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Application of Simple Factorial Design and Response Surface Methodology to Optimize Biodiesel and V_E Production from Soybean Deodorization Distillates

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ABSTRACT

The production of fatty acid methyl esters (FAME) used as a diesel substitute (biodiesel) was studied. The reaction of soybean deodorization distillates (SDDs) and methanol was carried out in a two-step reaction, the first step being the methyl esterification of free fatty acid (FFA) and the second step being the transesterification of triglycerides. The process of biodiesel production and the process of biodiesel and V_E production from esterified SDDs were optimized using a simple factorial design and response surface methodology. A second-order model was obtained to predict the optimum extraction conditions. The optimum conditions for methyl esterifications of FFA were 65°C for 3 h at a catalyst concentration of 4.2%; the optimum conditions for transesterification of triglycerides were 74.4°C for 2.1 h with a catalyst quality of 0.49%. FAME and Vitamin E (V_E) were extracted with supercritical CO_2 at 120 and 250 bar, respectively. Optimum conditions for the extraction of biodiesel were extraction pressure 120 bar, extraction temperature 60°C and a CO_2 flow rate of 15.0 kg/h; optimum conditions for the extraction of V_E were extraction pressure 250 bar, extraction temperature 40°C and a flow rate of 25.0 kg/h. Under the optimum conditions, FAME and V_E were successfully extracted. FAMA concentration and yield rate in the extraction were 87 and 82%, respectively, and the V_E concentration and yield rate in the extraction were 48 and 0.84%, respectively.

Keywords: esterification, extraction, fatty acid methyl ester, optimization, supercritical CO_2, tocopherol

INTRODUCTION

There is an increasing worldwide concern for environmenttal protection and for the conservation of non-renewable natural resources (Ayhan 2003). For this reason the possibility of developing alternative energy sources to replace traditional fossil fuels has been receiving much attention over the past few decades (Wang *et al*. 2008). Fatty acid methyl esters (FAME) show large potential as diesel substitutes, and are known as biodiesel. Biodiesel can be used directly or mixed with conventional fuel for diesel engines as a heating fuel (Fang 1999). Biodiesel is synthesized through direct transesterification from vegetable oils. Triglycerides in oils react with a short-chain alcohol, usually methanol, and a catalyst is needed in this reaction (Fang 2007; Sun 2008). FAME is also synthesized from free fatty acid (FFA) of vegetable oils with methanol (Zhou 2006). The transesterification reaction occurs through three steps described by Sharma *et al*. (2008), and glycerol is the product of the final step, transesterification.

Tocopherol, also known as Vitamin E (V_E), is mainly present in the seeds of ordinary crops and in green parts of higher plants. Recently, there has been increasing interest in V_E in the food industry due to its antioxidant activity and other nutrient effects (Babu *et al.* 2000; Georgantelis *et al.* 2007; Abramovic *et al.* 2007). Antioxidants are nutrients that block some of the damage caused by toxic by-products released when the body transforms food into energy or fights off infection (Barros *et al.* 2008). These by-products are mainly responsible for the aging process and the development of various diseases such as heart disease, cancer, Alzheimer's disease and a host of inflammatory conditions like arthritis (Bostyn *et al*. 2008).

Supercritical fluid extraction (SFE) has many advantages as an extraction technology (Lang and Wai 2001; Tura and Robards 2002; Zhou 2004). SFE can penetrate into the pores of solid materials more effectively than techniques based on liquid solvents, so it enables a much faster mass transfer, resulting in faster extractions (Zhou *et al.* 2004). Also, in SFE, fresh fluid is continuously pumped through the samples, so it can provide quantitative or complete extraction, and the solvency of the fluid can be manipulated by changing pressure and/or temperature, facilitating a remarkable high selectivity (Smith 1999; Zhou and Sheng 2004). Carbon dioxide (CO_2) is the mostly used solvent in the field of SFE. The most important advantage of utilizing supercritical CO_2 (SC–CO_2) is the easy separation of the solvent from the extracted material; moreover, it operates at an ambient temperature that does not destroy heat-sensitive compounds. SC–CO_2 also provides lower mass transfer resistance than conventional separation processes. These advantages have attracted increasing interest of researchers, especially from the food, pharmacy and environmental-engineering industries (Seda *et al*. 2007; Martínez *et al*. 2008).

