KOREAN J. FOOD SCI. TECHNOL.
Vol. 14, *No.* 2 (1982)

Isolation and Purification of Tocopherols and Sterols from Distillates of Soy Oil Deodorization

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(Received April 1, 1982)

대두유 탈취 증류분에 함유된 토코페롤 및 스테롤의 분리정제

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(1982년 4월 1일 수리)

Abstract

Various separation methods such as solvent extraction, chemical treatment and molecular distillation were tested for the separation of tocopherols and sterols from soy oil scum. The end products of these methods were tocopherol concentrates and sterol crystals. In the solvent extraction, purity and yield of tocopherols were 21.2% and 28.3%, and those of sterols were 69.2% and 2.6%. In the chemical treatment, purity and yield of tocopherols were 11.8% and 76.4%, and those of sterols were 85.1% and 34.3% respectively. In the molecular distillation, purity and yield of tocopherols were 45.0% and 68.0%, and those of sterols were 49.3% and 57.0% respectively. The end products from the methods were characterized by HPLC. Based on the results of this study, the molecular distillation method was found to be more efficient than any other method tested.

Introduction

Tocopherols and sterols are complex alcohols widely used as natural antioxidant in food industry and raw materials used in cosmetics and pharmaceutical industries respectively[1~6]. A considerable amount of tocopherols and sterols is contained in the scum which is a by-product from soy oil deodorization process[5]. Scum is a very complex mixture. In addition to tocopherols and sterols, it contains usually a substantial amount of fatty acids, esters of sterols, mixed fatty acid glycerides, hydrocarbons and other materials[4]. For pharmaceutical and other uses, concentrates of tocopherols and sterols should be of high purity. There are a number of processes to separate tocopherols and sterols from the scum. While some of these processes have been commercially acceptable, none have been completely satisfactory for a number of reasons[6,7]. One type of these processes consists of a series of chemical and physical treatments such as saponification and esterification followed by molecular distillation. But this process involves admixing large quantity of alkali in order to hydrolyze the esters. This reduces the tocopherol yield substantially because the tocopherols are extremely labile under basic condition[1,6,7]. Another type of separation method is solvent extraction. This process consists of the two basic steps involving polar and nonpolar liquid solvent pairs.

In this process, the separtion of tocopherols and sterols from other components of the scum is based on the polarity of each component, which can be extracted with the solvent having appropriate polarity. However, disadvantage of this process is that it requires very large amounts of solvents and that tocopherols and sterols may migrate to the raffinate fraction of the solvent pairs[6]. Molecular distillation is also one of the separation processes. This method is based on the molecular weight difference of the materials to be separated in the relatively low temperature and high vacuum condition[5,8]. In this condition, tocopherols are protected from degradation. On the other hand, in order to make such a high vacuum condition a special equipment must be used. In this research, a molecular distillation process was studied and compared with other processes in order to establish an adequate process for the separation of tocopherols and sterols from the soy oil scum.

Meterials and Methods

Meterials Scum obtained from soy oil refinery of Cheil Sugar Co., Inchon was used as starting material. The standard materials for tocopherols (α-, β-, γ-form), sterols (stigmasterol, β-sitosterol), naphthalene, and fatty acids (oleic acid, stearic acid) and glycerides (mono-, di-, tri-olein) were obtained from Supelco, ICN, Kasei, Sigma, respectively. Other reagents were purchased from Kanto, Waco, Hayashi, Junsei. GPC column packing material (Sephadex LH-20) was obtained from Sigma. The solvents for HPLC were of chromatographic grade (Burdick & Jackson, USA), and all other solvents, extra-pure or analytical grade, were purchased from Kanto, Waco, Kasei and Junsei.

