

Concentrating Alpha-tocopherol from Oil Byproduct with Supercritical Methanol and CO₂

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ABSTRACT

Oil deodorizer distillate (DOD) is a kind of byproduct from soybean oil refining process and is rich in high-value compounds, such as natural tocopherols (vitamin E) and sterols. Generally, DOD is a very complex system. To simplify the composition of DOD, methyl esterification and methanolysis are the necessary pretreatment processes, converting most of fatty acids and glycerides into fatty acid methyl esters (FAMES) and releasing some sterols from sterol esters. However, the two reactions of esterification and methanolysis are separately carried out because of catalyst difference. Such processes cause many problems, for instance high energy consumption, long period time and the wasted water. Consequently, we attempted to employ supercritical methanol as the reactive agents without any catalyst. Simultaneously, the possibility of tocopherols' alpha conversion was specially discussed. Additionally, the novel process was compared with conventional pretreatment in terms of the contents of target compounds.

1. INTRODUCTION

The byproduct of soybean oil industry, de-odorizer distillates (DOD) can be used to produce high-value natural products, tocopherols (vitamin E) and sterols. Generally, the producing process of natural tocopherols is composed of two steps: pretreatment and concentration.

The goal of pretreatment is to simplify the composition of DOD. Methyl esterification and methanolysis are two important reactions, as shown in **Figure 1**.

The former converts most of free fatty acids (FFA) into fatty acid methyl esters (FAMES) and the latter converts most of glycerides and sterol esters into FAMES and sterols, respectively. After such pretreatment, the product is called MEDOD (methyl esterified DOD), which mainly contains FAMES, tocopherols, sterols and other impurities. In the course of pretreatment, sulfuric acid and sodium methoxide are applied for esterification and methanolysis, respectively. Such processes cause many problems, for instance high energy consumption, long period time and the wasted water. Consequently, it is very interesting whether the two reactions could be completed during one process. In this work, we tried to use supercritical methanol (SC-MeOH) as the reactive agents without any catalyst since SC-MeOH can simultaneously react with FFA (methyl esterification) and glycerides (methanolysis) into FAMES [1-3]. Additionally, there is a possibility for alpha conversion of β -, γ -, δ -tocopherols into α -tocopherol [4]. We attempted to use SC-MeOH to treat DOD and compared the novel method with conventional pretreatment.

2. MATERIALS AND METHOD

2.1 Materials DOD was supplied by Kaidi Fine Chemical Industries Co. Ltd, P. R. China. Methanol, methyl palmitate, methyl linoleate, methyl oleate, α -tocopherol, campesterol, stigmasterol and β -sitosterol were obtained from Wako Pure Chemical Industries Inc. Japan. MEDOD was prepared from DOD according to the process shown in **Figure 1**.

2.2 Apparatus and procedure An experimental apparatus [5] was used for the process of SC-MeOH (Akico Co., Tokyo, Japan). In brief, the apparatus consists of a batch reactor (about 8.8 ML inner volume) and a heating furnace.

