Separation of Phytosterol and Synthesized V_E Succinate from Rapeseed Oil Deodorizer Distillate

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ABSTRACT

A method to separate phytosterol and synthesize V_E succinate from rapeseed oil deodorizer distillate was presented in this paper. It consisted of methyl esterification, crystallization and centrifugation to separate phytosterol followed by crystallization to recover synthesized V_E succinate from rapeseed oil deodorizer distillate. The reaction temperature, time, methanol/material ratio, catalyst and transesterification were investigated systematically and a model of esterification/transesterication was developed on the basis of analysis of response surface methodology(RSM). Meanwhile, the V_E succinate was synthesized in the residuals to prevent heat –sensitive tocopherol from damage. The results showed that the content and total recovery of phytosterol is above 85%, 80% after one crystallization, and 95%, 45% after twice crystallization, respectively. The high conversion from vitamin E to vitamin E succinate was 71.3%.

Keywords: phytosterol ; V_E succinate; deodorizer distillate; response surface methodology

1. INTRODUCTION

Deodorizer distillate (DOD) was produced during the refining steps that consisted of deodorization, bleaching, and refining. The sludge was composed of high aggregated compounds, such as tocopherols (Vitamin E), sterols (β -sitosterol, campesterol, stigmasterol), squalene, and fatty acids (M.F. Mendes et al., 2002).

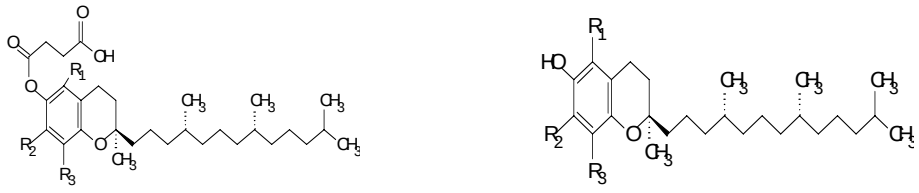
The sterols, presented in the deodorizer distillate, were very important raw material in the production of hormones and the biosynthesis of cholesterol. Most animal and human studies showed that phytosterol reduced serum and plasma total and lower density lipoprotein (LDL) cholesterol levels without causing serious side effect (Hubert Schaller, 2003. Mohammed H. 2000). The structural forms of sterols were presented in the Fig.2 (M.F.Mendes et al., 2002). The tocopherol concentrated in the deodorizer distillate was used as antioxidants and as additives in the food additives (C.J. Pollard et al., 1960). Vitamin E was composed of four isomers (α β γ δ -tocopherol). The differences among the isomers were in their structural forms shown in Table 1. The structural forms of the four isomers were presented in the Fig.1 (M.F. Mendes et al., 2002).

Although tocopherols were only 2-15% of deodorizer distillate by weight, they were considered being the most valuable compounds due to their vitamin E activity and antioxidant properties. Their esters such as Vitamin E succinate, were solid in room temperature and more stable against heat and oxidation than corresponding tocopherols. Recently, the cancer and atherosclerosis, the two most common causes of death in developed countries have increased. So the potential anti-atherogenic and anti-neoplastic activity of vitamin E succinate has been studied extensively (Ana Cristina Rego et al., 1998. Paulo Ottino et al., 1997). Therefore there has been an intensive search about vitamin E succinate.

The separation of sterols was involved a series of chemical and physical processing steps. These techniques were used alone or in combination with others, including solvent extraction, chemical treatment, crystallization, complexation, and molecular distillation (Peng Ying et al., 2002. Sun deng-wen. et al., 1993). In most literature, Vitamin E succinate was synthesized by pure vitamin E and succinate acid. But the procedure mentioned was labor-intensive, energy-consuming and low recovery. In this paper, a method to separate phytosterol and synthesize V_E succinate from rapeseed oil deodorize distillate was presented by analysis of response surface methodology and orthogonal design. It consisted of methyl esterification, crystallization and centrifugation to separate phytosterol followed by crystallization to recover synthesized V_E succinate from rapeseed oil deodorizer distillate.

