Research Article

Effect of Temperature on Lignin Extraction from Cocoa Shells using Deep Eutectic Solvent: Optimization and Characterization

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ABSTRACT

Lignin has been reported as a natural UV protectant due to its chemical structures. Deep Eutectic Solvent (DES) has shown potential for the lignin extraction method. This research aimed to study the effect of temperatures on the lignin extraction using DES (composed of choline chloride and lactic acid) from *Theobroma cacao* L. c.v. I.M.1, in which the employed parts were cocoa pod shells (CPS) and cocoa bean shells (CBS). The extracts were determined for %yield and lignocellulosic compositions (i.e., lignin, cellulose, and hemicellulose). The compositions were evaluated using TAPPI T222-om-88, acid chlorite, and TAPPI T203-om-88 methods. Fourier Transform Infrared Spectroscopy (FT-IR) was employed to characterize the chemical structure of lignin. The investigation found that the extracts from both biomasses had a color ranging from dark brown to black. The extracts from CBS (CBS-LE-T) prepared at 80 °C, 120 °C, and 150 °C had lignin contents of 80.0±2.1%, 79.7±3.4%, and 76.4±2.1%, respectively. These values were greater than those of the extracts from CPS. Consequently, more research was done on the CBS-LE-T extracts. Yield increased with elevated temperatures: 80 °C (4.5±0.7%), 120 °C (17.4±3.9%), and 150 °C (19.7±3.7%). The contents of cellulose and hemicellulose were 0.6±0.1% to 1.0±0.9% and 4.3±2.3% to 6.3±2.5%, respectively. FT-IR spectra illustrated the functional groups of lignin structure including hydroxy group and aromatic rings. Hence, DES possessed the efficient ability to extract lignin from biomass. Higher lignin content was provided by CBS, and 120 °C was shown to be the optimal temperature for both a high yield and lignin content from CBS.

Keywords:
Lignin extraction; Deep Eutectic Solvent (DES); Cocoa pod shells (CPS); Cocoa bean shells (CBS); The potential UV protective application of lignin; Natural UV protectant; Biomass sources

1. INTRODUCTION

Long-term exposure to ultraviolet (UV) rays and blue light can lead to dermatological problems[1]. Protecting skin against UV and blue light exposure is an important health issue. Sunscreen is a simple and effective way to protect the skin from direct sunlight. Sunscreen agents are mostly synthetic substances that can cause side effects such as allergy and irritant contact dermatitis[2].

Synthetic sunscreen is non-photostable and degrades after sunlight exposure[3]. Applying high-concentrate organic UV filters on the skin layer can cause skin irritation and allergy[4, 5]. The Institute of Dermatology, Thailand[6] reported that pregnant women should avoid several organic UV filters, especially Oxybenzone (Benzophenone: BP-3), Octinoxate (Octyl methoxycinnamate: OMC), Avobenzone (Butyl Methoxydibenzoylmethane: BMDM), Dioxybenzone, Octocrylene (OCR), and Para-aminobenzoic acid (PABA).

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These organic UV filters can penetrate human skin due to the lipophilicity of the UV filters, resulting in danger to the fetus. The Department of National Parks, Wildlife and Plant Conservation[7] prohibited using sunscreen containing organic UV filters such as BP-3, OMC, and 4-Methylbenzylid Camphor (4-MBC) in the national park. Also, academic research has revealed that these organic UV filters contribute to the deterioration of coral reefs because those chemicals destroy coral larvae, obstruct the reproductive system, and cause coral bleaching[7]. Consequently, natural ingredients, including lignin, have recently received more attention and are believed to be safer with fewer side effects[8].

Lignin is the most abundant aromatic and heterogeneous polymer present in nature[9]. Lignin has been promoted as a safer and biodegradable alternative to synthetic sunscreen. The safety of different lignins was demonstrated through cytotoxicity tests using two cell lines, including HaCaT human keratinocyte and 3T3 murine fibroblast cell lines, which proved lignin is safe and able to be used in cosmetic and pharmaceutical fields[10]. Commonly, lignin combines with cellulose and hemicellulose to form a lignocellulosic matrix that provides strength and rigidity to the cell wall[11, 12]. The chemical structure of lignin contains several functional groups, including phenolics, ketones, carboxylic acids, and quinoid structures[13,14]. The structural units of lignin can be classified into three main types, including p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), which differ in the methoxy group on the aromatic ring. These structural units are linked by β-O-4, β-β, β-5, β-1, 5-5, and 4-O-5 linkages. The UV protection properties of lignin also relate to the compositions of the structural units of lignins[15]. The highest UV absorbability of subunits is shown in the S unit, followed by the G and H units, because of the methoxylation degree on the aromatic ring, resulting in better UV protection performance[16]. The ratio of these units depends on the biomass source[17]. The mechanisms of UV absorbance are as follows. The UV photon energy activates electron transition from bonding or nonbonding orbitals into empty antibonding orbitals that vary with chemical structure and electronic state. The possible electron transitions include (1) $\sigma \rightarrow \sigma^*$ transition, (2) $\pi \rightarrow \pi^*$ transition, (3) $n \rightarrow \pi^*$ transition, and (4) $n \rightarrow \sigma^*$ transition. Only electron transition types 2, 3, and 4 can absorb in the UV and visible light ranges (200-800 nm), found in the chromophore groups (e.g., benzene ring conjugated with double bonds and/or carbonyl groups, free and etherified hydroxyl groups) of lignin structure[18].

