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Selective Separation of Tocol Homologues by Liquid-Liquid Extraction Using Choline-Based Deep Eutectic Solvents

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Abstract

In this paper we examined the potential of choline-based deep eutectic solvents (DESs) for selective extraction of tocol homologues from crude palm oil (CPO) through liquid-liquid extraction (LLE). Distribution of tocol homologues (α-tocopherol, α-, β, γ-, and δ-tocotrienol) presence in CPO when subjected to DES-assisted LLE has not been fully understood in the past. The effect of increasing the amount of DES on the distribution and selectivity of were investigated. It was found that tocol homologues were distributed in the order of their hydrophilic power, with tocols of higher polarity distributed more into the stripping phase compared to the less polar tocols. Distribution coefficients for α-tocopherol, α-, β, γ-, and δ-tocotrienol were 7.8, 13.1, 19.8, 22.1 and 29.6, respectively, when equal weight of CPO and choline chloride-malonic acid eutectic mixture (DES1) were used. The distribution of each tocol homologues also increased with increasing DES1, due to the increase in polarity of the stripping phase that attracted more tocols. Selectivity of δ-tocotrienol, which is the most polar tocol, was always higher than other homologues (α-tocopherol: 3.81, α-tocotrienol: 2.22, β-tocotrienol: 1.50, γ-tocotrienol: 1.34). Role of hydrophilic power of the tocols using selected DESs to selectively separate tocol homologues established in this paper has a potential for palm oil industry as tocotrienols are better antioxidants, thus more favorable, than α-tocopherol.

Keywords: Choline chloride, Deep eutectic solvent, Distribution coefficient, Oil palm tocol, Selective separation

Introduction

Vitamin E, also known as tocols (abbreviated from tocopherols and tocotrienols), is one of the antioxidants occurring naturally in vegetable oils [1]. Tocotrienols are the major composition of tocols in palm oil, with the remaining being α-tocopherol. Conventional high-end techniques are capable of concentrating palm tocols for nutritional capsules and dietary products formulations [2]. Studies also shown that tocotrienols have higher antioxidant effect than tocopherols [3]. Besides that, α-tocopherol may suppress the bioavailability of α-tocotrienol and is thus, less desired [4]. Therefore, it is important to find a new approach that can selectively recover the desired tocol homologues. Deep eutectic solvent (DES) showed promising role in removal of trace metals, purification of fuels and extraction of bioactive compounds [5-8].

DES is a new class of molten salts, substituting ionic liquids (IL), that has relatively low melting point, usually below 100 °C [9]. Like IL, DES has been the choice as alternative volatile organic solvents with wide selections of hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) [10]. DESs are gaining popularity as they are derived from renewable natural resources and are easy to prepare; usually by heating or grinding the 2 components until a homogenous liquid appears [11]. Biodegradable choline chloride salt is widely used as HBA in the development of DES with a variety of HBDs from natural resources, such as carboxylic acids, amides, alcohols and carbohydrates [9,12,13]. Abbott *et al*. (2004) recommended a DES formed between choline chloride and carboxylic acids as versatile alternative to conventional ILs [14]. The authors reported that a chloride ion (CL-) in choline chloride complexes with 2 carboxylic acid (O=C–OH) molecules to form a eutectic solvent as illustrated in **Figure 1(a)**.

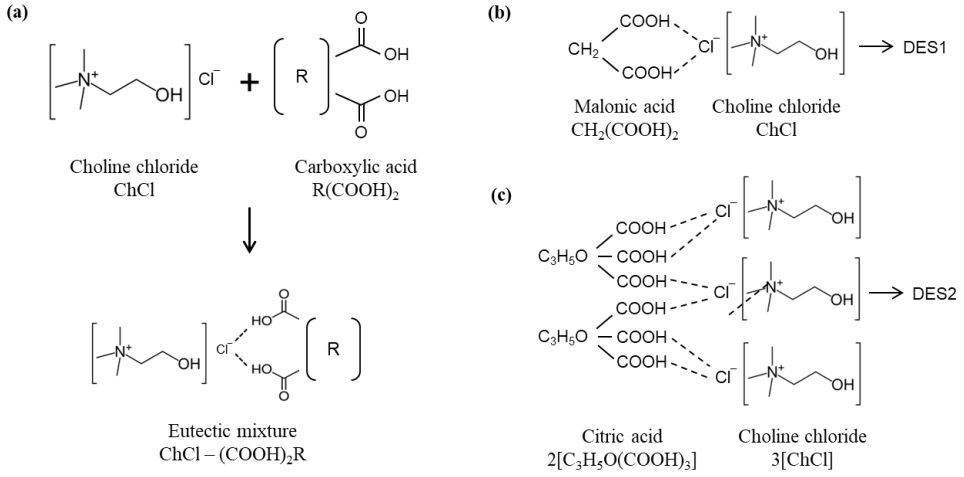

Figure 1 Schematic illustration for the (a) general development of eutectic mixture from choline chloride and carboxylic acid, (b) formation of DES1 using choline chloride and malonic acid and (c) formation of DES2 using choline chloride and citric acid.