Equipment Fig. 1 shows the molecular distillation system used in this study. By operating the mechanical vacuum pump (Duo-Seal Vacuum Pump, Sargent-Welch, Skokie, IL, USA) the pressure in the vacuum line was reduced to 1×10^{-3} torr, and by operating both mechanical and oil diffusion pumps, the pressure was reduced to 1×10^{-5} torr. This oil diffusion pump is of two stage type made by a glass blowing laboratory at Korea Advanced

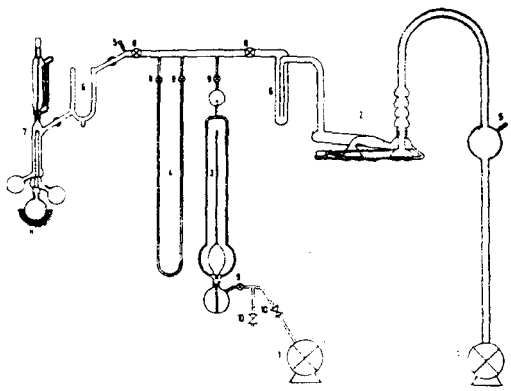

Fig. 1. Vacuum line and molecular distillation apparatus
1. Mechanical vacuum pump; 2. 2-stage oil diffusion vacuum pump; 3. Mc-Leod vacuum gauge; 4. U-manometer; 5. Vacuum release apparatus; 6. Trap; 7. Molecular distillation apparatus; 8. Vacuum cock, 6 *mm*; 9. Vacuum cock, 2 *mm*; 10. Needle valve

Institute of Science and Technology (KAIST). Mc-Leod gauge and other glass parts in this system were also made by the glass blowing laboratory. Molecular distillation apparatus (a falling film type, K-285600) was purchased from Kontes (Evanston, IL, USA). Its capacity is 60 *ml* and the distance between heater and condenser is 6 *mm*.

Analytical methods Tocopherols, sterols and free fatty acids were analyzed by spectrophotometry[9,10], titration[11], and HPLC[12~14]. HPLC was performed withWaters Associates ALC/GPC-244 equippedwith R 401 detector and μ-Porasil column. The solvent system of HPLC was 5% (v/v) diethyl eth er inhexane, flow rate was 2 *ml*/min and detector w as fixed at 280 *nm*. Beckman Spectrophotometer Mo del 20 was used for the measurement of absorbancy. RC-5 superspeed refrigerated centrifuge (Sorvall) was employed for the sterol crystal separation.

Separation methods of tocopherols and sterols from scum Separations by solvent extraction and by chemical treatment were based on Brown's[6] and Smith's methods[1], respectively. In the molecular distillation method[5], the scum was degassed before the first molecular distillation step. In order to determine the condition of the first molecular distillation step, temperature control was performed

at relatively low vacuum ($3\sim7\times10^{-3}$ torr). After the first distillation under predetermined condition, distilland of the first step was subjected to the second distillation step. In the second molecular distillation step, a higher vacuum condition was required. Therefore, the oil diffusion pump was operated in addition to the mechanical pump. To determine the conditions of the second molecular distillation, temperature was also controlled at a high vacuum (4×10^{-4} torr). Distillate of the second molecular distillation step was separated into the sterol crystals and tocopherol concentrate by crystallizing the sterols.

Sparation of tocopherols by GPC A sample containing 14.1% tocopherols was loaded on the GPC column. The packing material of the GPC column was Sephadex LH-20. The eluting solvent was 1,2-dichloroethanol, and the working temperature was 35°C[15].

Results and Discussion

Analysis of total tocopherols, sterols and free fatty acids

Total tocopherols, sterols and free fatty acids of the scum used as a starting material were 7.79%, 27.9% and 27.0%, respectively.

Separation of tocopherols and sterols from scum

1. Determination of the molecular distillation condition

In the first distillation step, the pressure was $3\sim7\times10^{-3}$ torr and the temperature range was 100~140°C. As shown in Fig. 2, approximately 90% free fatty acids contained in the scum was removed at temperature 131.5°C, but at this condition 13.4% tocopherols and 9.4% sterols in the scum were also distilled off. Therefore, these were considered as a loss in the first distillation step. The first distillation was performed at 131.5°C and distilland was used as a feed to the second distillation. In the second distillation step, the pressure was 4×10^{-4} torr and the temperature range was 100~200°C[16]. As shown in Fig. 3, the maximum yields of tocopherols and sterols were obtained at 177°C. In the sterol crystallization step, distilland of second distillation step was also subjected to the sterol crystallization because the amount of sterols in the distilland was larger than in the distillate.