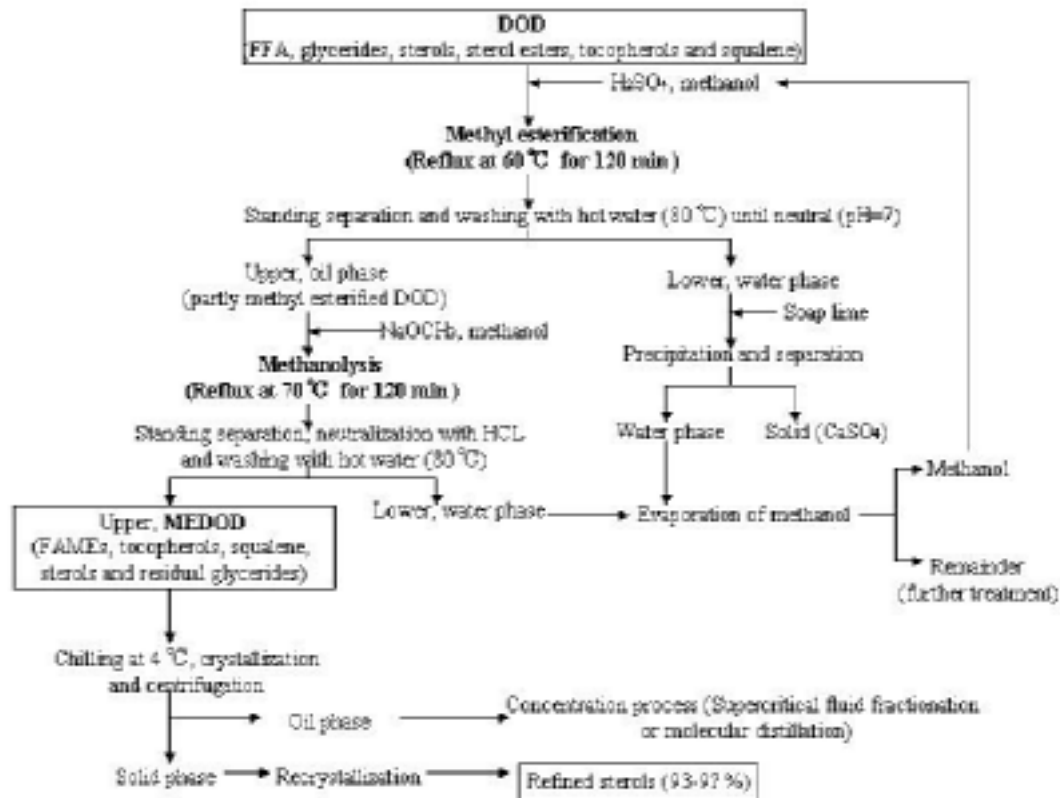


Figure 1. Conventional pretreatment of DOD

2.3 Analysis methods

2.3.1 HPLC analysis. Analysis of tocopherol was performed by HPLC [6].

2.3.2 GC-MS analysis. The samples were analyzed with GC-MS (Hewlett Packard-5890 series), coupled with a mass selective detector (HP 5972). The column used was a HP-5MS phenyl methyl siloxane capillary (30 m×0.25 mm i.d., film thickness 0.25 μm). The GC conditions were: oven temperature programmed from 150 ° to 180 ° at 10 °/min, to 200 ° at 3 °/min, to 265 ° at 10 °/min and then to 300 ° at 3 °/min, finally at 300 ° for 5 min, injector temperature 300 °. As for the main components, methyl palmitate, methyl linoleate, methyl oleate, α -tocopherol, campesterol, stigmasterol and β -sitosterol were identified by comparison of mass spectra and retention time with those of pure standards. For other components, the probability-based matching (PBM) algorithm was employed for finding the most probable match in the reference library (NIST library of mass spectra and subsets, HPG 1033A).

2.3.3 GC-FID analysis. The contents of FAMEs and sterols were determined with GC-FID, which was operated with the similar temperature program to that of GC-MS. First of all, through analyzing MEDOD, GC-MS was used for identifying every peak's order and retention time of every FAME and sterol, and then GC-FID was employed for determining the contents of these

compounds in samples, with methyl oleate and β -sitosterol as the external standards for FAMES and sterols, respectively.

3. RESULTS AND DISCUSSION

3.1 Composition of DOD and MEDOD. As a kind of byproduct from oil industry, DOD is relatively complicate system, mainly containing free fatty acid (FFA), glycerides (Gly.), tocopherols and sterols, as shown in **Table 1**. According to AOCS methods [7], the approximate contents of FFA and glycerides were calculated from acid and saponification values (A.V. and S.V.), and expressed as the contents of oleic acid and triolein.

Table 1. Characteristics of DOD

A.V. (mg KOH/g)	S.V. (mg KOH/g)	FFA (%)	Gly. (%)	Tocopherols (%)	Isomer percentage(%)			Sterols (%)	Isomer percentage (%)		
					α -	β + γ -	δ -		Campesterol	Stigmasterol	β -Sitosterol
92.2	166.6	46.6	29.1	7.7	13.1	60.2	36.7	9.6	33.6	23.6	43.0

According to the analysis of GC-MS, 27 compounds were identified MEDOD. The composition was 78.84 % fatty acid esters, 1.18 % squalene, 9.23 % tocopherols and 10.75 % sterols. The main FAMES were methyl palmitate (13.7 %), methyl linoleate (33.4 %) and methyl oleate (22.7 %) and the three compounds took 88.5 % of all fatty acid esters. With the analysis of HPLC and GC, the accurate contents of tocopherols, FAMES and sterols were 7.8 %, 67.3 % and 10.18 %, respectively.

As described above, the oil phase obtained with the conventional pretreatment was called as MEDOD, for convenience, that obtained with SC-MeOH process was called as NMEDOD (new MEDOD). In addition, after every run, the obtained NMEDOD was about 95-96 % weight of DOD, the amount was very close to that of MEDOD, as a result, the recovery difference can be analyzed by comparing the compound content in NMEDOD with that in MEDOD.