Molten salts as extracting media in liquid-liquid extraction (LLE) can enhance the extraction ability and selectivity. Selective separations of synthetic tocopherols mixtures (α-, β-, γ- and δ-tocopherol) by LLE using imidazole-based ILs have been reported where ILs strongly interact with tocopherol homologues, resulting in specific distribution trend [15]. Similar experiments conducted by Mohammadi *et al*. (2021) also demonstrated selective separation of α-tocopherol using choline chloride-based DES in various solvents such as ethylene glycol, ethanolamine and sucrose; tocotrienols were not included [16]. In a separate study, the extraction of tocols (α-tocopherol and tocotrienols) from palm oil using imidazolium-based ILs and choline-based DESs doubled the concentration of tocols in the product compared to that in control without ILs [17]. However, the distribution and selectivity of tocols were not discussed [17].

LLE is one of the most common separation techniques in which 2 immiscible liquids are mixed to allow transportation of solutes between the 2 liquid phases. It has the advantage of quick separation due to 1 phase being in direct contact with the other. Separation using pure liquids, such as methanol-heptane LLE system, could be improved with addition of DES to increase the polarity of the methanol phase. In this study, malonic acid and citric acid, each with 2 and 3 –COOH functional groups, were used as HBDs (**Figure 1**). Hydrogen bond formed between the carboxyl groups with chloride ion creates strong hydrophilicity (polarity), thus might have influenced the extraction of tocols from palm oil as the tocols are weak-polar compounds.

We investigated the distribution and selectivity of palm tocol homologues (α-tocopherol, α-, β-, γ- and δ-tocotrienol) by using choline-based DESs via LLE. These distribution data enabled us to understand the "partitioning" behaviour of polar components (e.g., tocol homologues) into aqueous-like stripping phase and less polar components into immiscible organic (feeding) phase. This "partitioning" phenomenon is guided by the distribution coefficient. The distribution coefficient also shows the selectivity, in which a liquid-liquid separation system selectively separates 1 compound from another. This is the first-time selectivity of tocol homologues in palm oil towards selected DESs are being reported, the others usually experimented with only tocopherols mixtures prepared using pre-owned synthetic substances. Results from this study will serve as knowledge bridge in tocols separation mechanism in LLE and the prospects of LLE in the recovery of valuable phytonutrients from palm oil.

Materials and methods

Materials

Methanol (95 %) and hexane (95 %) were purchased from R&M (Essex, U.K). Ethyl acetate (99.8 %) and heptane (99 %) for analysis were of chromatographic grade and purchased from Merck (Darmstadt, Germany). α-Tocopherol concentrate (≥ 96%), citric acid (99 %), malonic acid (99 %) and choline chloride salt (98 %) and were purchased from Sigma Aldrich (St. Louis, USA). Crude palm oil (CPO) - which was used as extraction feed - was obtained from a local palm oil mill in Malaysia.

Synthesis of DESs

Choline chloride and malonic acid were mixed in a molar ratio of 1:1 to produce DES1. In a similar fashion, choline chloride and citric acid (1.5:1 molar ratio) were used to synthesize DES2. The mixtures were heated at 85 °C while stirring until a homogenous, clear liquid was formed. **Figures 1(b) - 1(c)** illustrate the formation of DESs based on their respective molar ratio which showed each chloride ion in choline chloride complexed with 2 carboxylic acid molecules as recommended by Abbott *et al*. (2004). Here, choline chloride is taken as salt i.e. hydrogen bond acceptor (HBA) and carboxylic acids as hydrogen bond donor (HBD).

Characterization of DESs

Viscosity (measured at 30 °C) and water content (measured at 25 °C) of the DESs were determined by rotational viscometer (Anton Paar DV-2 P) with an TL7 spindle and Karl Fischer titrator (Mettler Toledo V10S Volumetric KF Titrator), respectively.

Functional groups of the prepared DESs, HBDs (malonic acid and citric acid) and choline chloride salt were analysed at 25 °C using a Fourier-transform infrared spectroscopy (FTIR) spectrometer (PerkinElmer FTIR Frontier).

Solubility of selected diluents (hexane, heptane, ethanol and methanol) in DES was measured as follows: 0.5 mL (gradual increased to 10.5 mL) diluents were added into a calibrated test tube containing 100 mL DES and shaken at constant temperature (30 °C) [18]. Solubility was defined as the volume of diluents dissolved in 100 mL of DES. These values were obtained based on visual evaluation. Solubility below 0.5/100 mL DES and above 100/100 mL DES is regarded as "immiscible" and "miscible", respectively.

Liquid-liquid extraction (LLE) experimental manipulation

LLE was performed by creating 2-immiscible-phase liquid system to keep DES and CPO in contact but separated throughout the extraction. CPO was heated to 60 °C to ensure homogeneity before used in experiment. 10 g DES in 100 mL methanol (stripping phase) was mixed with 10 g CPO in 100 mL hexane (feeding phase) and shook at 200 rpm with temperature maintained at 25 °C for 3 h. The mixture was then transferred into a separating funnel and left for 2 h where the stripping phase (lower layer) is separated from the feeding phase (upper layer). Both phases were collected. The extraction was repeated using DES/CPO weight ratio of 2/1 - 5/1.

Oil raffinate in the feeding phase was recovered by removing hexane using vacuum evaporator. To recover tocol extract, 10 mL water-hexane mixture (1:4, v/v) was added to the stripping phase where tocol extract is extracted into hexane layer. Extraction using water-hexane was conducted twice to maximize tocol extraction from the stripping phase. The tocol-rich hexane layer was collected and the hexane was removed by vacuum evaporation. The recovered oil raffinate and tocol extract are kept for HPLC analysis.

The above method of extraction of tocols from palm oil using was patented.