In the present study, FAMA was produced through a two-step esterification reaction from soybean deodorization distillates (SDDs), which is a by-product during vegetable oil production. Each of the two reaction processes was optimized by using a simple factorial design and response surface methodology. SFE was used to extract FAME and V_E from esterified SDDs. Extraction conditions were studied in order to obtain the highest yield of FAME and V_E, and the influence of the extraction conditions of the method were examined. The results obtained were compared with the results obtained using solid–liquid extraction.

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Table 1 Characteristics of SDD sample.

Moisture (%)	AV (mgKOH/g)	FFA (wt%)	UC (wt%)	V_E (wt%)	Sterols (wt%)
0.3	11.7	58.3	22.1	4.7	16.8

AV: acid value; FFA: free fatty acid; UC: unsaponification compounds
FFA wt%: the value of the percent ratio of FFA weight to SDD weight
UC wt%: the value of the percent ratio of UC weight to SDD weight
V_E wt%: the value of the percent ratio of V_E weight to SDD weight

Table 2 Design of factors levels in esterification and transesterification experiments.

Levels	X_1 (°C)	X_2 (h)	X_3 (W/W)	X_4 (°C)	X_5 (h)	X_6 (W/W)
-1	60	2	0.02	65	1	0.04
0	65	3	0.03	70	2	0.05
1	70	4	0.04	75	3	0.06

Note: W/W is the rate of vitriol/sodium methoxide quality to SDD weight.
X_1: Esterification temperature; X_2: Esterification time; X_3: Vitriol acid volume; X_4: Transesterification temperature; X_5: Transesterification time; X_6: Sodium methoxide quality

Table 3 Different levels of factors and arranged in esterification and transesterification experiments and content of V_E and FFA or glycerol in esterification experiments.

Number	X_1	X_2	X_3	V_E (%)	FFA (%)	V_E (%)	Glycerol (%)
1	-1	-1	0	2.68	3.28	4.84	1.13
2	-1	0	-1	3.07	3.03	5.24	1.36
3	-1	0	1	3.23	2.74	5.25	1.53
4	-1	1	0	3.28	2.93	5.54	1.74
5	0	-1	-1	3.48	2.84	5.64	1.84
6	0	-1	1	3.68	2.42	5.78	2.41
7	0	1	-1	3.79	2.53	5.92	2.63
8	0	1	1	3.91	2.33	5.94	2.72
9	1	-1	0	3.92	3.12	5.95	2.93
10	1	0	-1	3.90	2.64	6.04	2.55
11	1	0	1	3.88	3.06	5.96	2.83
12	1	1	0	3.93	3.00	6.04	2.83
13	0	0	0	3.92	1.67	6.07	2.75
14	0	0	0	3.92	1.78	6.09	2.67
15	0	0	0	3.93	1.65	6.09	2.69

FFA: free fatty acid

MATERIALS AND METHODS

Material

SDDs were provided by Dongfang Long Oil Co, Binzhou, China. The characteristics of SDD samples are listed in **Table 1**. All other chemicals were of analytical grade. V_E (99% tocopherol) was obtained from Merck (Germany). The moisture content of SDD was determined according to the China State Standard, GB/T 5528-1985, the FFA content according to GB/T14489.3-1993, unsaponified compounds content according to GB/T5535-1985, and glycerol content according to GB/T GB/T13216.6-91. Sterols, methyl esters and V_E were determined according to the method described below.

GC analysis of methyl ester

A Shimadzu (Japan) GC-9A with a flame-ionization detector (FID), and a 30 m × 250 µm i.d. HP-5 (d_f= 0.25 µm) was used. The temperature program used was: 80°C (1 min), 80-140°C (10°C/min), 140-180°C (5°C/min), 180-265°C (25°C/min) and 265°C (10 min). The injector and detector temperatures were 260 and 280°C, respectively. The carrier gas (N_2) flow rate was 4.0 mL/min.