3.2 Influence of reaction temperature on target compounds' contents

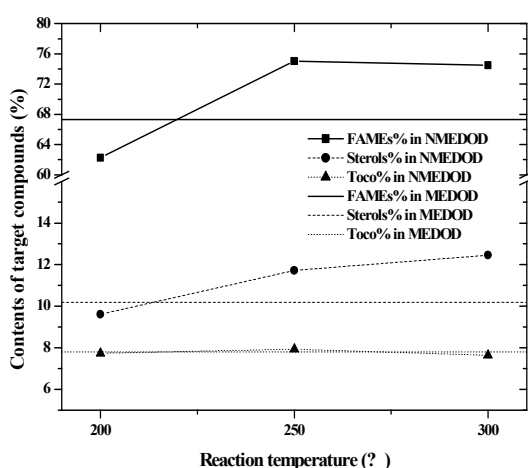


Figure 2. Influence of reaction temperature

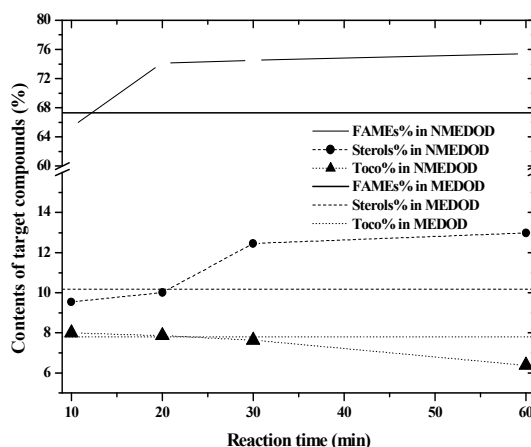


Figure 3. Influence of reaction time

3 mL DOD and 5 mL methanol was charged into the reactor, and then the reaction was carried out at different temperatures for 30 min. Totally, three temperatures were investigated and the result is shown in **Figure 2**. For comparison, three lines were drawn and represented the content levels of FAMES, sterols and tocopherols in MEDOD, respectively.

Obviously, the contents of FAMES and sterols were greatly influenced by temperature, as higher temperature led to higher contents, especially 250 and 300 °C resulted in higher contents in NMEDOD than those in MEDOD, indicating that more fatty acid (FA) in glycerides and more sterol esters were reacted with SC-MeOH. Different from FAMES and sterols, tocopherols were not influenced by temperature. Actually, such results were very interesting and meaningful. Most important was that the tocopherols in DOD were not damaged with SC-MeOH process and such process can be considered as a feasible choice for pretreatment. Additionally, compared with conventional pretreatment, SC-MeOH released more FAMES and sterols. It means less impurity and simpler composition in NMEDOD.

3.2 Influence of reaction time on target compounds' contents. Besides temperature, reaction time is another important parameter need to be investigated. Furthermore, some experiments were run for different period ranging from 10 to 60 min, other parameters were the temperature of 300 °C and the DOD to methanol (D/M) ratio of 3:5 (0.6). As shown in **Figure 3**, on one hand, the reaction result at 10 min was not as good as that of conventional pretreatment. On the other hand, the reaction result at 60 min, the longest period, led to the highest FAMES and sterols' contents, but the damage to tocopherols was very remarkable, just because of the damage, a reaction period longer than 30 min does not seem to be reasonable.

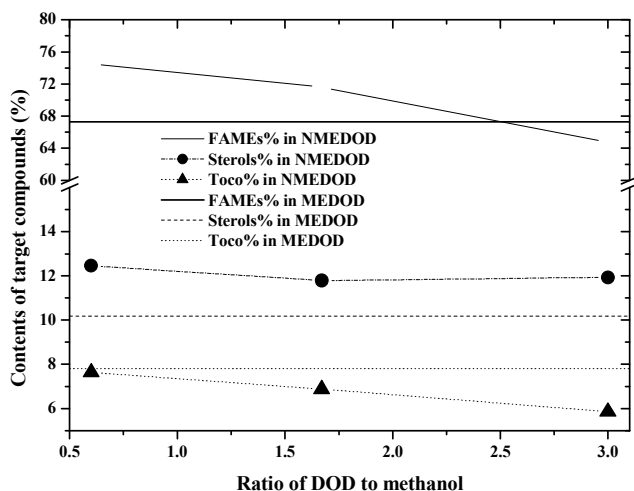


Figure 4. Influence of the DOD to methanol ratio

3.3 Influence of the DOD to methanol (D/M) ratio on target compounds. Other two D/M ratios of 5:3 (1.67) and 6:2 (3.0) were chosen for comparison. The reaction temperature and time were 300 °C and 30 min, respectively. The data shown in **Figure 4** indicated that less methanol passively influenced the reaction efficiency, resulting in more damage to tocopherols and less formations of FAMES and sterols. The possible reason is that less methanol leads to lower pressure,