Analysis of tocols

Analysis of tocols was performed using a Waters LC system installed with a 600-series pump, a 2707-series autosampler and a 996-series photodiode array. Separations were carried out on a 5 µm silica column (150×4.6 mm i.d.; Phenomenex). Mobile phase used was heptane and ethyl acetate (96:4, v/v) with a flow rate at 0.7 mL/min. UV absorbances of the individual tocols were measured at wavelengths ranging from 290 - 300 nm. Concentrated tocols were used to identify the tocol peaks. An external calibration curve (fitting linear regression) was prepared using α-tocopherol standard with 6 different concentrations. The curve's slope, M was obtained and used to quantify concentration of tocols in extracts and raffinates. The regression equation is as follows:

$$Y = 75307X \qquad (R^2 = 0.9995)$$

where Y is peak area (µV.s.) and X is sample's concentration (mg/kg).

Analysis of tocols' concentration, distribution coefficient and selectivity

0.02 g product and 0.05 g raffinate from LLE were dissolved separately in 5 mL heptane. 100 µL of the prepared sample was then injected into the HPLC. Concentration of each tocol homologue, C_i, was calculated as follows:

$$C_i = \frac{1}{M} \times A_i \times \frac{v_i}{m_i} \quad (1)$$

where M is the linear regression's slope carrying the unit of µV.s.mg/kg, A_i is the peak area (µV.s), v_i is the volume of sample (mL) and m_i is the weight of sample (g). The distribution coefficient of tocol i, D_i was calculated as follows:

$$D_i = C_i^{ex}/C_i^{ra} \quad (2)$$

where C_i^{ex} is the concentration of tocol i in the extract (from stripping phase) and C_i^{ra} is the concentration of tocol i in the raffinate (from feeding phase). The selectivity of tocol i to tocol j, $S_{i/j}$ can then be determined:

$$S_{i/j} = D_i/D_j \quad (3)$$

Statistical analysis

Statistical analysis (analysis of variance) using SPSS software (IBM, Malaysia) was carried out to compare the mean of results and determine significant changes in tocols distribution and selectivity. A 95 % confidence level ($p < 0.05$) was used to evaluate significant differences between means.

Results and discussion

Viscosity and water content

Viscosity and water content of neat DES, CPO and DES upon dilution with methanol (hence known as stripping phase or DES-methanol phase) are presented in **Table 1** and **Figure 2**. CPO and DES were diluted in hexane and methanol respectively prior to extraction as CPO is semi solid at ambient and DES is highly viscous, which make them difficult to handle in their pure forms. Water content in DES may alter the structure of DES due to the transition from ionic mixture to aqueous solution which usually happened when DES is prepared through water mixing. However, DES structure was reported to be stable at remarkably high level of water (about 42 wt. %), while at 51 wt. %, structure of DES began to disrupt [19]. Castro *et al.* (2021) also reported that the effect of water is significant when it is present at over 0.9 mole fractions [20]. Water content in DES1 and DES2 were 1.45 and 4.30 wt. % respectively, while water content in DES-methanol phase reduced gradually to less than 1.0 wt. % as a result of dilution with methanol (**Figure 2**).

Table 1 Viscosity and water content of DES and CPO.

Chemical	Viscosity at 30 °C (MPa s)	Water content at 25 °C (wt. %)
DES2	17,460	4.30
DES1	1,216	1.45
CPO	47.2	0.20

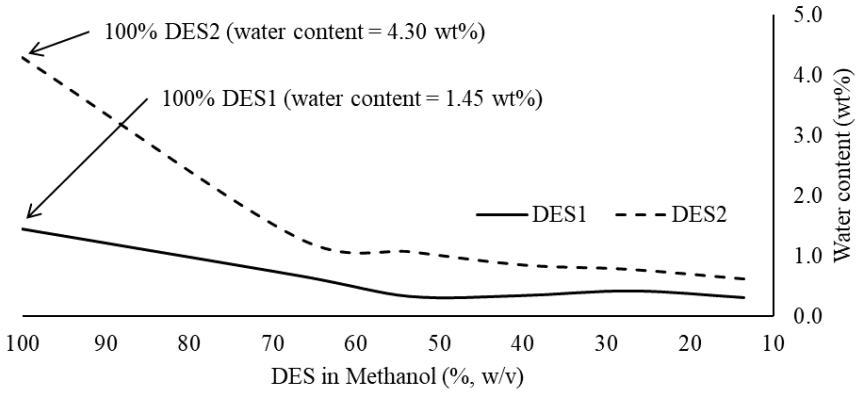

Figure 2 Water content in DES after dilution with methanol (DES-methanol phase).

Hydrogen bond has considerable influence on the O-H stretching vibration which could be observed at between 3,600 and 3,200 cm^{-1} in FTIR. **Figure 3** shows the FTIR spectra of choline chloride, malonic acid, citric acid and DESs formed. O-H stretching vibration in choline chloride (3,318 cm^{-1}) shifted to 2,923 cm^{-1} in DES1 and DES2, whereas a broad stretching vibration at 3,278 cm^{-1} (DES1) and 3,304 cm^{-1} (DES2) indicated intermolecular H bonds. A strong peak at 1,717 - 1,716 cm^{-1} vibrational band corresponds to carbonyl compound and C=O stretching in the DESs. Vibrational bands at 1,480 cm^{-1} refers to the CH_2 bending of alkyl group, the prominent group in choline-based DES. These spectra are in agreement with prior reports [21-23].