GC analysis of sterols

A Hewlett-Packard model HP-5890 GC (Palo Alto, CA) with a hydrogen flame ionize detector (HFI), and a 30 m × 250 µm i.d. HP-5 (d_f= 0.25 µm) fused-silica capillary column was used. Phytosterol standard (w>99%) was supplied by Dr. Maobin Chen. Injection and HFI detector were carried out at 320 and 300°C, respectively. The oven temperature was 285°C. The carrier gas was nitrogen (100 KPa). Split ratio was 1:30 and trail blow was 40 ml/min. Squalane (w>99%) bought from Dikma was used as internal standard.

LC analysis of V_E

A Shimadzu (Japan) HPLC equipped with an ultraviolet-visible array detector was used to analyze V_E in the SDDs, esterified SDDs and the extracts obtained using the SFE system described above. The separation column was a supelcosil LC-18 of φ 4.6 mm × 15 cm and 5-µm particle diameter. The samples were injected with no previous treatment into the 20-µL injection loop of the HPLC system. The mobile phases were 98% methanol and 2% distilled water at a flow rate of 1.0 mL/min over 15 min. Identification of compounds was achieved by comparing their retention times and spectra with those of the standards. For quantitative analysis, calibration curves were made by analyzing different concentrations of tocopherol standards and by representing peak area vs. concentration (% (w/w)); correlation coefficients were 0.99. Detection of V_E was performed at its maximum absorption in the UV, i.e. at 292 nm.

Operating procedure of esterification of SDDs

Esterification of SDDs included two steps. In the first step, the SDDs containing 4% (w/w) V_E were mixed with methanol and vitriol acid and shaken in a 500 mL distilling bottle; the bottle was then placed into a thermostatic bath to reflux the mixture for 3 h. During the reaction, the mixture was continuously shaken. After the reaction, the mixture was washed with 60-70°C water whose volume was five times of mixture until neutral, then held at 4°C for 12 h. The sterols in the SDDs were removed by filtering under vacuum. In this step, the free fatty acid was converted to FAMA. In the second step, the sterol-removed SDDs were mixed with methanol and sodium methoxide in a 500 mL distilling bottle, which was then placed into a water bath to reflux the mixture for 2 h. During the reaction, the mixture was continuously shaken. The product was evaporated under vacuum to remove methanol; in this step, the triglycerides were converted to FAMA. After removing the sterols the esterified SDDs contained about 5% (w/w) V_E and about 85% (w/w) FAME. Design of factors levels in esterification and transesterification experiments is listed in **Table 2**. Different levels of factors and arranged in esterification and transesterification experiments and content of V_E and FFA or glycerol in esterification experiments are listed in **Table 3**.

SFE of FAME and V_E from esterified SDDs

The SFE system used in this study was purchased from Huaan Supercritical Supplement Ltd. model HA231-50-025 and had the following features: an extraction cell and a separator column with four different temperature levels (2 m in length, 17.5 mm i.d.).

After chemical modification, the FAME in SDDs that were produced showed improved solubility in SC-CO_2. Since FAME were more volatile, they were extracted preferentially over V_E and other higher molecular weight compounds. Two steps were applied in the SC-CO_2 extraction. 50 g esterified SDDs were placed into the extraction cell. A continuous 20 kg/h flow of CO_2 was introduced into the whole supercritical system. When the operating pressures and temperatures reached the set value, static extraction was performed for 10 min, then the extration of FAME began. FAME was dissolved by CO_2 in the extraction cell and was separated in the separator column. Fractionates were collected every 10 min. When the weight of all fractions did not increase, the extraction of FAME was considered to be complete. Then the pressure of the extraction cell was increased from 120 to 250 bar to extract V_E. The extraction temperature and the separator column temperature for both V_E and FAME were 40°C and 40-55-70-80°C (from bottom to top), respectively. Meanwhile, the CO_2 flow rate was 25.0 kg/h, whereas the separator pressure was 8.0 bar. When all the working conditions of the second extraction were reached, 2~6% cosolvent (methanol, ethanol, mixed ethanol (methanol: ethanol = 2:1)) was pumped into the extraction cell and mixed with CO_2.

