

COUNTERCURRENT EXTRACTION FOR THE FRACTIONATION OF NATURAL TOCOPHEROLS WITH SUPERCRITICAL CO₂

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1. INTRODUCTION

Oil de-odorizer distillate (DOD) is a kind of byproduct of oil refining process and is an important resource of natural tocopherols (vitamin E) and sterols, which are widely used in food and medical industries [1]. Vacuum or molecular distillation has been applied to commercial production of tocopherols from DOD. Since there are some drawbacks such as high production cost, unstable quality and thermal degradation of tocopherols caused by high processing temperature [2], developing new alternative isolation techniques has been desired.

Supercritical CO₂ (SC-CO₂) extraction makes it possible to selectively extract many heat-sensitive components. Although some researchers concentrated tocopherols from DOD by SC-CO₂ [1, 3 - 5], the operation parameters, especially pressure, are different from author to author. In this paper, we used methyl esterified DOD (ME-DOD) as the feed for SC-CO₂ fractionation. Methyl esterification and alcoholysis were used to convert most of free fatty acids and glycerides into fatty acid methyl esters (FAMES) and most of sterols were easily removed because of their low solubilities in FAMES. After such treatments, ME-DOD mainly contains FAME (65-80 wt.%), tocopherols (10-15 wt.%) and other impurities (such as sterols, glycerides, squalene, pigments, long chain paraffins, all of these hold about 10-15 wt.%).

According to our previous work on the phase equilibrium for the ME-DOD and SC-CO₂ system [6], the distribution coefficient of tocopherols is much smaller than that of FAMES below 20 MPa, in particle, when pressure is lower than 16 MPa, the separation factor between tocopherols and FAMES is less than 0.2, it means that tocopherols and FAMES can be well separated by SC-CO₂ at a pressure lower than 16 MPa. Based on these conclusions, the basic experimental strategy was adopted in this study: first of all, SC-CO₂ was employed to remove most of FAMES from ME-DOD at 16 MPa and then the operation pressure was increased for extracting tocopherols.

2. MATERIALS AND METHODS

2.1 Materials

CO₂ was supplied from Praxair Inc. with the purity of 99.9 %. DOD was supplied by Hubei Zhongchang Oil and Fat Co. and contained 9.45 wt.% tocopherols and 8.53 wt.% sterols. DL- α -tocopherol was purchased from Merck Co. with a reported purity above 98 %.

For methyl esterification, a mixture of methanol and DOD (0.8 volume: 1 weight) reacted at

60 °C for 2 hours, with 4.5 wt.% (DOD basis) H₂SO₄ as the catalyst. As for acoholysis, sodium methoxide was applied as the catalyst with an amount of 0.5 wt. % (oil basis), the ratio of methanol to oil (volume to weight) was 1:1, the reaction temperature 70 °C with reflux and the reaction time 2 hours.