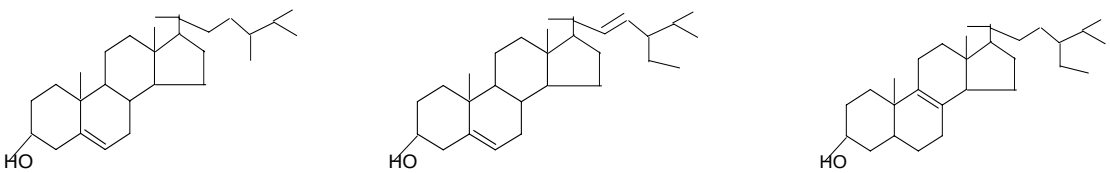
Table 1 Differences between the isomers in R_1, R_2, R_3

isomers	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3



(a) (b)

Figure1. General structural formulas of vitamin E succinate (a) and vitamin E(b)



(a) (b) (c)

Figure 2. Structural formulas of campesterol (a), stigmasterol (b) and β -sitosterol (c)

1.1 Safety emphasis

In Anhui province, China, there was a much large potential market for the production of rapeseed oil. The high production of rapeseed oil produced large quantities of a by-product known as rapeseed oil deodorizer distillate. Therefore, it was necessary for us to develop a simple, economical processes to separate sterols, vitamin E and their esters from rapeseed oil deodorizer distillate. It was a promising measure for further utilization of agricultural products.

2. MATERIAL AND METHODS

2.1 Chemicals

Rapeseed oil deodorizer distillate (sterols, 13.3%; vitamin E, 4.47%; acid value, 46.5), obtained from Fengda Oils Factory (Hefei, China), was kept in a cold room while not in the use. β -sitosterol (99%), α -tocopherol (99%), were purchased from Sigma Chemical(USA). Methanol, acetone, acetic anhydride, succinic anhydride, n-hexane, sulfuric acid, phosphate acid and 2,2-bipyridyl were all the analytical reagent.

2.2 Apparatus

Refrigerator centrifuge, GL-20G, manufactured by AnTing Factory, Shanghai; UV-1201 spectrophotometer, manufactured by LvLi Analytical Factory; Refrigerator, manufactured by Roystar; constant temperature bath cabin, HH4, manufactured by GuoHua Co.Ltd. Rotary evaporator manufactured by Henan, Gongyi Factory; electronic balance with a precision of 0.1mg, manufactured by Balance, Shanghai ; Capillary melting point instrument.

2.3 Procedure

The procedure was depicted in the Fig.3. Methyl esterification of rapeseed oil deodorizer distillate with methanol in the presence of sulfuric acid as catalyst was carried out. The esterified deodorizer distillate was cooled to room temperature, then was taken in the refrigerator at -3℃. After 12 hrs, the esterified deodorizer distillate was centrifuged at 3000r/min and the precipitate appeared at the bottom of the centrifuge tubes .The wet cake was raw sterols. The filtrate was used as the raw material to synthesize vitamin E succinate.

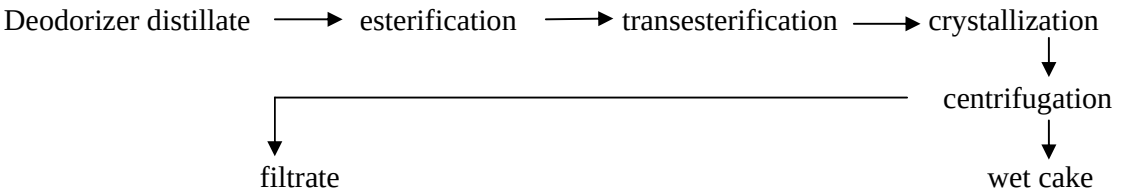


Figure 3. Schematic diagram for the separation of sterols and synthesized $V_E S$

2.4 Analysis of Physical Chemistry Characteristics

2.4.1 Colorimetric Analysis of Sterols

Certain standard sterols were dissolved in the acetic anhydride and a drop of sulfuric acid was added in it. The solution's color changed from colorless, red to dark green and took several minutes for color to develop. With blank solution as reference solution, absorbance (Y) at Vis 650nm with a 10mm quartz cell was used to calculate the concentration of sterols (X) (GAO Yu-ying et al,.2001). The errors could be controlled to less than 0.7% through duplicated experiments and analysis.

Standard curve equation: Y=487.99435X+0.00545(n=5). correlation coefficient R : 0.99487, linear range: $7\times10^{-5}\sim5\times10^{-4}$g/ml

2.4.2 Colorimetric Analysis of Vitamin E

Certain standard vitamin E ethanol solution, 1ml standard 2,2-bipyridyl and 1ml $FeCl_3$ ethanol solution were mixed in the 10ml volumetric flask. The mixed solution stood for 10min. After that, 1ml H_3PO_4 solution and ethanol solution were fed to the flask and it was limited to terminate reaction. With blank solution (prepared with solvent without the material) as reference solution, the solution was measured at 520nm with 10mm quartz cell.