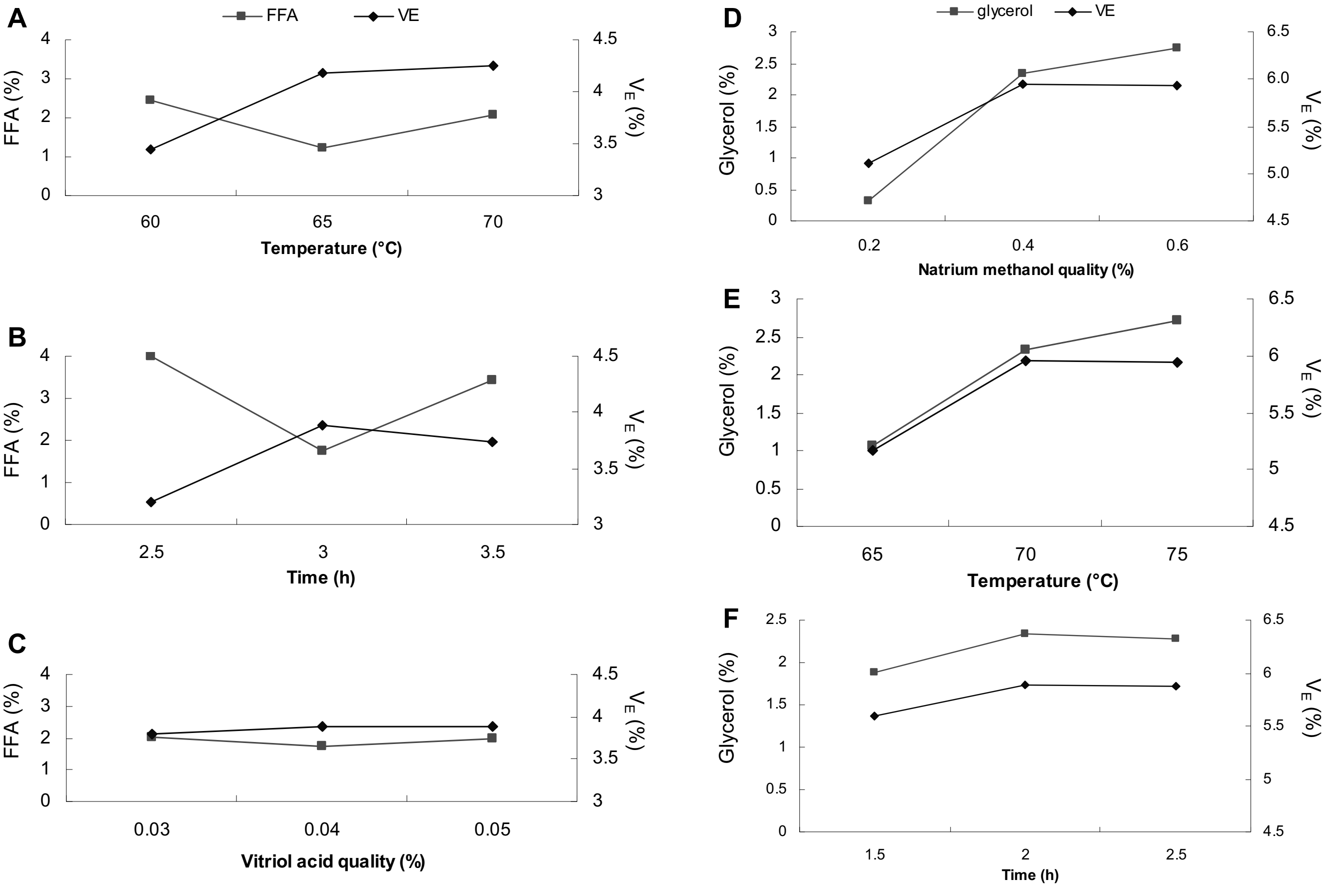

Fig. 1 Effects of methyl esterification factors on FFA esterification. (**A**) methyl esterification temperature; (**B**) methyl esterification; (**C**) methyl esterification; (**D**) natrium methanol quality; (**E**) transesterification temperature; (**F**) transterification time.