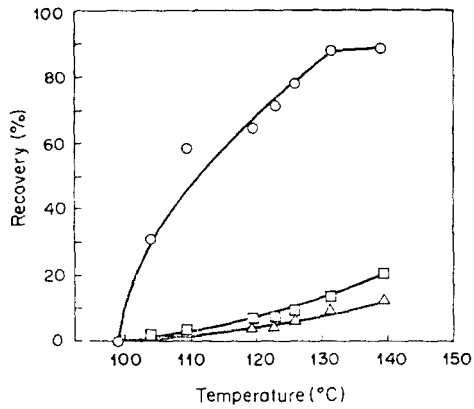

Fig. 2. Recovery of free fatty acids (○), tocophers (□) and sterols (△) in the distillate of first molecular distillation

$$\text{Recovery } (\%) = \frac{\text{FFA or tocopherols or sterols in distillate}(g)}{\text{FFA or tocopherols or sterols in scum}(g)} \times 100$$

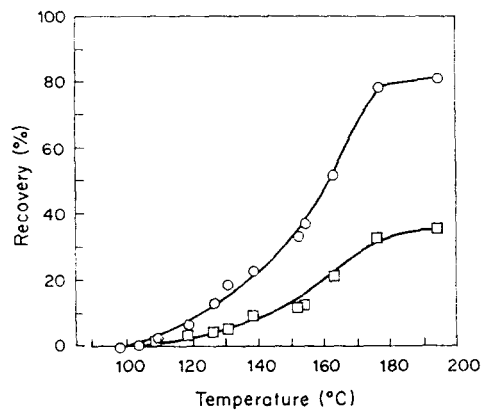

Fig. 3. Recovery of tocopherols (○) and sterols (□) in the distillate of second molecular distillation

$$\text{Recovery } (\%) = \frac{\text{Tocopherols or sterols in distillate}(g)}{\text{Tocopherols or sterols in first distillated oil}(g)} \times 100$$

2. Comparison of the separation processes

The purity and yield of tocopherols and sterols of the end products from the three separation processes are shown in Table 1. In the sterol crystallization step, after the first centrifugation, volume of the supernatant was reduced by solvent evaporation and sterols were found to be crystallized again, and at on ce. Therefore, further crystalliza-

Table 1. Purity and yield of tocopherol and sterol of the end products

Separation process	Purity(%)		Yield(%)	
	Tocopherol	Sterol	Tocopherol*	Sterol**
Solvent extraction	21.2	69.2	28.3	2.6
Chemical treatment	11.8	85.1	76.4	34.3
Molecular distillation	45.0	49.3	68.0	57.0

$$* \text{ Tocopherol yield}(\%) = \frac{\text{Tocopherols in tocopherol concentrate}(g)}{\text{Tocopherols in scum}(g)} \times 100$$

$$** \text{Sterol yield}(\%) = \frac{\text{Sterols in sterol crystals}(g)}{\text{Sterols in scum}(g)} \times 100$$

tion of the supernatant would increase the yield of sterols, and the purity of the sterol crystals could be increased easily by recrystallization[1,6]. However, selection of the separation method should be based on yield and purity of tocopherols of the end products. In the solvent extraction method, a considerable amount of tocopherols and sterols migrated into raffinate portion of the first and second solvent extraction steps, thus resulting in the reduction of the yield of tocopherols and sterols. While tocopherol yield of the chemical treatment process was high, purity of the tocopherol concentrate was very low. On the other hand, the tocopherol yield and the purity of the tocopherol concentrates from the molecular distillation process were both high. In the molecular distillation process, tocopherol and sterol mixture was obtained by only three steps including a degassing step. Furthermore, while the color of the tocopherol concentrates from the molecular distillation was yellow, those of the tocopherol concentrates from the chemical treatment and the solvent extraction process were dark brown and redish yellow, respectively. Sterol crystals were obtained as white powder in all three cases. Consequently, in the separation of tocopherols and sterols from the scum, the molecular distillation method was found to be more efficient than any other method tested in this study. HPLC was used for comparing the processing effect on the composition of tocopherol isomers. Because of the unavailability of δ-tocopherol, E peak in the HPLC chromatogram (Fig. 4) was not identified, but E peak appears to correspond to tocopherol[11]. From