2.2 Apparatus and procedure

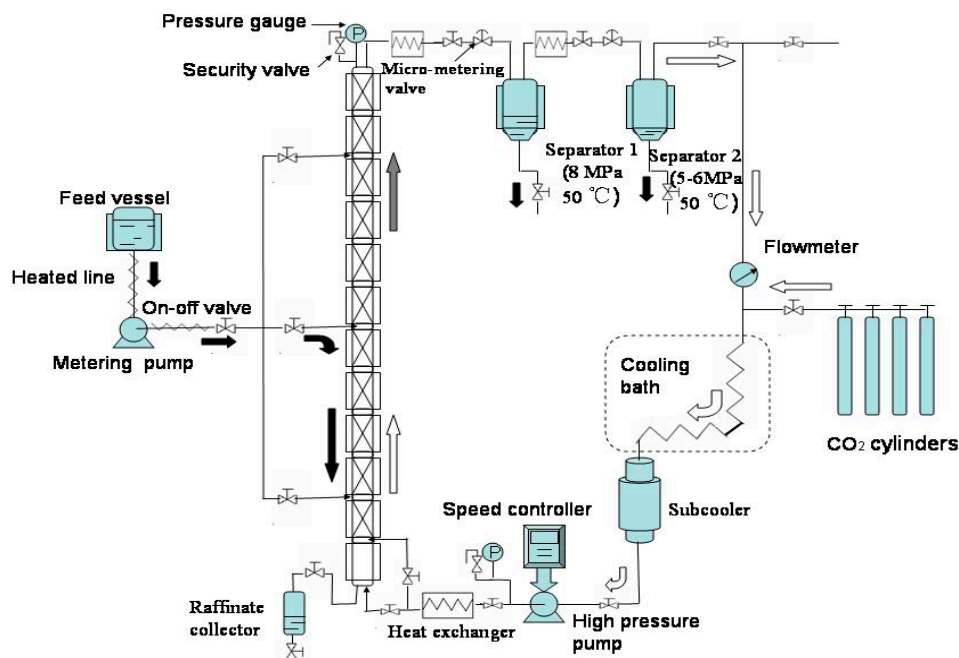


Figure1. Experimental apparatus

A SC-CO₂ fractionation system shown in **Figure 1** was applied in this study. A 9.6 m column with the inner diameter of 48 mm was the pivotal part and CY1200 standard packing was loaded inside, the temperature gradient was controlled by 12 PID controllers, the pressure and CO₂ flowrate were controlled by adjusting the speed controller of the high-pressure pump and two micro-metering valves connected with the separators.

In each run, 6 kg preheated ME-DOD was charged into the middle of the column at 6 kg/hr and the fraction from the separators was weighted and analyzed in every 30 min. The first step was to remove most of FAMEs (about 70 wt. % feed) at 16 MPa (initial pressure). During ME-DOD was charged into the column, the operation was in the countercurrent mode, where CO₂ was the continuous phase and feed oil was dispersed phase, whereas after feeding, the operation was in batch mode. When the total yield from the column top reached about 70 wt.% of the feed, the second step began for separating tocopherols from other impurities by increasing the column pressure to a higher value (end pressure) . The experiment was terminated when the massflow per 30 min was less than 50 g.

The total and tocopherol yields were calculated from the massflow and tocopherol content in the fractions. We mainly investigated the effects of end pressure, temperature gradient and CO₂ flowrate on the average tocopherol content (ATC) and tocopherol recovery (TR) in the fractions collected during the second step.

2.4 HPLC analysis of tocopherols

The tocopherol analysis was performed by Waters 2690 HPLC, 996 photodiode array

detector with a silica-gel column 200×4.6 mm (5 μm, HYPERSIL, Waters), the mobile phase was 99 *n*-hexane : 1 propanol at 1 ml/min, the detection wavelength 295 nm and the column temperature 25 °C. The result was calculated with the external standard of DL- α -tocopherol.

2.5 GC-MS analysis

The GC-MS analysis was carried out using a Finnigan Trace GC-MS ThermoQuest (Finnigan Co. U.S.A.). Typical electron energy was 70 eV with ion source temperature maintained at 200 °C. The individual components were separated using an OV1701 capillary column (30 m×0.25 μm×0.25 mm), which was operated in the splitless mode. The initial temperature was set at 240 °C for 3 min and programmed to 265 °C at 10 °C/min and subsequently isothermal at 265 °C for 40 min. The injector temperature was set at 250 °C.

3. RESULTS AND DISCUSS

3.1 Tocopherol content in ME-DOD

After methyl esterification and alcoholysis, ME-DOD contained 10.19 % tocopherols and the proportion of α - β - γ - δ -isomers was 12.05:1.28:60.29:26.38 as shown in **Figure 2**.