After static extraction for 15 min, SFE began. Fractionates were collected every 10 min (three times in total) until the weight of all fractions increased to 12 (w/w) of the esterified SDDs. Extraction and separation conditions were kept constant throughout the experiments. The extraction time for each step of all experiments was recorded.

RESULTS

Esterification of FFA and transesterification of triglyceride

It was found that methyl esterification temperature, time and catalyst concentration have positive effects on the esterification of FFA, and the effect of time is smaller than that of temperature and larger than that of catalyst concentration.

The optimization of the variables affecting the esterification of FFA was carried out following the factorial design of experiments and response surface methodology. The parameters of the second-order model were determined by multiple regression.

V_E regression equation of esterification of FFA:

$$Y = 3.92 + 0.42X_1 + 0.14X_2 + 0.06X_3 - 0.33X_1^2 - 0.14X_2^2 - 0.07X_3^2 - 0.15X_1X_2 - 0.05X_1X_3 - 0.02X_2X_3 \quad (1)$$

Regression equation of esterification of FFA:

$$Y = 1.70 - 0.02X_1 - 0.11X_2 - 0.06X_3 + 0.86X_1^2 + 0.52X_2^2 + 0.31X_3^2 + 0.06X_1X_2 + 0.18X_1X_3 + 0.06X_2X_3 \quad (2)$$

As shown in **Fig. 1A-C**, below 65°C, the vitriol acid volume and esterification time had a considerable effect on V_E content, whereas above 65°C, they affected V_E content less. There is a close relationship between vitriol dosage, esterification time and temperature. As shown in **Fig. 1D-F**, as for acid value, the optimal esterification condition is around the center of the range of esterification temperature, esterification time and vitriol acid volume.

The optimal conditions resulted from two regression models: 65°C (esterification temperature), 3 h (esterification time), 4.2% vitriol dosage.

Catalyst concentration, transesterification temperature and transesterification time were found to have a positive effect on transesterification of triglycerides, and the effect of transesterification temperature is smaller than that of catalyst concentration and larger than that of transesterification time. The rate of transesterification increases with increasing temperature. However, the maximum operating temperature cannot exceed the boiling point of the reactants.

The optimization of the variables affecting the transesterification of glycerol was carried out following the factorial design of experiments and response surface methodology

The parameters of the second-order model were determined by multiple regression: V_E regression equation:

$$Y = 6.08 + 0.39X_1 + 0.15X_2 + 0.34X_3 - 0.01X_1^2 - 0.15X_2^2 - 0.12X_3^2 - 0.15X_1X_2 - 0.02X_1X_3 - 0.03X_2X_3 \quad (3)$$

Glycerol regression equation:

$$Y = 2.70 + 0.67X_1 + 0.20X_2 + 0.14X_3 - 0.44X_1^2 - 0.11X_2^2 - 0.20X_3^2 - 0.18X_1X_2 + 0.03X_1X_3 - 0.12X_2X_3 \quad (4)$$

Fig. 2A-C show the response surface analysis of V_E content after transesterification temperature over 70°C. Natrium methanol dosage and transesterification time have less effects on V_E content while temperature below 70°C, natrium methanol dosage and transesterification time have considerable effects on V_E content. Dosage of natrium methanol used depends on transesterification temperature and time.

Fig. 2D-F show the response surface analysis of glycerol content after transesterification. The higher the transesterification time is within the range used in experiment, the greater the glycerol content.

The optimal conditions combined resulted from two regression models: 74.4°C (transesterification temperature), 2.1 h (transesterifiction time), 0.49 g/ 100 g SDDs natrium methanol.