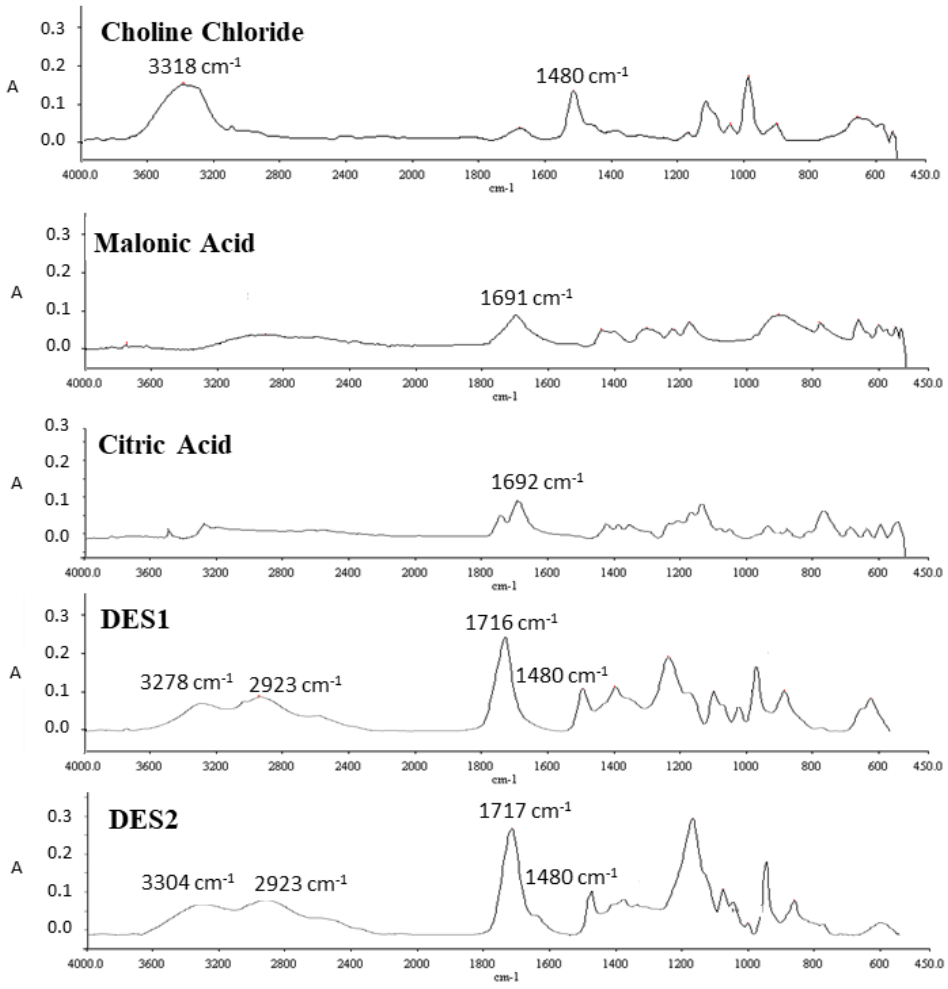

Figure 3 FTIR spectra of the DES1 and DES 2 at room temperature in the region of 4,000 - 400 cm^{-1}.

Solubility of DESs

Differences in solubility between molecules are often manipulated to selectively draw and isolate desired compounds. Preparations of feeding and stripping phases are therefore crucial to carry out liquid-liquid extraction (LLE) which depended on the miscibility of the 2 liquid phases. The properties (miscibility in DES, solubility in water and polarity index) of diluents examined in this study are depicted in **Table 2**. Solubility in water and polarity index of various solvents were obtained from literature [24,25]. Polar diluents (ethanol, methanol, water) have high solubility in DES and their tendency to be dissolved in DES is similar to that of water. Compounds with good solvability in the DES also indicate that they are polar molecules. Methanol was used in this study as it has good solubility in DES, as well as being the simplest form of alcohol with highest polarity index (5.1), after water (polarity index: 10.2). *n*-Hexane (solubility in water 0.001 %) was selected as CPO's diluent as it has moderate solubility value compared to *n*-heptane which has nearly zero solubility in water (0.0003 %), as well as meeting the purpose of creating immiscible phase with DES.

Table 2 Solubility of diluents in DES and water.

Diluents	DES1	DES2	Solubility in water % [24]	Polarity index [25]
n-Heptane	Immiscible	Immiscible	0.0003	0.1
n-Hexane	Immiscible	Immiscible	0.001	0.1
Ethanol	Miscible	Miscible	100	5.2
Methanol	Miscible	Miscible	100	5.1
Water	Miscible	Miscible	100	10.2

Effect on tocol concentrations with different contact times

Figure 4 shows the concentration of tocols in the extract over 6 h extraction. Four sets of preliminary LLE experiments with contact time varied at 10 min, 1, 3 and 6 h were carried out. The recovered stripping phases were clarified and the tocol-rich extract analysed for its tocols content. Control experiment using only methanol (without DES) was also conducted. Concentration of tocols increased from 3,406 mg/kg in control to 5,922 mg/kg in DES1 and 8,314 mg/kg in DES2, indicating significant role of DESs in tocols extraction. The CPO used in this study contained 808 ± 6 mg/kg tocols which is within normal range. It was found that methanol alone could increase tocols concentration in the extract by 3 - 4 times. Near total extraction equilibrium was achieved within 10 min. 3 h phase contact time was employed throughout the study to ensure complete separation. LLE could achieve equilibrium much faster rate when compared to extractions with supported liquid membrane (SLM) or emulsion liquid membrane (ELM) [26]. This could be due to the larger contact area between the feeding and stripping phases in the LLE. The 2 immiscible phases, which were in direct contact within the system, allowed for higher surface area and therefore closer contact, resulting in rapid extraction. In experiments using SLM, where feeding and stripping phases were separated by a membrane, permeation of solutes occurred continuously, yet slowly, to up to 25 h to achieve equilibrium [18,27]. The equilibrium contact time in this study correlated with the outcome reported by Yang *et al.* (2009) when LLE was used to separate tocopherol homologues where the equilibrium was achieved within 10 min of extraction [15]. Another study by Ni *et al.* (2012) also demonstrated the application of ionic liquids in the extraction of phenolic compounds using LLE by employing 3 h shaking time to achieve extraction equilibrium [28].