which is probably an important factor influencing the process, consequently the reaction degree and efficiency were weakened. As for the decrease in tocopherols' content, we assumed that the sufficient existence of methanol could protect tocopherols, because molecular and vacuum distillations (>220□) were reported to be disadvantageous to tocopherols' recovery [8] because the polymerization of tocopherols was caused by high temperature. However, in our experiment, there was no remarkable damage of tocopherols' content during a relatively short reaction period (10-30 min) even though higher temperatures than that of distillation were employed. Perhaps, the process of distillation just puts tocopherol molecule in a hot and 'dry' surrounding, while the process of SC-MeOH provides a hot but 'wet' surrounding for tocopherols, there are many methanol molecules between the space of two tocopherol molecules, consequently the polymerization of tocopherols can be prevented at high temperature. Similarly, the decrease of tocopherols' content shown in **Figure 4** can also be explained by the hypothesis.

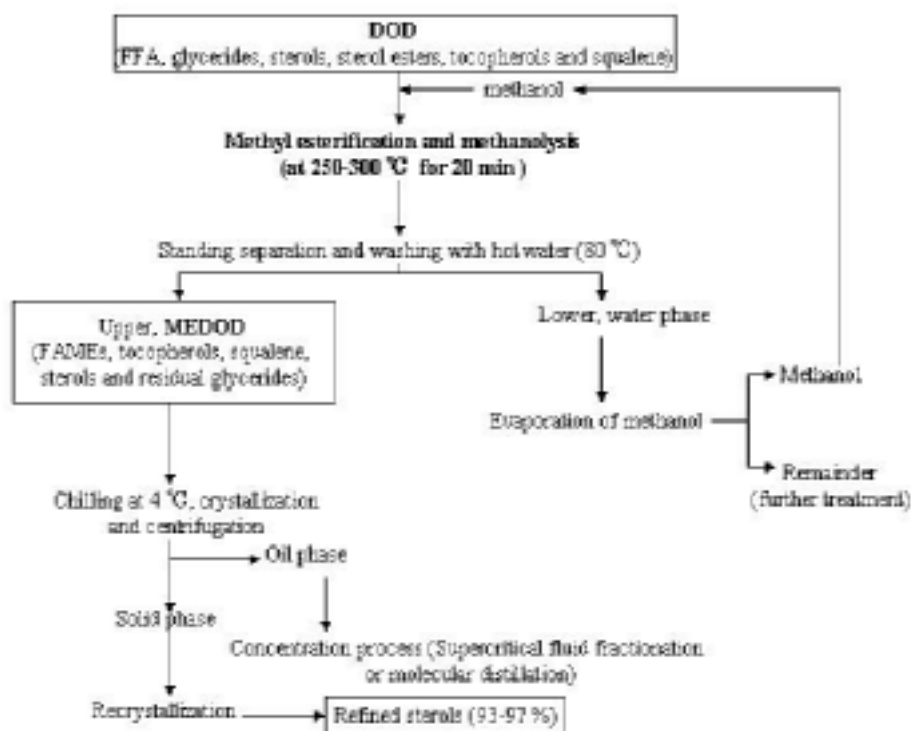


Figure 5. Schematic process of Sc-MeOH as the pretreatment method

3.4 Other phenomena during the process of SC-MeOH. The HPLC analysis also showed that the tocopherol isomers' proportion really changed at different conditions. The ratio between tocopherol isomers after reaction changed more or less. For example, at the reaction conditions of 300□, 30 min and the D/M ratio of 0.6, the α -tocopherol proportion was the highest, about 26.28 %, twice more than that in DOD. More obvious changes are a decrease in β -+ γ - isomers' percentages (in the range of 50.2 - 32.6) and an increase of δ -isomer percentage (in the range of 32.81 - 54.68). Compared with tocopherols, the composition changes of FAMES and sterols were slighter, almost

being the same proportions as those in MEDOD.

4. CONCLUSION

According to the obtained results, the process of SC-MeOH leads to more FAMES and sterols than those with conventional pretreatment, moreover there is no damage to tocopherols at high temperature (250 – 300 °C) for relatively long period (20-30 min) and with sufficient existence of methanol (5/3 v/v DOD). In addition, partial conversion of tocopherol isomers takes place in the process. On the basis of our experiments, a schematic process of SC-MeOH was put forward, as shown in **Figure 5**. Undoubtedly, the novel process seems to be simpler and more effective than the conventional pretreatment shown in **Figure 1**. Especially, the water amount for washing is far smaller than that of conventional method, and the wasted water is almost neutral and easier to be treated than that from conventional pretreatment.

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