The above optimal experiments were repeated 3 times

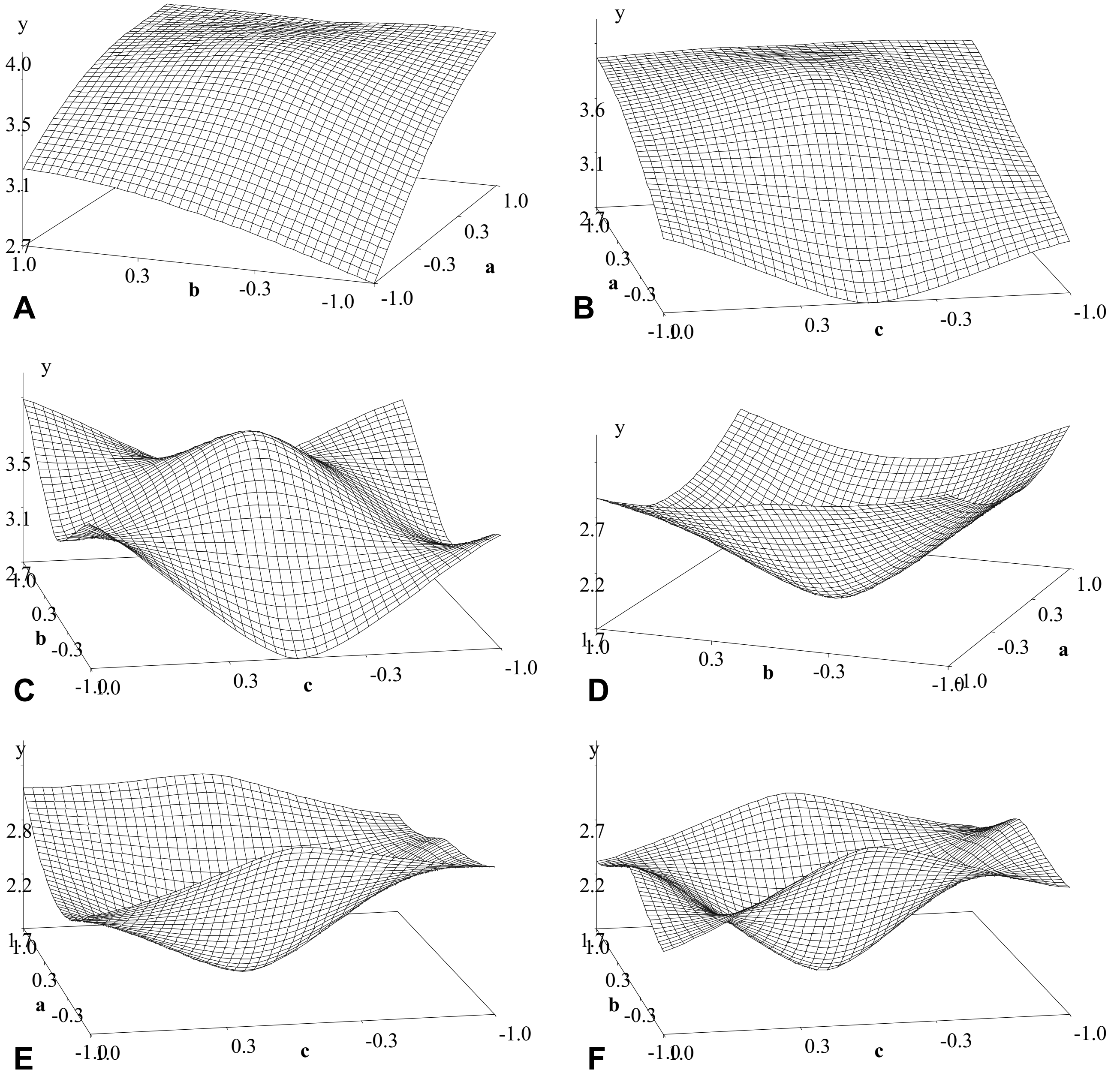

Fig. 2 Response surface analysis of FFA esterification. (**A**) Response surface plot showing the effect on temperature and time and their mutual effect on the V_E content during FFA esterification. (**B**) Response surface plot showing the effect on temperature and vitriol acid quality and their mutual effect on the V_E content during FFA esterification. (**C**) Response surface plot showing the effect on time and vitriol acid quality and their mutual effect on the V_E content during FFA esterification. (**D**) Response surface plot showing the effect on temperature and time and their mutual effect on the FFA content during FFA esterification. (**E**) Response surface plot showing the effect on temperature and vitriol acid quality and their mutual effect on the FFA content during FFA esterification. (**F**) Response surface plot showing the effect on time and vitriol acid quality and their mutual effect on the FFA content during FFA esterification. In all cases (**A-F**), other variables are held at the zero level.

