Extraction of Tocopherol from Soybean Oil Deodorizer Distillate by Deep Eutectic Solvents

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Abstract: Natural tocopherols have strong antioxidant and physiological functions, which are mainly produced from vegetable oil deodorized distillates. In this work, a simple and green solvent extraction method based on deep eutectic solvent has been developed to simultaneously extract three isomers of tocopherols (α, γ and δ-tocopherols) from soybean oil deodorizer distillate (SODD). The key factor to affect the solvent extraction efficiency was proposed that phenolic deep eutectic solvents interacted with targeted tocopherols mainly through π-π bonds interaction. This sustainable extraction process included two steps. Firstly, total tocopherols were extracted from SODD at room temperature by phenolic deep eutectic solvent composed of ChCl and p-cresol. Subsequently, tocopherols were successfully separated from deep eutectic solvent phase by re-extraction with n-hexane, and tocopherols products could be simply recovered. Under the optimum extraction conditions, the extraction efficiency of total tocopherols (α, γ and δ-tocopherols) from SODD was 77.6% after extraction with phenolic deep eutectic solvent.

Key words: deep eutectic solvents, extraction, tocopherol, soybean oil deodorizer distillate

1 Introduction

The vegetable oil deodorizer distillate is a by-product obtained in the deodorization process of vegetable oils, which contains abundant beneficial ingredients such as natural tocopherol, sterol, and squalene[1]. Therefore, the deodorizer distillate is the main raw material for the production of natural tocopherols, sterols and so on. Tocopherol contains four isomers, named α, β, γ, and δ-tocopherol, and a mixture of α, γ, and δ-tocopherol is widely found in vegetable oils[2]. As an active substance with strong antioxidant and physiological function, tocopherols have attracted much more attention in many fields, such as food, pharmacy and cosmetics[3-6].

In order to obtain more natural tocopherols, the natural tocopherols obtained from the deodorizer distillate have been extensively studied. Nowadays, the main industrial method of recovering tocopherol from deodorizer distillate is molecular distillation[7, 8]. Although molecular distillation can obtain tocopherol with higher purity, it has low separation efficiency and harsh operating conditions such as high temperature and high vacuum. As an alternative, the extraction of tocopherol by supercritical fluids is a hot research topic. Supercritical CO\textsubscript{2} has the characteristics of non-toxic and non-corrosive, and the obtained tocopherol has the advantages of good quality and high purity. However, supercritical CO\textsubscript{2} requires specialized and expensive equipment. And the supercritical methanol is also used to pretreatment of the soybean oil deodorizer distillate (SODD) for obtaining concentrated tocopherols[9]. Compared with the conventional pretreatment methods, supercritical methanol has less methanol consumption and less pollution, but further processing is required to obtain tocopherol products. In addition, the solvents extraction and ion-exchange resin[10] have been also reported to purify natural tocopherols. Notably, almost all of the above methods need pretreatment of the deodorizer distillate, that is, esterification of free fatty acids with methanol to reduce the boiling point of free fatty acids and the viscosity of the whole system. These techniques are easy to operate, but they consume a large amount of organic reagents (e.g., methanol). Therefore, it is necessary to find an environmentally friendly extraction solvent which can effectively extract tocopherols from deodorizer distillate (e.g., SODD).

At present, an emerging solvents called deep eutectic solvent (DES) as green solvent has received great attention due to its unique physicochemical properties such as incombustibility, biodegradability, non-toxic, low melting point and volatility[16, 17]. As a general rule, DES is a uniform
liquid obtained by simply mixing a quaternary ammonium salt and a hydrogen bond donor (HBD) under a mild temperature condition (60-80°C), which can be directly used without further purification. Most DESs are inexpensive and environmentally friendly because the compositions of DESs are widely sourced and inexpensive such as choline chloride (ChCl), an inexpensive, non-toxic, biodegradable quaternary ammonium salt. In addition, many HBDs are available from nature, such as urea, carboxylic acids, and polyols. Another attraction of DES is that it is a tunable reagent, and researchers can adjust its performance by choosing right composition and proper ratio of each component. Considering these characteristics, DESs can be applied in many fields such as separation, extraction, catalysis, organic synthesis, and electrochemistry. However, the extraction of natural tocopherol from deodorized distillates using deep eutectic solvents as extractants have not been extensively studied. The extraction of tocols from crude palm oil using choline-based DESs was proposed in a recent report.

In this work, we have synthesized a series of choline-based DESs and applied them into tocopherol extraction from soybean oil deodorizer distillate (SODD). What’s more, when we chose phenols as hydrogen bond donors, and choline chloride as a hydrogen bond acceptor to afford a phenol-based DES, the three isomers of tocopherols could be simultaneously extracted from SODD. The merit of tocopherols extraction based on DESs is time saving and reducing the usage of toxic and volatile conventional solvents.