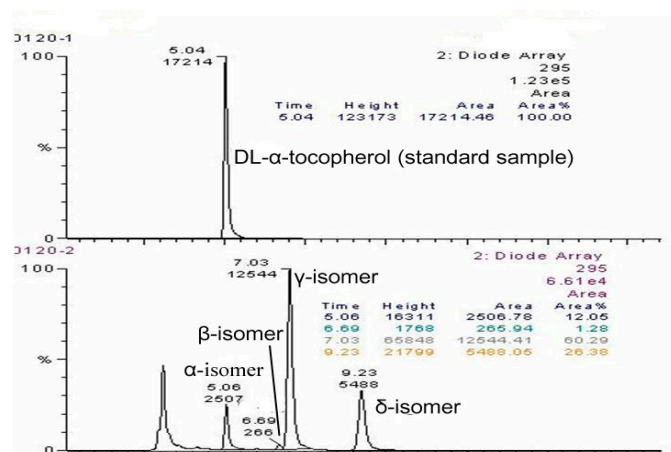


Figure 2. HPLC profile of tocopherols

3.2 Effect of end pressure

Figure 3 shows the change of the fractions as a function of time at different end pressures. In these experiments, other operation parameters were the same, the temperature gradient was a linear distribution from 40 °C at the bottom to 80 °C at the top, the ratio of CO₂ to feed was 120 to 6. There was no distinct change in the first step of 16 MPa, the massflow was about 2 kg per 30 min (**Figure 3. a**), and after 1.5 hr the yield reached about 70 wt.% of the feed (**Figure 3. b**), and the tocopherol yield in this step was only 7-8 %, which favored the following concentration of tocopherols at higher pressure. In the second step of higher pressure, the massflow and the total yield increased with an increase in the end pressure, but the change of tocopherol content at 22 MPa was not as sharper as that at 20 or 18 MPa (**Figure 3. c**). It indicated that a higher pressure leads to an increase in the total solubility but simultaneously a decrease in selectivity. The ATC of all fractions at 22 MPa was only 39.55 %, although the corresponding TC was 89.28 % (**Figure 3. d**). As for 18 MPa and 20 MPa, both obtained a high ATC about 50 %, but the TC at 18 MPa was only 70.34 %, lower than 80.60 % at 20MPa. Moreover, 18 MPa resulted in a longer fractionation process because of the low

solubility. Therefore, 20 MPa was more reasonable to be selected as the end pressure.