and the average rate of conservation of V_E being 90.5% and transesterification rate 89.4%.

Extraction of biodiesel and V_E

Extraction pressure, extraction temperature and flow rate were found to have a positive influence on concentration of biodiesel, and the effect of extraction temperature was smaller than that of extraction pressure and larger than that of flow rate. Extraction pressure, extraction temperature and flow rate were found to have a positive influence on extraction of V_E, the effect of extraction temperature is smaller than that of extraction pressure and larger than that of catalyst concentration.

The concentrate obtained at 120 bar was 87% FAME, and yield rate for FAME was 82%. Meanwhile, the extracts obtained at 250 bar had 48% V_E, and the yield rate for V_E was 0.84%.

DISCUSSION

V_E content, V_E recovery and the weight of target extract (those extracts with the concentration of V_E above 40%) increased when extraction time decreased. FAME content in extracts by SC-CO_2 under 120 bar depends on the extent of esterification of FFA and transesterification of triglyceride. The response surface corresponding to the second-order model (**Fig. 3D**) indicates that for low temperatures, ester conversion increases with increasing catalyst concentration. Maximum ester conversions are therefore obtained for large catalyst concentrations within the lower temperature range. This is due to the fact that the most significant factor is catalyst concentration, and its effect is positive. At higher temperatures, however, a different behaviour is observed. Conversion increases initially, reaching a maximum at intermediate catalyst concentrations, and then decreases at high catalyst concentrations. This is the result of a negative tem-