2 Experimental

2.1 Material

The soybean oil deodorizer distillate (SODD) used in this study was provided by Yihai Kerry Group (Yihai Kerry, Qinhuangdao, China). α-Tocopherol, γ-tocopherol and δ-tocopherol were purchased from Sigma-Aldrich (Sigma Aldrich, Shanghai, China). Choline chloride, m-cresol, o-cresol and malonic acid were purchased from Macklin (Macklin, Shanghai, China). Phenol and p-cresol were purchased from Sinopharm Chemical Reagent Co., Ltd (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Ethylene glycol, glycerol, n-hexane and citric acid were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin Kemiou Chemical Reagent Co., Ltd, Tianjin, China). Acetic acid and methanol used were of HPLC grade, and other chemicals were of analytical reagent grade. Besides, the experimental water was redistilled.

2.2 Preparation of deep eutectic solvents (DESs)

The choline chloride and hydrogen bond donors (HBD) were accurately weighed in an appropriate molar ratio, and the mixture was heated and stirred at 60°C until a transparent, uniform and liquefied DES was formed. The types of DESs prepared in this experiment were showed in Table 1, including their abbreviated names and the molar ratio of components.

2.3 Extraction of tocopherols from SODD by DESs

A certain amount of soybean oil deodorizer distillate (SODD) was accurately weighed in a test tube, and then a certain volume of DESs were added in the test tube (Fig. 1). The mixture was treated with vortex-assisted extraction for 5 min and then centrifuged at 6000 rpm for 3 min until two phases was obtained. Then 200 μL of DES-rich phase was transferred to a 2 mL volumetric flask with methanol. The solution was filtered through a 0.45 μm microporous filter for high performance liquid chromatography (HPLC) analysis of tocopherols.

2.4 Recovery of tocopherols from DES extracts

A certain amount of lower DESs phase was added in a test tube, and followed by the addition of n-hexane or water (Fig. 1). The mixture solution was treated with vortex-assisted anti-extraction for 5 min, and centrifuged to obtain two phases. 200 μL lower DES phase was transferred to a 2 mL volumetric flask with methanol. After fil-

<table>
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<th>Abbreviation</th>
<th>HBA</th>
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<th>Molar ratio</th>
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<tr>
<td>DES-1</td>
<td>Choline chloride</td>
<td>Acetic acid</td>
<td>1:1</td>
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<tr>
<td>DES-2</td>
<td>Choline chloride</td>
<td>Malonic acid</td>
<td>1:2</td>
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<td>DES-3</td>
<td>Choline chloride</td>
<td>Ethylene glycol</td>
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<td>Choline chloride</td>
<td>Glycerol</td>
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<td>Choline chloride</td>
<td>Phenol</td>
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<td>Choline chloride</td>
<td>α-Cresol</td>
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<td>Choline chloride</td>
<td>m-Cresol</td>
<td>1:2</td>
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<tr>
<td>DES-8</td>
<td>Choline chloride</td>
<td>p-Cresol</td>
<td>1:2</td>
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2.5 HPLC-UV analysis

The three isomers of tocopherols were quantified using a Waters e2695 HPLC analysis system with a C18 reversed-phase column (4.6×250 mm×5 μm, Waters SymmetryShield™ RP18, Waters, Shanghai, China). The system was equipped with a UV detector (Waters 2489). The mobile phases were methanol (A) and 0.5% (v/v) acetic acid aqueous solution (B). A gradient elution with the flow rate of 0.8 mL/min was performed as follows: 0-13 min, 96%A; 13-20 min, 96%-100%A; 20-24 min, 100%A; 24-24.5 min, 100%-96%A; and 24.5-30 min, 96%A. The injection volume was 10 μL; the column temperature was set at 35 °C; the tocopherols was quantified at wavelength of 294 nm.

The specific formulas for calculating the concentration of individual tocopherol in SODD were as follows:

\[ C_i = \frac{1}{G} \times A_i \times R_i \times 2 \]

where \( C_i \) = concentration of individual tocopherol (g/kg), \( G \) = response factor from the linear regression of the linearcu, \( A_i \) = area of sample, \( 2 \) = volume, and \( R_i \) = enrichment factor. Enrichment factor was defined as the weight of sample divided by volume of extraction solvents:

\[ R_i = \frac{V_{DES}}{200 \times m_i} \]

where \( V_{DES} \) = volume of DES-rich phase (μL), 200 = partial volume of DES-rich phase was injected into the HPLC system, \( m_i \) = the weight of sample (g).