Standard curve equation: Y=0.603X+0.086(n=9), where Y: the concentration of vitamin E, X: the absorbance. Correlation Coefficient R: 0.9928. linear range: 0.4×10^{-4}~4×10^{-4}mol/L.

2.4.3 Acid Value Analysis

Acid value was determined by the literature (Huang Wei-Kun, et al,. 1995)

2.4.4 Melting Point Analysis

The melting point of refined sterol and Vitamin E succinate were determined by the Capillary melting point Instrument. As determined, the range of refined sterol's melting point was 135.8℃~136.4℃ and Vitamin E succinate 75.4℃~75.8℃.

2.5 Experiment Design

2.5.1 Response Surface Methodology of Esterification of DOD

Response surface methodology（RSM）was a statistical-mathematical method which used quantitative data in an experimental design to determine, and simultaneously solve, multivariate equations, to optimize processes or products (Giobanni, M.1983, Junsoo Lee, et al.2000). The central composition rotational design for K=3 was used. The independent process variables were methyl esterification time, catalyst, methanol /material（ml/g）. The levels used were summarized in Table 2. The actual design of the experiments was presented

in the Table 3. This design was preferred because relatively few experimental combinations of the variables were adequate to estimate complex response function.

For each of the experiments done, the total recovery of sterol was determined.
Recovery of sterols (%) = sterols obtained (g)/ sterols in material (g)×100%

The response function used was a quadratic polynomial equations as given below.
Recovery of sterols (%)
$=A_0+A_1X_1+A_2X_2+A_3X_3+A_4X_1X_2+A_5X_1X_3+A_6X_2X_3+A_7X_1X_1+A_8X_2X_2+A_9X_3X_3$

Where A_0=constant.
A_1, A_2, and A_3=linear coefficients.
A_4, A_5, A_6=cross-product coefficients.
A_7, A_8 and A_9=quadratic coefficients.

Table 2. Levels of variables chosen

Variables	Levels		
	+1	0	-1
X_1: methanol/DOD (ml/g)	120/100	100/100	80/100
X_2: esterification time (h)	2.5	1.5	0.5
X_3: catalyst/DOD (g/g)	6%	4%	2%

Table 3. Experimental design with three independent variables

Experiment Number	X_1	X_2	X_3
1	-1	-1	0
2	-1	0	-1
3	-1	0	1
4	-1	1	0
5	0	-1	-1
6	0	-1	1
7	0	1	-1
8	0	1	1
9	1	-1	0
10	1	0	-1
11	1	0	1
12	1	1	0
13	0	0	0
14	0	0	0

15	0	0	0

The parameters of the response equation, their statistical significance and optimum value of solubility of total oil were evaluated using MATLAB 6.1 software (Matlab 6.1,.2000). A multiple regression analysis was performed to obtain the coefficients and the equation was used to predict the response.

2.5.2 Experiment Design of the Synthesis of Vitamin E Succinate

150 ml acetone/methanol (4:1, v/v) was added to the rapeseed oil deodorized distillate 50g. The solution was heated at 60℃ for 2 min to prompt dissolving, then cooled to room temperature and held at -8℃ for 4 hrs first. The crystals rich in phytosterol were collected by filtration, the filtrate was held at -8℃ for last 20 hrs to get rid of phytosterol as much as possible and reduced the loss of vitamin E as little as possible. The sterol-removed deodorized distillate was used to synthesize the Vitamin E succinate. The reaction of succinylization with succinate acid was carried out in the presence of potassium acetate as a catalyst at 120℃-160℃ in the nitrogen environment (Kuo-Min Lin, 2002; Zhao Ya-Ping, 2001). Schematic diagram for the synthesized vitamin E succinate was depicted in Fig.4.

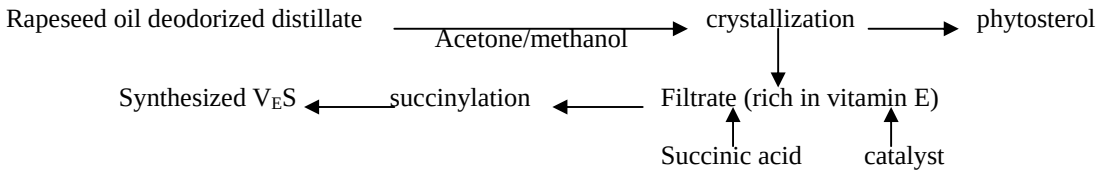


Figure 4 .Schematic diagram for the synthesized vitamin E succinate

Table 4. Factors and treatments

	Succinic anhydride(g)	Reaction temperature(℃)	Reaction time (h)	Catalyst(g)
	A	B	C	D
1	4%	120	1	1%
2	5%	140	2	2%
3	6%	160	3	3%