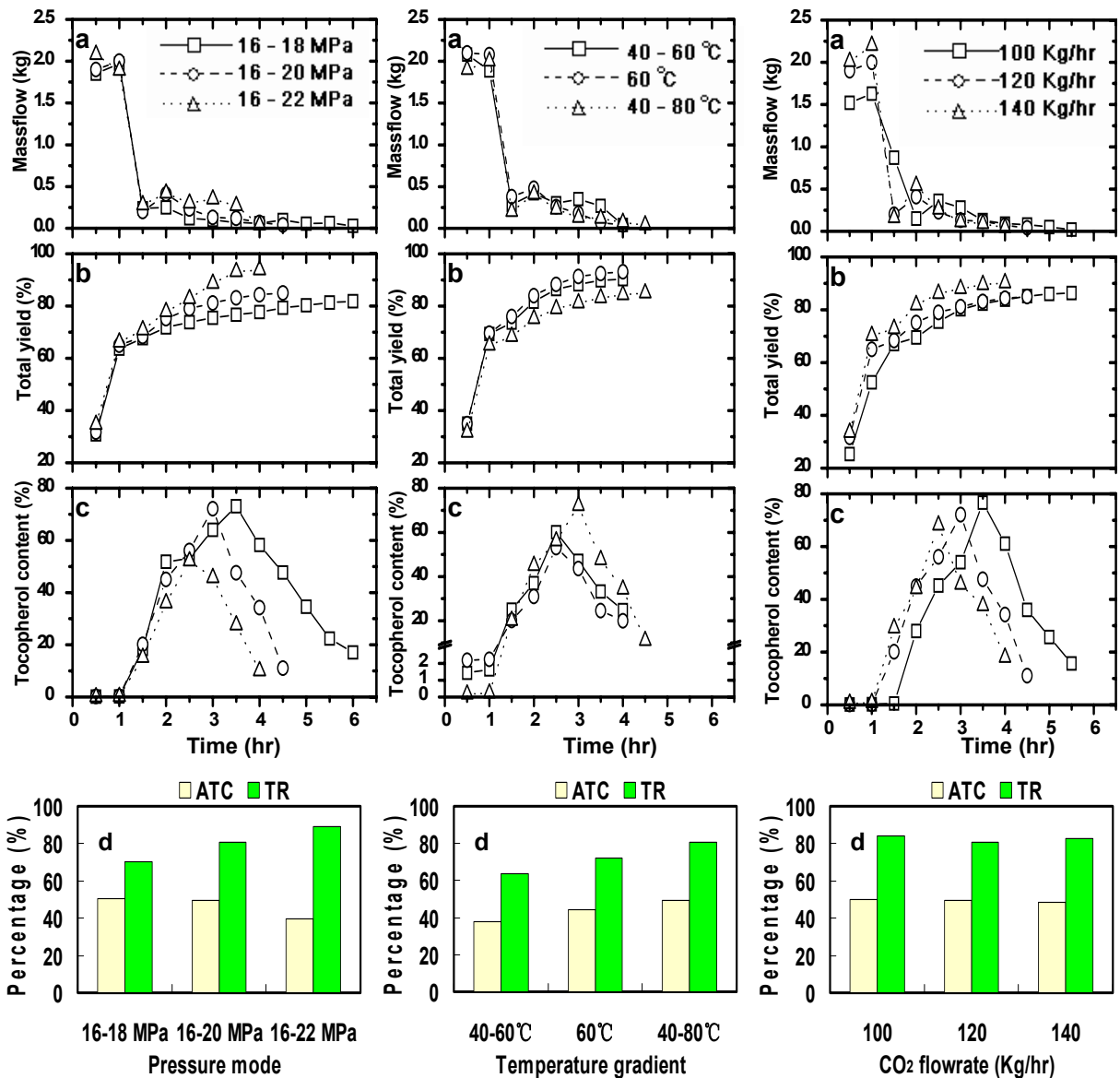


Figure 3. Effect of end pressure on fractionation

Figure 4. Effect of temperature gradient on fractionation

Figure 5. Effect of CO₂ flowrate on fractionation

3.3 Effect of temperature gradient

After establishing the pressure mode from 16 to 20 MPa, different temperature gradients were investigated at the CO₂ flowrate was of 120 kg/hr. The results shown in **Figure 4** indicated that larger temperature difference improved the tocopherol content in the fractions at 20 MPa (**Figure 4. c**), although the total yield of 40 - 80 °C was lower than that of the uniform gradient of 60 °C or the smaller gradient of 40 - 60 °C (**Figure 4. b**). Generally, the temperature increase along the column causes an internal reflux, where less soluble components condense and drop back when the supercritical fluid containing dissolved solutes at lower temperature flow into the higher temperature zone due to the solubility decrease of solutes with the increase in temperature. These internally refluxed drops countercurrently

contact with the fluid flowing up in the column, resulting in the rectification. In addition, compared with the uniform temperature of 60 °C, 40 - 60 °C didn't lead to a manifest difference in the tocopherol content of 20 MPa fractions. The possible reason was that the small difference of 20 °C didn't cause enough internal reflux for improving the selectivity of the whole process. Moreover, the two temperature modes remarkably increased the tocopherol content of 16 MPa fractions (**Figure 4. c**). As a result there were more than 20 % tocopherol yield existing in 16 MPa fractions, decreasing the ATC and TR of 20 MPa fractions, which were 37.93 % and 63.9 %, respectively, when the column was kept at 60 °C.

3.3 Effect of CO₂ flowrate

The effect of CO₂ flowrate on the fractions was investigated at constant feed rate of 6 kg/hr. **Figure 5** shows the changes in fractions with the pressure mode of 16 - 20 MPa and temperature gradient of 40 – 80 °C. As shown in **Figure 5. a** the extraction rate increased with an increase of CO₂ flowrates and consequently a higher total yield was obtained in a shorter time (**Figure 5. b**), while CO₂ flowrate didn't influence remarkably on the distribution of tocopherol in 20 MPa fractions (**Figure 5. c**). In the range of CO₂ flowrate investigated, the ATCs and TRs at 20 MPa were about 50 % and 80 – 84 %, respectively (**Figure 5. d**). Additionally at the CO₂ flowrate of 100 kg /hr, an ATC of 50.03 % was obtained and the corresponding TR was 84 %. The result was the best one among all these experiments and especially there was a 76.66 % fraction at 3.5 hr, which was also the highest content obtained in this study.

3.4 GC-MS analysis

Under the temperature gradient of 40 – 80 °C and 100 kg/hr CO₂ flowrate, 50.03 % tocopherols were obtained by mixing all fractions of 20 MPa, and then the tocopherol concentrate was analyzed with GC-MS for determining other impurities.