In order to obtain higher purity tocopherol, it was necessary to carry out re-extraction of tocopherols from the DES phase. The re-extraction efficiency was calculated using the following formula:

\[ \text{Re-extraction efficiency} (\%) = \frac{M_{total} - M_{remain}}{M_{total}} \times 100\% \]

where \( M_{total} \) and \( M_{remain} \) were the amount of the total tocopherol and the remaining tocopherol.

All results are expressed as mean ± standard deviation (SD). Data evaluation was performed using one-way analysis of variance (ANOVA) and Duncan for comparisons between groups using Statistical Product and Service Solutions (SPSS). \( P<0.05 \) was considered to be statistically significant.

3 Results and Discussion

3.1 Optimization of the factors to affect the extraction efficiency

3.1.1 Extraction method

Different extraction methods will produce different contents of tocopherol from SODD. At present, the methods commonly used in liquid-liquid extraction mainly include vortex-assisted extraction, ultrasonic-assisted extraction and so on. In order to extract more amount of tocopherols (α, γ and δ-tocopherols) through DESs (choline chloride/\( p \)-cresol), two assisted extraction methods were compared in this work. As shown in Fig. 2, the concentration of vortex-assisted extraction (87.1 g/kg total tocopherols) was better than that of ultrasonic-assisted extraction (47.4 g/kg total tocopherols) under the same extraction time (5 min). This was because the vortex could fully mix the two phases in a shorter time, and the contact area between SODD and DES phases was increased, which was beneficial to the extraction of tocopherols (α, γ and δ-tocopherols) simultaneously by DESs. However, due to the high viscosity of SODD and DESs, ultrasonic-assisted extraction showed lower efficiency for mixing of the two phases. Thus, the vortex-as-
sisted extraction based on DESs was selected in the following experiments.

3.1.2 Extraction solvents

The extraction solvent plays an important role in affecting the extraction efficiency of tocopherols. In this study, ChCl-based deep eutectic solvents were synthesized by combining ChCl with different HBDs (Table 1), which were considered as none or lower toxic compounds in food or chemical industry. As shown in Fig. 3, eight different deep eutectic solvents (DES-1~DES-8) were used to extract three tocopherols from SODD. As for acid-based DESs (DES-1:ChCl-acetic acid, DES-2:ChCl-malonic acid) and polyols-based DESs (DES-3:ChCl-ethylene glycol, DES-4:ChCl-glycerol), the extraction concentration of total tocopherols (α, γ, and δ-tocopherol) (0.2~3.8 g/kg) were both lower than phenol-based DESs (DES-5~DES-8) (7.4~87.1 g/kg total tocopherols). Therefore, it could be concluded that the acid-based DESs and the polyols-based DESs was not suitable for the tocopherol extraction from SODD. In fact, acid-based DESs and polyols-based DESs were more suitable for extracting organic compounds with large polarity, while phenol-based DESs were suitable for extracting low-polar aromatic compounds.

Among all the tested phenols-based deep eutectic solvents, ChCl- p-cresol (DES-8) showed the highest extraction concentration of total tocopherols (87.1 g/kg). We speculated that the extraction principle of DES-8 might be the principle of similar compatibility, or that phenol-based DESs could form π-π bonds with phenolic substances. And electron-rich phenol-based DESs (DES-6, DES-7, DES-8) showed higher extraction concentration of total tocopherols than that of DES-5 (ChCl-phenol). Thus DES-8 can simultaneously extract three tocopherols from SODD through high π-π interaction (Fig. 4). For comparison, commonly used organic solvents, such as methanol (CH₃OH), showed much lower extraction concentration of total tocopherols (59.4 g/kg) from SODD than DES-8 under the same extraction condition. Therefore, phenol-based DES composed of choline chloride and p-cresol (DES-8) was selected as the suitable DES in the following experiments.

3.1.3 The ratio of DES to SODD

The amount of DES-8 affected the extraction content of tocopherols from the SODD. Thus, the ratio of DES to SODD was evaluated in the range from 4 mL/g to 10 mL/g while the extraction time was set as 5 min. As seen from Fig. 5, the tocopherol concentration increased when the ratio of DES-8 to SODD was increased from 4 mL/g to 6 mL/g (from 66.3 g/kg to 92.4 g/kg total tocopherols), and then changed slightly over 6 mL/g. With the increase of the ratio of DES-8 to SODD, the extraction process could reach equilibrium, but the further increase of the amount of DES-8 would lead to the waste of extraction solvent. Thus, 6 mL/g was selected as the optimal DES-8 to SODD ratio.