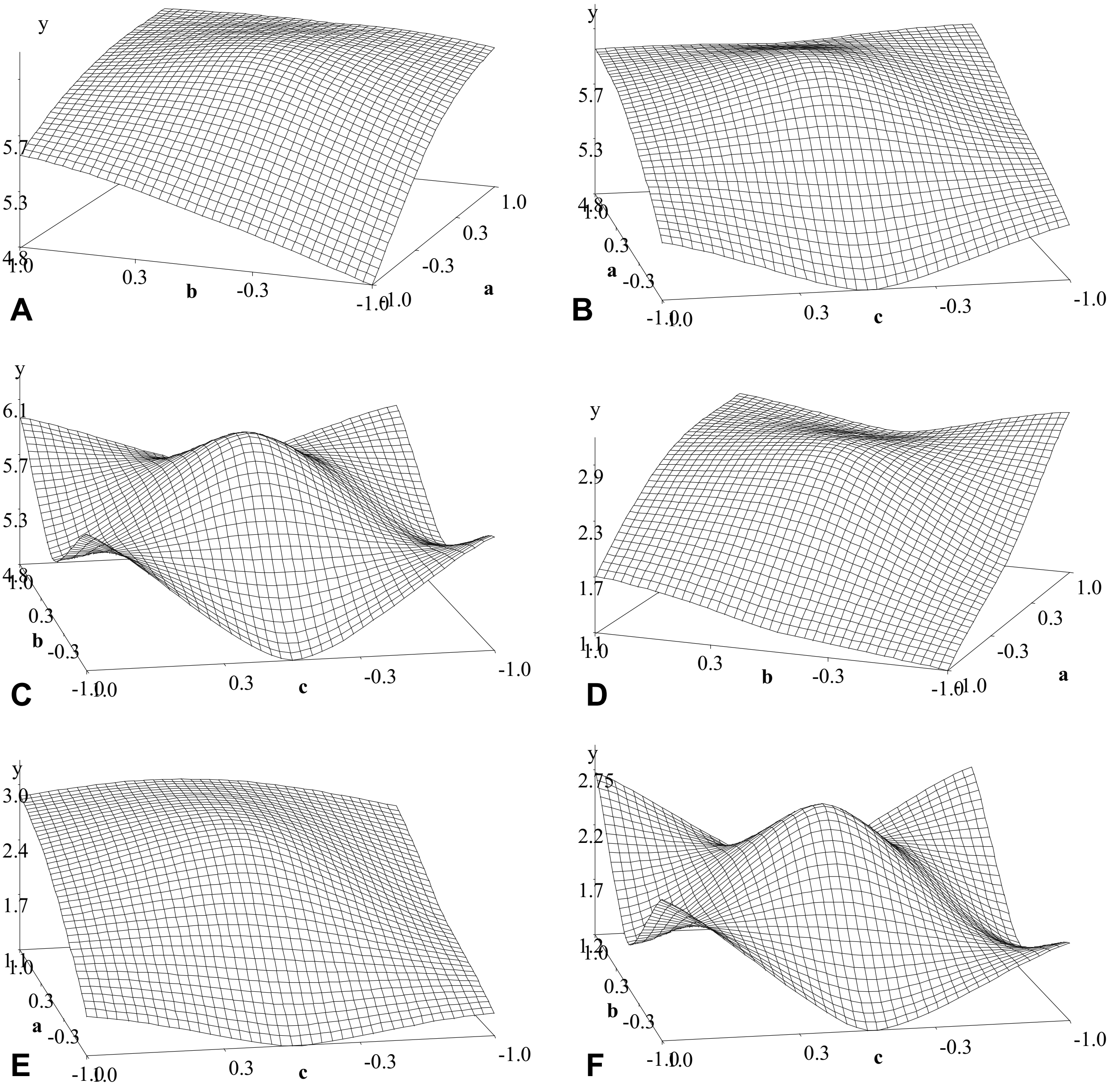

Fig. 3 Response surface analysis of triglyceride transesterification. (**A**) Response surface plot showing the effect on temperature and time and their mutual effect on the V_E content during triglyceride transesterification. (**B**) Response surface plot showing the effect on temperature and natrium methanol quality and their mutual effect on the V_E content during triglyceride transesterification. (**C**) Response surface plot showing the effect on time and natrium methanol quality and their mutual effect on the V_E content during triglyceride transesterification. (**D**) Response surface plot showing the effect on temperature and time and their mutual effect on the glycerol content during triglyceride transesterification. (**E**) Response surface plot showing the effect on temperature and natrium methanol quality and their mutual effect on the glycerol content during triglyceride transesterification. (**F**) Response surface plot showing the effect on time, natrium methanol quality and their mutual effect on the glycerol content during triglyceride transesterification. In all cases (**A-F**), other variables are held at the zero level.

perature-concentration interaction and a negative concentration quadratic coefficient, probably caused by soap formation side reactions. These experiment results are in agreement with findings from other studies (Fang 1999; Ayhan 2003; Zhou *et al*. 2004; Zhou and Sheng 2004; Zhou 2006).

At low catalyst concentrations there is a moderate increase in conversion with temperature, since the temperature effect is positive and smaller than that of concentration. For larger catalyst concentrations, the increase of conversion with temperature becomes smaller and finally, conversion becomes almost constant with temperature at high catalyst concentrations, as a result of the negative interaction. Soap formation is an undesirable side reaction, which lowers FAME yield. Therefore the centration interaction effect was found to be negative, probably due to side reactions, such as soaps formation.

Figs. 2 and **3** show the response surface corresponding to the second-order model (**Figs. 2, 3**). **Fig. 2** indicates that temperature and time have less effects on V_E content. **Fig. 3** indicates that V_E content decreases with increasing catalyst concentration.

A two-step extraction with SC-CO_2 is very beneficial for biodiesel extraction and V_E concentration. The pressure margin between biodiesel extraction and V_E concentration is so big that other components can not be extracted and the extract is highly pure. This technique is very fit for industry-scale production of both V_E and biodiesel.

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