA in the distillate stream. To increase the deacidification of the SODD, the operating range needs to follow evaporator temperature increasing and feed flow rate decreasing. For SODD deacidification, it is not interesting to carry out the experiments at low evaporator temperature. The high evaporator temperature promotes the evaporation of lighter molecules from SODD, and consequently, increases the FFA content. Figure 5 shows the response surface for total tocopherol in the residue stream. In order to concentrate tocopherols, it is necessary to increase the evaporator temperature and to decrease feed flow rate. At high evaporator temperature, all the FFA were distilled and collected as distillate. But in this case, some loss of tocopherol can be observed.

For all the responses, the maximum value can be obtained at high evaporator temperature and low feed flow rate. To FFA at distillate stream, a large range can be used to get a maximum deacidification of SODD. The

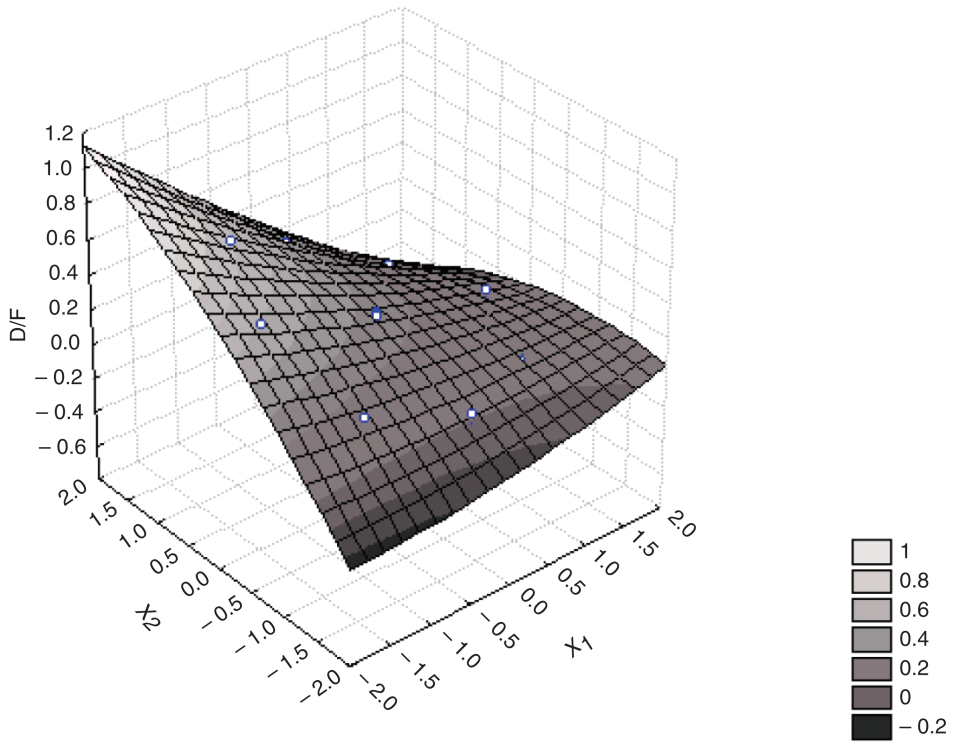

Fig. 3. Response surface for the D/F split ratio.