The orthogonal design for k=4 was used. The various process parameters involved in the synthesis of Vitamin E succinate were: succinic anhydride (A), reaction temperature (B), reaction time (C), catalyst (D). Three levels (low, medium and high denoted as 1,2,3 respectively.) of variables chosen for the experiments were given in Table.4. The orthogonal design plans was depicted in Table 7**.** The conversion of vitamin E to its succinate was calculated based on the amount of tocopherol reduced after the reaction, assuming the loss of tocopherol during preparation of tocopherol succinate was not significant. The equation for conversion percentage was as follows:

Conversion (%) = (amount of tocopherol before reaction — amount of tocopherol after reaction)/amount of tocopherol before reaction×100%

3. RESULTS AND DISCUSSION

3.1 Response Surface Optimization of Methyl Esterification

An optimization of methyl esterification of rapeseed oil deodorizr distillate was tried using RSM. In order to develop response surface equation to predict the percentage of recovery of sterols in the range of studies conducted, the experimentally recovery of sterols was fitted to quadratic polynomial equations and the parameters of the equation were evaluated. The recovery of sterols was listed in Table 5. The regression coefficients of the response function with statistical analysis were given in Table 6. The response equation fitted the experimental data with a high degree of significance.

Table 5. Experimental results of recovery of sterols

Experiment Number	X_1	X_2	X_3	Recovery	Purity
1	−1	−1	0	0.286	98.2%
2	−1	0	−1	0.343	97.9%
3	−1	0	1	0.323	99%
4	−1	1	0	0.414	95.6%
5	0	−1	−1	0.398	97.1%
6	0	−1	1	0.383	98.6%
7	0	1	−1	0.353	95.8%
8	0	1	1	0.434	96.4%
9	1	−1	0	0.263	97.9%
10	1	0	−1	0.414	99.2%
11	1	0	1	0.368	95.2%
12	1	1	0	0.308	96.5%
13	0	0	0	0.448	97.1%
14	0	0	0	0.448	98.4%
15	0	0	0	0.441	98.5%

Table 6. Coefficient of the response function

Coefficient	Values
A_0	0.44567
A_1	-0.010375
A_2	0.023625
A_3	0.0075
A_4	-0.02075
A_5	-0.019

A_6	0.0265
A_7	-0.071458
A_8	-0.056458
A_9	0.0052917

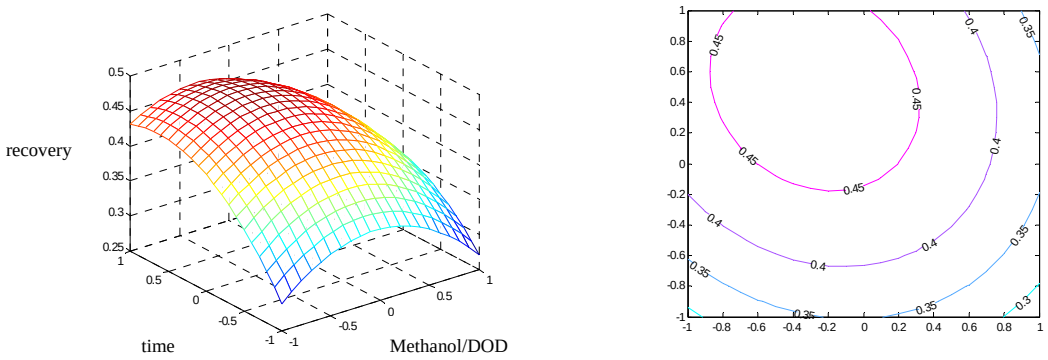


Figure 5. Response surface curve and contour plot showing predicted response surface of recovery of sterols as a function of methanol/DOD and time (catalyst=6%)