3.1.4 Extraction time

Extraction time would directly affect the extraction effect of total tocopherols from SODD. To obtain the ap-
appropriate extraction time, the extraction time was studied in the range from 0.25 min to 5.0 min. As shown in Fig. 6, the total tocopherols concentration (from 88.2 g/kg to 93.2 g/kg) was increased with the increasing of extraction time (0.25 min to 0.50 min). However, further prolonging the extraction time had no significant effect on the improvement of extraction efficiency of the three tocopherols. Therefore, 0.5 min was selected as the optimized extraction time.

3.1.5 Extraction temperature

As we all know, temperature has significant effect on the viscosity and solubility of the extraction solvent. Thus, the effect of the extraction temperature on tocopherols was studied (Fig. 7). However, the results showed that the extraction temperature (25°C, 40°C, 55°C) had no significant effect on the content of three tocopherols due to the high efficiency of vortex-assisted extraction, which afforded total tocopherols ranged from 103.6 g/kg to 105.5 g/kg. Therefore, 25°C (room temperature) was chosen as the optimized extraction temperature.

Under the optimized extraction conditions, the content of total tocopherols in SODD could be decreased from 133.5 g/kg to 103.6 g/kg (Table 2). The extraction efficiencies of three tocopherols (α, γ, and δ-tocopherol) were 70.7% ~ 80.4%. And the extraction efficiency of total tocopherols was 77.6% after extraction with DES. With extraction for two times, the extraction efficiency of total tocopherols could be increased further.

3.2 Re-extraction process for tocopherols

3.2.1 Effect of the amount of n-hexane

Based on above optimized conditions, three tocopherols could be extracted from SODD and concentrated in DES phase. In order to afford the natural tocopherols product, it is necessary to separate the three tocopherols from the DES phase. DESs have the physicochemical properties of low melting point and volatility, which was unsuitable to be removed through conventional evaporation at reduced pressure. In this work, n-hexane was used as a good anti-solvent for separating three tocopherols from the DES phase. Thus, the amount of n-hexane in re-extraction from DES phase was investigated in this work. Figure 8 showed that the re-extraction efficiency of total tocopherols was increased rapidly when the volume ratio of n-hexane to DES-8 was increased from 2:1 to 6:1. The extraction efficiencies of α-tocopherol, γ-tocopherol and δ-tocopherol were increased from 91.0% to 100%, 73.4% to 90.2%, and 56.7% to 78.1%, respectively. With the volume ratio of n-hexane to DES-8 increasing, the re-extraction efficiency was changed slightly. Considering the cost of anti-solvent,
3.2.2 Effect of the water dosage

As we all know, DES is a uniform liquid through formation of hydrogen bond between the components. And introducing a certain amount of water in the DES phase will decrease the hydrogen bond of each component (e.g., ChCl and p-cresol), which will affect the solubility of original DESs\(^{17}\). On the above results of using a small amount of \textit{n}-hexane as anti-solvent, the effect of water addition on the re-extraction efficiency was studied as well. Figure 9 showed that the re-extraction efficiency of total tocopherols was increased rapidly when the volume ratio of water to DES-8 was increased from 0:1 to 6:1. As for \(\gamma\) and \(\delta\)-tocopherols, their extraction efficiency were increased from 90.2\% to 98.6\% and from 78.1\% to 98.2\%, respectively. And the re-extraction efficiency showed no significant change when the volume ratio of water to DES-8 was over 6:1. Therefore, the volume ratio of water to DES-8 of 6:1 was chosen was the best water addition amount.

4 Conclusions

The deep eutectic solvents could be used as green solvents to simultaneously extract \(\alpha\), \(\gamma\) and \(\delta\)-tocopherols from the soybean oil deodorizer distillate (SODD). In the presence of the DES composed of choline chloride and p-cresol (mol/mol = 1:2), higher concentration of total tocopherols could be extracted by vortex-assisted extraction at room temperature (25\(^\circ\)C) for only 0.5 min. The key factor to affect the solvent extraction efficiency was proposed that phenolic deep eutectic solvents interacted with targeted tocopherols mainly through \(\pi-\pi\) bonds interaction. Furthermore, in order to get pure natural tocopherol products, \textit{n}-hexane and water were selected as the re-extraction solvents. And the ratio of \(\textit{n}\)-hexane to DES (6:1, v/v) and water to DES (6:1, v/v) led to near 100\% extraction efficiency of total tocopherols, which showed the optimized re-extraction recovery of \(\alpha\), \(\gamma\) and \(\delta\)-tocopherols were up to 100\%, 98.6\%, and 98.2\%, respectively. This developed extraction method based on DES will provide a simple and sustainable alternative to separate active natural compounds from vegetable oil deodorized distillates.

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