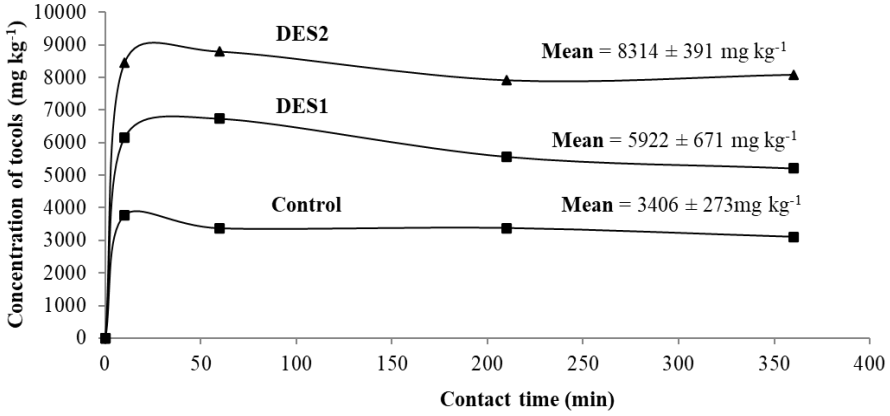

Figure 4 Concentration of tocols at different contact times using 1/1 DES/CPO weight ratio.

Distribution of tocols

Tocols is a collective term frequently used to designate tocopherols and tocotrienols. Tocols are amphipathic (hydrophilic and lipophilic) compounds. Their hydrophilic property is attributed to the polar chromanol ring attached to the hydrophobic 16-carbon side chain via C-2 atom as shown in **Figure 5**. Saturated phytyl side chain in tocopherols and unsaturated isoprenyl (consists of 3 double bonds) side chain in tocotrienols determine their lipophilic properties. Tocopherols and tocotrienols usually occur as α-, β-, γ- and δ-homologues that differ by the number and position of methyl groups in the chromanol ring as showed in **Figure 5**.

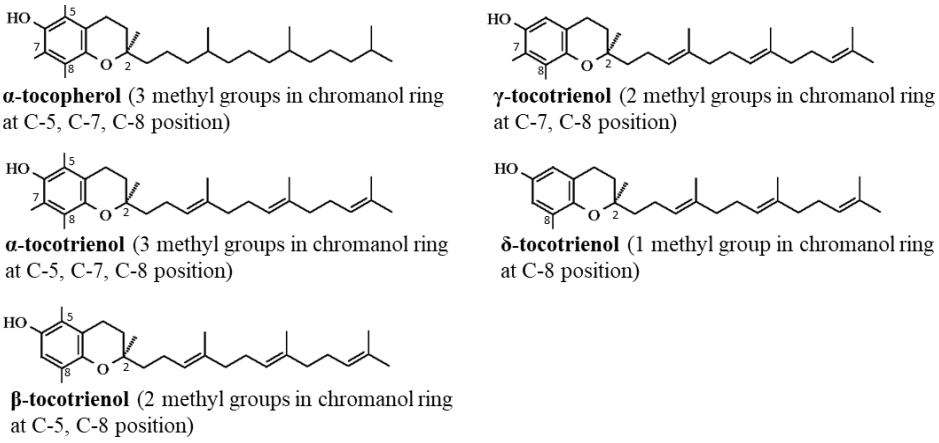

Figure 5 Molecular structure of tocol homologues commonly found in palm oil with 16-carbon side chain attached to C-2 position.

Extraction can be facilitated by understanding the polarity of the desired compound so that suitable stripping phase can be introduced to the receiving phase. Polarity of the tocols is mainly influenced by the number of methyl groups in the chromanol ring, and to a lesser extent, by steric effects of the methyl groups and the slightly increased polarity of the unsaturated side chain of tocotrienols compared to tocopherols. The most difficult compounds to be separated were the β- and γ-tocols, as both have 2 methyl groups in their ring structure albeit different positions. The polarity of the tocols can be arranged in the following order: α-tocopherol < α-tocotrienol < β-tocotrienol < γ-tocotrienol < δ-tocotrienol [29,30].

The distribution coefficients of each individual tocols were determined by analysing concentration of tocols in the extract (from stripping phase) and raffinate (from feeding phase). The distribution coefficient (D_i) of tocol homologue was defined in Eq. (1).

Figure 6 illustrates the transportation mechanism of tocols from the receiving phase to the stripping phase. Tocols are lipid-soluble compounds and are slightly polar due to the chromanol ring attached to the long side carbon chain. Tocols in the CPO can be transported into the polar phase by introducing the tocols solution into a polar stripping phase. While extraction of all tocols is impossible due to the side chain that remained in the non-polar feeding phase, partitioning of tocols can be achieved by manipulating the hydrophilicity (polarity) of the stripping phase.