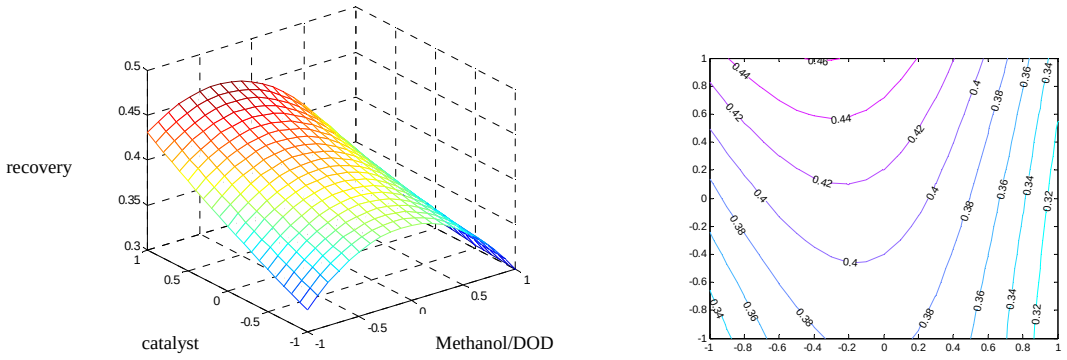


Figure 6. Response surface curve and contour plot showing predicted response surface of recovery of sterols as a function of methanol/DOD and catalyst (time=2.5hrs)

Fig.5 showed the sterols recovery as a function of methanol/DOD and esterification time in the presence of catalyst 6%. It could be seen in Fig.5 that at the amount of catalyst 6%, the recovery of sterol increased as the methanol/DOD increased with respect to methanol/ DOD (ml/g). The optimum methanol/DOD for the maximum recovery of sterols was around 100ml/100g. It may be concluded that too much methanol could make the methanol esterification reverse and it was not enough to generate reaction when methanol was little. So it could be seen in Fig.5 that at 6% of catalyst, the optimum esterification time and methanol/DOD for recovery of sterols were around 1.5hrs and 100ml/100g.

Fig.6 was the three dimensional plot showing the response surface of recovery of sterols as a function of methanol/DOD and catalyst at a constant esterification time 2.5hrs.The sterols

recovery as a function of methanol/DOD and catalyst were shown in Fig.6. It could be seen in Fig.4 that at reaction time 2.5hrs, the optimum catalyst and methanol/DOD for recovery of sterols were around 6% and 100ml/100g.

By partial differential coefficient operation to this model, and equal to zero, then the point of curved surface was determined (X_1=0, X_2=0, X_3=-0.7450,Y=0.453). According to RSM's result, an experiment of methanol/DOD, esterification time, catalyst was conducted in order to investigate the effect of RSA. The content and total recovery of phytosterol were above 85%, 80% after one crystallization. The experiment recovery of sterols was found to be 46.4% after twice crystallization and was in good agreement with the predicted one. The R^2 and RMSE value of the models developed was 0.796 and 0.0513, thus the predicted results according to models were close to observed values. The model equation developed could be used for predicting recovery of sterols.

3.2. Optimization of Synthesis of Vitamin E succinate

An optimal process route to synthesize vitamin E succinate was determined by orthogonal design. L_9 (3^4) orthogonal design was selected. The experimental results could be seen in Table.7. The factors affecting the conversion of Vitamin E were shown in Table 4.That was: reaction temperature > succinic anhydride > reaction time > catalyst. The more conversion of vitamin E will be obtained according to design $A_2B_2C_1D_3$. In our experiment, the conversion of vitamin E was 71.3%.

Table.7. Orthogonal experiment results

Experiment Number	A	B	C	D	Conversion of vitamin E (%)
1	1	1	1	1	63.9
2	1	2	2	2	65.7
3	1	3	3	3	62.6
4	2	1	2	3	67.5
5	2	2	3	1	68.8
6	2	3	1	2	67.1
7	3	1	3	2	59.7
8	3	2	1	3	70.4
9	3	3	2	1	67.1
Ⅰ	192.2	191.1	201.4	199.8	The optimum result is $A_2B_2C_1D_3$
Ⅱ	203.4	204.9	200.3	192.5	
Ⅲ	197.2	196.8	191.1	200.5	
R	11.2	13.8	10.3	8.0	

4. CONCLUSIONS

- A model of methyl esterification of rapeseed oil deodorized distillate was developed on the basis of the analysis of response surface methodology. The reaction temperature, time, liquid/solid ratio, catalyst and transesterification were investigated systematically. The content and total recovery of phytosterol is above 85%, 80% after one crystallization and 95%, 45% after twice crystallization.
- The V_E succinate was synthesized in the residuals to prevent heat –sensitive tocopherol from damage and high conversion of Vitamin E 71.3% were attained. It is very important for us to find out a suitable crystallization solvent of Vitamin E succinate from complex mixture.

5. ACKNOWLEDGEMENTS

This work was supported by Natural Science Foundation of Anhui Province in China (No. 03041302). The authors wish to thank assistant professor Zhao for her lively discussions and comments.

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