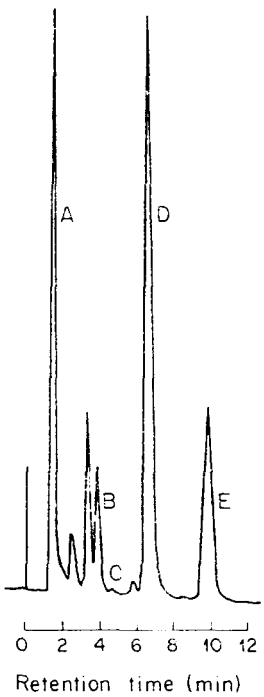

Fig. 4. HPLC chromatogram of tocopherol concentration from molecular distillation
Column, μ-Porasil; solvent, 5% (v/v) diethyl ether in haxane; flow rate, 2 *ml/min*; detector, UV 280 *nm*; Peak: (A) naphthalene, (B) α-tocopherol, (C) β-tocopherol, (D) γ-tocopherol, (E) δ-tocopherol

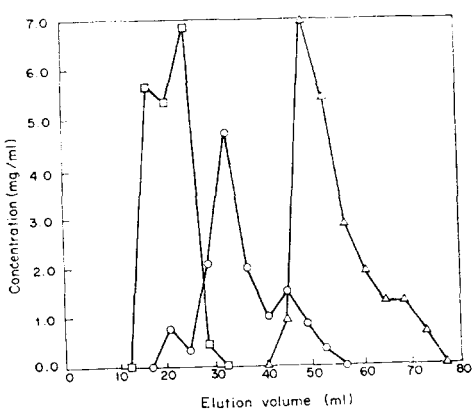

Fig. 5. GPC chromatogram
Peak: (□) sterol, (○) tocopherol, (△) free fatty acid. Sample: A mixture of intermediates from molecular distillation process

the result of HPLC, it could be said that the effect of processing on the tocopherol composition was negligible.

Separation of tocopherols by GPC

The yield and purity of tocopherols in GPC were 83.2% and 43.8%. As shown in Fig. 5, tocopherols were relatively easily separated from sterols and free fatty acids. Therefore, it is possible to upgrade the purity of the tocopherol concentrate, the end product of the molecular distillation process, by GPC.

요 약

대두유의 탈취 증류분으로 부터 토코페롤과 스테롤을 분리해내기 위하여 분자 증류법, 용매추출법, 화학처리법 등을 이용하였으며 최종 산물로 토코페롤 농축물과 스테롤 결정을 얻었다.

용매추출법의 경우, 얻어진 토코페롤의 순도와 회수율은 각각 21.2%와 28.3%였으며 스테롤의 순도와 회수율은 각각 69.2%와 2.6%였다. 화학처리법의 경우, 토코페롤의 순도와 회수율은 11.8%와 76.4%였으며 스테롤의 순도와 회수율은 85.1%와 34.3%였다. 한편 분자증류법의 경우 토코페롤의 순도와 회수율은 45%와 68%였으며 스테롤의 순도와 회수율은 각각 49.3%와 57%로 나타났다. 또한 얻어진 토코페롤 농축물을 HPLC로 비교하였다.

이 연구의 결과로 분자증류법이 실험한 다른 방법보다 더 효과적임을 알았다.

Acknowledgement

One of the authors (JSR) wishes to express his appreciation to Korean Traders Scholarship Foundation for their financial support.

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