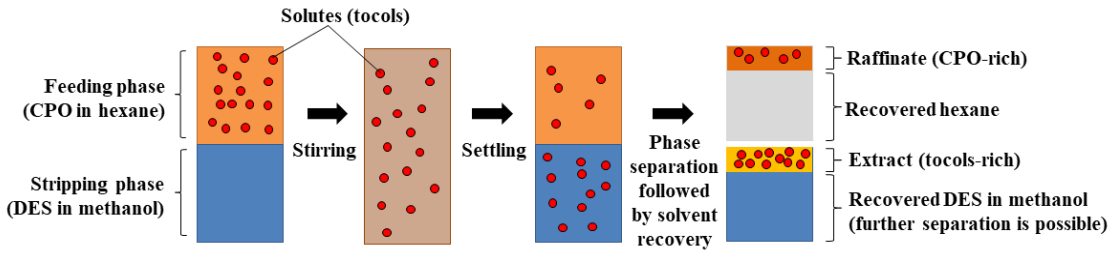

Figure 6 Illustrative mechanisms on the separation of tocols in liquid-liquid extraction assisted by deep eutectic solvent.

Table 3 shows the distribution coefficient of each tocol homologues when DES1 was used. D_i value of more than 1 indicated that tocol *i* was distributed into the stripping phase. All tocol homologues were distributed into the stripping phase, except for α-tocopherol (D_i = 0.2) when DES1/CPO ratio with 5/1 was used. Distribution coefficient of each individual tocol in DES1/CPO (ratio 1/1) increased significantly following the order of their polarity. The distribution coefficients of α-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol in DES1/CPO (ratio 1/1) were 7.8, 13.1, 20.0, 22.1 and 29.7 respectively. Higher D_i value indicated that the tocols distributed more easily into the stripping phase.

Table 3 Distribution coefficient of individual tocols from feeding into stripping phase in extraction using DES1.

Tocol homologue, *i*	Polarity [29,30]	Distribution coefficient of tocol homologue, D_i				
		DES1/CPO (w/w)				
		1/1	2/1	3/1	4/1	5/1
α-tocopherol	Lowest	$7.8 \pm 0.3^{a}{}_{d}$	$5.9 \pm 0.7^{a}{}_{c}$	$8.5 \pm 0.5^{a}{}_{d}$	$4.8 \pm 0.2^{a}{}_{b}$	$0.2 \pm 0.1^{a}{}_{a}$
α-tocotrienol	↓	$13.1 \pm 0.2^{b}{}_{b}$	$12.6 \pm 1.0^{a}{}_{b}$	$21.3 \pm 1.6^{ab}{}_{c}$	$13.0 \pm 0.2^{a}{}_{b}$	$3.6 \pm 0.2^{a}{}_{a}$
β-tocotrienol	↓	$20.0 \pm 3.5^{c}{}_{a}$	$35.2 \pm 3.4^{b}{}_{ab}$	$40.2 \pm 8.7^{bc}{}_{b}$	$41.1 \pm 11.0^{b}{}_{b}$	$42.4 \pm 4.5^{b}{}_{b}$
γ-tocotrienol	↓	$22.1 \pm 0.5^{c}{}_{a}$	$25.4 \pm 1.6^{b}{}_{a}$	$34.9 \pm 2.6^{ab}{}_{c}$	$29.7 \pm 0.4^{b}{}_{b}$	$25.4 \pm 1.2^{c}{}_{a}$
δ-tocotrienol	Highest	$29.7 \pm 2.1^{d}{}_{a}$	$62.2 \pm 7.9^{c}{}_{bc}$	$89.8 \pm 20.7^{d}{}_{d}$	$70.6 \pm 5.7^{c}{}_{bc}$	$50.1 \pm 2.5^{d}{}_{ab}$

Note: Means within a column with different superscripts are statistically different ($p < 0.05$)
Means within a row with different subscripts are statistically different ($p < 0.05$)

The distribution coefficient of the tocol homologues in extraction using DES1/CPO with ratio of 2/1 - 5/1 appeared to fluctuate and not in the order of its polarity (α-tocopherol < α-tocotrienol < β-tocotrienol < γ-tocotrienol < δ-tocotrienol). For instance, γ-tocotrienol, which has higher polarity than β-tocotrienol, was distributed less in the stripping phase than β-tocotrienol ($D_{\gamma\text{-tocotrienol}}$ = 25.4, 34.9, 29.7, 25.4; $D_{\beta\text{-tocotrienol}}$ = 35.2, 40.2, 41.1, 42.4). This might be due to the high composition of γ-tocotrienol (*ca.* 42 %) in feeding material (CPO) which might have hindered its distribution and transportation from the feeding into the stripping phase. Tocol homologues exist naturally in CPO as a result of plantation growth and breeding factor, thus, possess unequal tocols compositions. CPO contains *ca.* 159 mg/kg α-tocopherol, 216 mg/kg α-tocotrienol, 17 mg/kg β-tocotrienol, 340 mg/kg γ-tocotrienol and 76 mg/kg δ-tocotrienol (total *ca.* 808 mg/kg). Significant difference ($p < 0.05$) in distribution coefficient between the least polar (α-tocopherol) and the most polar (δ-tocotrienol) tocol can be observed in all cases; $D_{\alpha\text{-tocopherol}}$: 7.8, 5.9, 8.5, 4.8 and 0.2 compared to $D_{\delta\text{-tocotrienol}}$: 29.7, 62.2, 89.8, 70.6 and 50.1 with respect to DES1/CPO with 1/1 - 5/1 ratio.