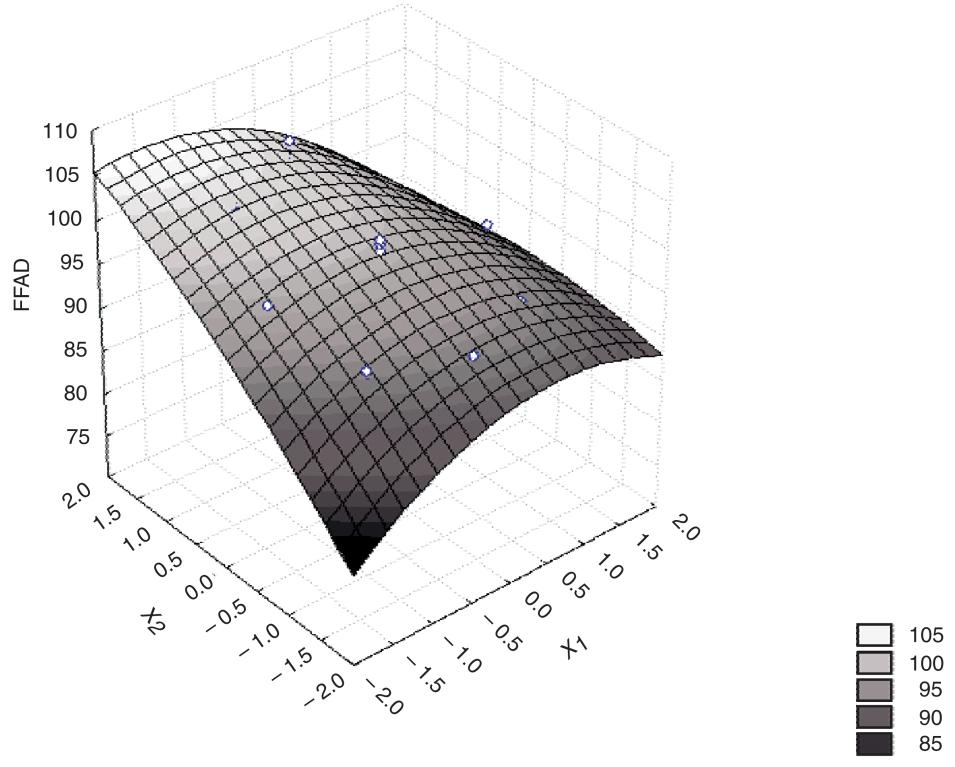

Fig. 4. Response surface for FFA content in the distillate stream.

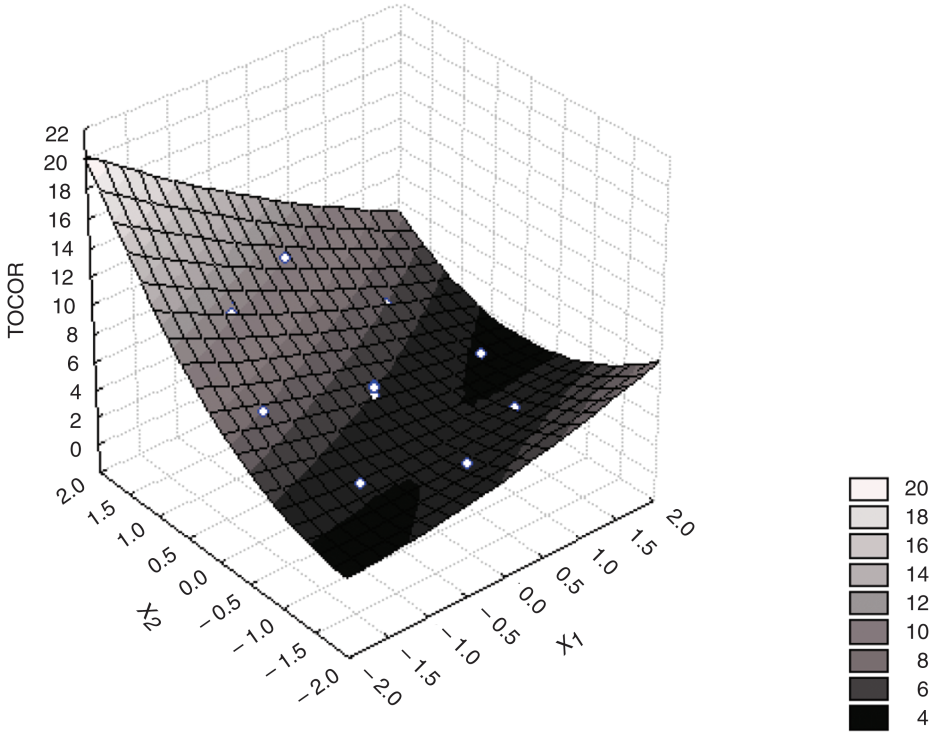

Fig. 5. Response surface for tocopherol content in the residue stream.

Table 5
Predicted and Experimental Responses for Molecular Distillation Process

Real value		Coded value		Response		
				Experimental	Predicted	SD
X_1	X_2	X_1	X_2		Y_1	
7.5	160	–0.17	0.2	22.6	30.9	5.87
10.0	160	0.69	0.2	10.4	17.0	4.67
					Y_2	
7.5	160	–0.17	0.2	96.4	99.6	2.26
10.0	160	0.69	0.2	93.3	97.6	3.04
					Y_3	
7.5	160	–0.17	0.2	4.5	5.1	0.42
10.0	160	0.69	0.2	5.1	5.3	0.14

Units for X_1 and X_2 are mL/min and °C, Y_1 is a dimensional, Y_2 and Y_3 are %(w/w).

results are validated through the independent experiments for the molecular distillation process. The predicted D/F ratio, FFA, and Tocopherols levels were close to the experimental data as shown in Table 5. The difference between the experimental and predicted responses is small. The data in Table 5 shows the validation of the fitted models through RSM.

Concluding Remarks

In this study, RSM successfully optimized the conditions used in the deacidification of SODD and concentration of tocopherols. The generated regression models could be used to predict the SODD deacidification and tocopherols concentration. Results of this study might serve as a guideline for a scale-up of the molecular distillation process, such as a pilot plant, for concentration of vitamin E from SODD.

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