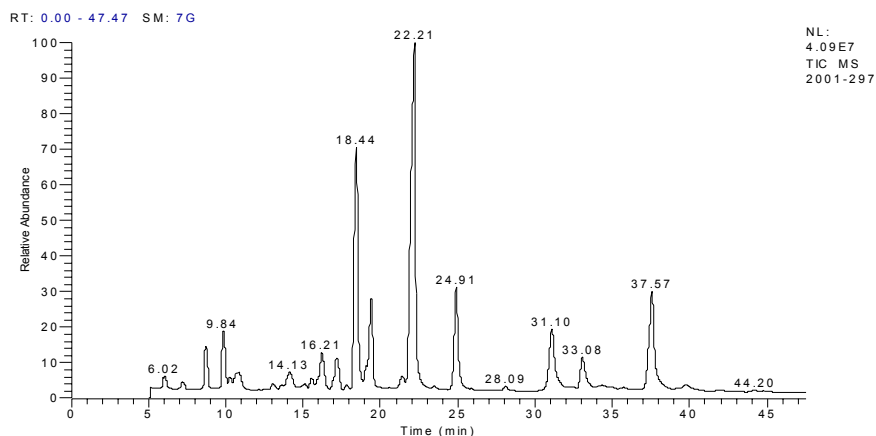


Figure 6. GC-MS TIC chromatogram of 50.03% tocopherol concentrate

The corresponding GC-MS TIC chromatogram and searching results from NIST and WILLY database were illustrated in **Figure 6** and **Table 1**. The main impurities were steroids (26.13%), squalene (3.94 %), long chain paraffins (4.4 %) and long chain FAMES (4.65 %). It should be noted that the area percentage in **Table 1** was different from the actual weight content because the relative abundances were different from component to component. There were still some components of large molecular weight or high boiling point, such as glycerides and pigments undeterminable under the present GC-MS conditions.

Table 1. GC-MS analysis result of 50.03 % tocopherols

Peak	Retention time (min)	Component	Area (%)
1	6.02	Docosanoic acid, methyl ester	1.22
2	7.22	Tricosanoic acid, methyl ester	0.51
3	8.71	Tetracosanoic acid ,methyl ester	2.57
4	9.84	Squalene	3.94
5	10.72	Nonacosane	2.67
6	13.06	Hexacosanoic acid,methyl ester	0.35
7	14.13	Hentriacontane	1.73
8	16.21	Stigmast-4-en-3-one	3.66
9	17.19	Cholesta-6,22,24-trien,4,4-dimethyl-	2.69
10	18.44	Gamma-Tocopherol	16.45
11	19.40	Delta-Tocopherol	6.91
12	21.43	Beta-Tocopherol	0.86
13	22.21	Gamma-Tocopherol	28.81
14	24.91	Alpha-Tocopherol	7.68
15	28.09	Campest-5-en-3 β -ol	0.29
16	31.10	Campest-5-en-3 β -ol	6.47
17	33.08	Trans-stigmasta-5,22-dien-3beta-ol	3.42
18	37.57	Stigmast-5-en-3-ol, (3beta,24s)-	9.60

CONCLUSION

In this paper, SC-CO₂ fractionation was carried out for concentrating tocopherols from ME-DOD. The experimental results indicated the tocopherol contents in different fractions change as a peak-style. High average tocopherol content and tocopherol recovery were obtained at the end pressure of 20 MPa. As to the column temperature, a larger gradient of 40 - 80 °C is advantageous for improving internal reflux and consequently extracting tocopherols with a higher contents. Additionally the CO₂ flowrate didn't influence obviously on the fractionation of tocopherols. As to the tocopherol concentrate of 50.03 %, GC-MS analysis indicated that the main impurities were steroids, squalene, long chain paraffins and FAMES.

ACKNOWLEDGEMENT

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REFERENCE

- [1] MENDES, M., PESSOA, F., ULLER, A., J. Supercrit. Fluids, Vol. 23, **2002**, p. 257
- [2] LUCAS, A., MARTINEZ, E., RICON, J., BLANCO, M., GRACIA, I., J. Supercrit. Fluids, Vol. 22, **2002**, p. 221
- [3] LEE, H., CHUNG, B., PARK, Y., J. Am. Oil Chem. Soc., Vol. 68, **1991**, p. 571
- [4] KING, J., FAVATI, F., TAYLOR, S., Sep. Sci. Tech., Vol.31, **1996**, p.1843
- [5] BRUNNER, G., MALCHOW, T., STURKEN, K., GOTTSCHAU, T., J. Supercrit. Fluids, Vol. 4, **1991**, p. 72
- [6] FANG, T., SHI, X., YUN, Z., DING, X., China Fat and Oil, VOL.26, **2001**, p20