The distribution of tocols in extraction using DES2 was also evaluated. The distributions of individual tocols were expected to be similar to the distribution trend observed in extraction using DES1. This was confirmed by results in **Table 4** whereby the distribution coefficient of individual tocol in the extraction using DES2/CPO with 1/1 ratio was in the order of its polarity, except for γ-tocotrienol, which showed lower distribution coefficient compared to β-tocotrienols (17.7 and 26.4, respectively). It was perceived that the type of HBA (carboxylic acids) also influenced the distribution. Higher number of –COOH functional group in HBD of DES2 (citric acid) indicated that it could develop higher polarity, thus attracting more polar tocols. For example, distribution coefficient of δ-tocotrienol in DES2 (34.9) is significantly higher ($p < 0.05$) than in DES1 (29.7). The concentration of tocols in DES2 (8,314 mg/kg) was also higher than that of DES1 (5,922 mg/kg) by more than 2,000 mg/kg (**Figure 4**).

Table 4 Distribution coefficient of individual tocol from feeding into stripping phase in extraction using DES2.

Tocol homologue, *i*	Polarity [29,30]	Distribution coefficient of tocol homologue, D_i				
		DES2/CPO (w/w)				
		1/1	2/1	3/1	4/1	5/1
α-tocopherol	Lowest	$7.8 \pm 0.6^{a}{}_{bc}$	$8.8 \pm 0.9^{a}{}_{c}$	$6.7 \pm 0.6^{a}{}_{b}$	$4.2 \pm 0.3^{a}{}_{a}$	$2.9 \pm 0.0^{a}{}_{a}$
α-tocotrienol	↓	$13.4 \pm 1.3^{b}{}_{bc}$	$16.6 \pm 1.3^{b}{}_{c}$	$16.7 \pm 1.1^{b}{}_{c}$	$12.2 \pm 0.0^{ab}{}_{b}$	$7.8 \pm 0.3^{a}{}_{a}$
β-tocotrienol	↓	$26.4 \pm 1.4^{c}{}_{a}$	$34.9 \pm 0.7^{c}{}_{ab}$	$36.5 \pm 5.0^{c}{}_{b}$	$36.4 \pm 3.5^{c}{}_{b}$	$29.1 \pm 3.2^{b}{}_{ab}$
γ-tocotrienol	↓	$17.7 \pm 0.3^{d}{}_{b}$	$22.4 \pm 1.0^{d}{}_{c}$	$25.2 \pm 1.1^{d}{}_{d}$	$19.2 \pm 0.6^{b}{}_{b}$	$14.2 \pm 1.0^{c}{}_{a}$
δ-tocotrienol	Highest	$34.9 \pm 1.2^{e}{}_{ab}$	$46.7 \pm 1.6^{e}{}_{c}$	$46.7 \pm 1.3^{e}{}_{c}$	$40.5 \pm 4.7^{c}{}_{bc}$	$29.5 \pm 1.1^{b}{}_{a}$

Note: Means within a column with different superscripts are statistically different ($p < 0.05$)
Means within a row with different subscripts are statistically different ($p < 0.05$)

It was also observed that distribution coefficients of γ-tocotrienol was always lower than β-tocotrienol in DES2/CPO with ratio of 2/1, 3/1 and 4/1 ($D_{\gamma\text{-tocotrienol}}$ = 22.4, 25.2, 19.2 and $D_{\beta\text{-tocotrienol}}$ = 34.9, 36.5, 36.4) although they were theoretically inferred to be otherwise. The composition of γ-tocotrienol is highest among all tocols in CPO, which halted the extraction when equilibrium is reached. The polarity of β-tocotrienol is closed to γ-tocotrienol as a result of having similar molecular weight where their difference lies only on the position of methyl (CH_3) groups in the chromanol ring. The partitioning behavior of the tocols could be further understood by examining the selectivity parameters.

Selectivity of tocols

While distribution data provided a good insight on partitioning of a particular tocol homologue between feeding and stripping phase, selectivity further explains the partitioning of 1 tocol homologue from another. **Table 5** shows that the selectivity of each tocol homologues in relation to α-tocopherol increased significantly ($p < 0.05$) in the following order: δ-tocotrienol (3.82) > γ-tocotrienol (2.84) > β-tocotrienol (2.58) > α-tocotrienol (1.68). The order is also true for the polarity of each of these tocol homologues.

γ-Tocotrienol was slightly preferred over β-tocotrienol in DES1/CPO (ratio of 1/1), with selectivity of γ-tocotrienol to β-tocotrienol being 1.13 (selectivity value of 1 indicates fair distribution of both components). However, selectivity of γ-tocotrienol to β-tocotrienol was inverted, *i.e.* 0.72 (less than 1) with the increased in the amount of DES1 (**Table 6**). While it was reasonable to consider that tocol homologues were distributed according to their hydrophilic properties, unequal tocol compositions may cause some hindrance in the separation mechanism.

Selectivity of tocotrienols in relation to α-tocopherol tend to increase with the increase of DES in the stripping phase (**Table 6**) thus suggesting the key role of DES in extractive separation. For example, selectivity of δ-tocotrienol to α-tocopherol in DES1/CPO (2/1) was significantly higher ($S_{i/j}$ = 10.52) compared to in DES1/CPO (1/1) ($S_{i/j}$ = 3.82) ($p < 0.05$). This could be of great advantage for it was reported that tocotrienols with lower amount of α-tocopherol is needed for maximum antioxidant protection [3].

Table 6 Selectivity of homologue *i* to homologue *j* in extraction using DES1/CPO (2/1).

Selectivity of homologue *i* to homologue *j*, $S_{i/j}$					
i	***j***				
	α-tocopherol	**α- tocotrienol**	**β- tocotrienol**	**γ- tocotrienol**	**δ- tocotrienol**
α-tocopherol	-	*f-1*	*f-1*	*f-1*	*f-1*
α-tocotrienol	2.14 ± 0.14^{b}	-	*f-1*	*f-1*	*f-1*
β-tocotrienol	5.96 ± 0.37^{d}	2.79 ± 0.04^{b}	-	*f-1*	*f-1*
γ- tocotrienol	4.31 ± 0.31^{c}	2.02 ± 0.04^{b}	0.72 ± 0.03^{a}	-	*f-1*
δ- tocotrienol	10.52 ± 1.23^{e}	4.92 ± 0.29^{cd}	1.76 ± 0.10^{ab}	2.44 ± 0.17^{b}	-

Note: Means across the table with different superscripts are statistically different ($p < 0.05$)

f-1 means that the selectivity value of $S_{i/j}$ is the inverse function of $S_{j/i}$, e.g., $S_{\alpha\text{-tocopherol}/\alpha\text{-tocotrienol}}$ is 0.47 which is the inverse value of $S_{\alpha\text{-tocotrienol}/\alpha\text{-tocopherol}}$ (2.14)

Selectivity and distribution coefficient of tocotrienol homologues (α-, β- γ- and δ-tocotrienol) in this study were also in parallel with the findings by Yang *et al.* (2009) as shown in **Table 7** [15]. Distribution coefficient and selectivity of tocopherol homologues (α-, β/γ- and δ-tocopherol) in imidazolium-based ionic liquid ([bmim]Cl) was also reported as comparison. Average distribution and selectivity value for β- and γ-tocotrienol in DES extraction was calculated to make comparison with distribution and selectivity of β/γ-tocopherol in [bmim]Cl extraction (e.g., $D_{\beta\text{-tocotrienol}}$ = 20.0, $D_{\gamma\text{-tocotrienol}}$ = 22.1, thus $D_{\beta/\gamma\text{-tocotrienol}}$ = 21.1). The distribution and selectivity of tocol homologues using both [bmim]Cl and DES was in the order of α < (β and γ) < δ whereas the selectivity is in the order of δ/(β and γ) < (β and γ)/α < δ/α, respectively. Large difference in distribution coefficient between of δ-tocol and α-tocol was observed in both extraction using [bmim]Cl and DES (for [bmim]Cl: 2.34 > 0.11, 2.58 > 0.16 and for DES1: 29.7 > 13.1, 62.2 > 12.6), thus confirmed distribution of tocol homologues in accordance to their polarity.

Table 7 Selectivity of tocols in DES and ionic liquid.

Molten salt	Description	Distribution coefficient			Selectivity		
		α	β and γ	δ	δ/(β and γ)	(β and γ)/α	δ/ α
Imidazolium chloride ionic liquid ([bmim]Cl)[1]	1/1.3 mole ratio to methanol	0.11	0.95	2.34	2.5	8.6	21.3
	1/2.7 mole ratio to methanol	0.16	1.18	2.58	2.2	7.4	16.1
Choline chloride DES (DES1)	1/1 weight ratio to CPO	13.1	21.1	29.7	1.43	1.61	2.27
	2/1 weight ratio to CPO	12.6	30.3	62.2	2.10	2.41	4.92

Source: [1]Yang *et al*. (2009)
Note: Extraction using imidazolium chloride ionic liquid reported on selective separation of tocopherol homologues whereas extraction using DES in this study reported results for tocotrienol homologues

Selectivity of tocol homologues is comparatively low when using DES1 compared to [bmim]Cl; *e.g.* selectivity of δ/(β and γ) (1.43, 2.10 < 2.5, 2.2), (β and γ)/α (1.61, 2.41 < 8.6, 7.4) and δ/ α (2.27, 4.92 < 21.3, 16.1). This could be due to CPO samples used consisted of more than 90 % triglyceride and less than 0.1 % tocols. Tocopherol solutions prepared by Yang *et al*. (2009) using synthetic material (> 90 % purity) are less 'crowded' hence higher selectivity could be obtained. In the future, palm fatty acid distillate (PFAD), a by-product of palm oil refinery, could be use in actual application as it is cheaper than CPO and contains more tocols. PFAD consists of mainly free fatty acids (81.7 %) and about 0.5 % tocols, the remaining being glycerides (14.4 %) and other minor substances.

Conclusions

This study presents a complete data on distribution and selectivity of 5 tocol homologues (α-tocopherol, α-, β-, γ-, and δ-tocotrienol) present in palm oil using liquid-liquid extraction (LLE). The hydrophilicity of LLE's stripping phase was manipulated by using choline-based deep eutectic solvents (DESs) with malonic acid and citric acid as hydrogen bond donors. The distribution and selectivity of tocol homologues was found to be affected by the polarity of the tocols. The selective separation was influenced by the hydrogen-bonding interactions between the carboxylic's –OH group and the chloride anion. This caused most of the polar tocols to be distributed into the extract phase. For example, δ-tocotrienol was far better distributed in the stripping phase compared to α-tocopherol. Low selectivity of α-tocopherol could benefit nutraceutical formulations as it has less antioxidative property as well as lowering the bioavailability of the tocotrienols.

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