Cocoa (*Theobroma cacao* L.) beans are the seeds of cocoa fruit and are commonly used to produce chocolate powder, cocoa powder, and cocoa butter. In this study, cocoa pod shells (CPS) and cocoa bean shells (CBS) were the sources of lignin. They are classified as hardwood plants and are reported to possess high S and G units[19], which are good candidate biomass materials for use as UV absorbers[15, 20].

Several chemicals are used to separate lignin (e.g., strong, or diluted acids, alkalis, and toxic organic solvents) at increased temperatures and pressures for reduced processing time. The degradation of lignin is responsible for the cleavage of the aryl-ether linkage between lignin and the lignin-carbohydrate complex. These techniques obtain fragmented lignin, which is not only low-purity and has poor environmental credentials but also low commercial practicability[21]. Thus, novel lignin recovery techniques have become increasingly vital for the development of new lignin applications such as sunscreen formulation. The utilization of deep eutectic solvents (DES) in biomass extraction has received more attention in recent years. DES is a mixture of hydrogen bond donor (e.g., lactic acid) and hydrogen bond acceptor (e.g., choline chloride: ChCl) through hydrogen bond interaction to form a lower melting point than individual components[22]. It is becoming more attractive as an environmentally friendly alternative to organic solvents due to their benefits, including low volatility, non-flammability, high dissolution, and adaptability[23]. ChCl-lactic acid-based DES has proven to be a potential solvent for lignin extraction from several biomass materials[12, 24-31], which is responsible for the proposed mechanism as follows. (i) DES swells the cell walls, causing them to rupture which makes DES more permeable in the cell walls. (ii) The proton-catalyzed cleavage of ether (e.g., β-O-4 linkage) and the covalent bonds from lignin and lignocellulosic matrix degrades the lignin macromolecules into subsequent lignin fragments[32]. (iii) Cl⁻ has strong electronegativity, resulting in the formation of hydrogen bonds. When the ether bonds in the lignin macromolecule are cleaved, Cl⁻ attaches to the hydroxy group of the lignin fragment to form the strong hydrogen bonding in the DES solution. Enhancing DES performance in lignin extraction requires several factors, including the biomass-to-DES ratio[31-33], and time[30]. The treatment is usually conducted at a temperature of 80-160 °C[31]. Increasing lignin fractionation performance is affected by the elevated temperatures caused by the decrease of DES viscosity allowing the enhancement of heat and mass transfer[32, 33].

The objective of this study was to extract lignin using the ChCl:lactic acid (1:10 molar ratio) at different temperatures (80 °C, 120 °C, and 150 °C) to compare DES extraction ability. The sources of biomass were cocoa pod shells (CPS) and cocoa bean shells (CBS). The lignin extracts obtained determined the yield and lignocellulosic composition, as well as further characterized the chemical structure through Fourier Transform Infrared Spectroscopy (FT-IR). Finally, thermal stability, photostability, and elemental analysis were also evaluated to prove the properties and safety of the lignin extracts for cosmetic applications.

2. MATERIALS AND METHODS

2.1 Materials

CPS material was obtained from the local cocoa growing area in Phitsanulok Province, Thailand. CBS material was purchased from the Hill tribe cocoa cof Co. Ltd., (Chiangmai, Thailand). The materials were ground and sieved to 0.841 mm. ChCl (98%) and lactic acid (88%) were obtained from Loba Chemie (Mumbai, India). Ethanol (95%) was purchased from Krungthepchemi (Bangkok, Thailand). Acetic acid glacial (99.7%), Acetone (99.5%), and Sulfuric acid (98%) were obtained from RCI Labscan (Bangkok, Thailand). Sodium chlorite (80%) was purchased from DC Fine Chemicals (Cornellà de Llobregat, Spain). Sodium hydroxide was obtained from Ajax Finechem (New South Wales, Australia). All chemicals used in the experiments were used without further purification.

2.2 Preparation of DES

To extract lignin with high yield, DES was formulated using ChCl as a hydrogen bond acceptor and lactic acid as a hydrogen bond donor at a specific molar ratio of 1:10. They were mixed and magnetically stirred using a hot plate stirrer (C-MAG HS 7, IKA, Staufen, Germany) at 80 °C until a homogeneous and transparent liquid was obtained.

2.3 Extraction of lignin from CPS and CBS using DES

The diagram showing the summary process of the lignin extraction from cocoa shells is shown in **Figure 1**.

During the pretreatment step, the biomass powder was mixed with DES in a round-bottom flask at a mass ratio of 1:20, and the flask was then immersed in an oil bath at room temperature. The biomass-DES mixture was stirred continuously using the hotplate stirrer for 24 hr. Following the pretreatment step, the DES treatment was performed under the same condition, with the exception that the temperature was controlled at three distinct levels: 80 °C, 120 °C, and 150 °C. Following a 24-hr period, the treated solid (i.e., biomass) and liquid (i.e., DES) were separated by vacuum filtration through a Whatman No. 1 filter paper. The treated solid was washed by pouring 95% ethanol over it until the washing liquid turned clear. The ethanol was then removed from the liquid fraction under reduced pressure in a bath at 50 °C using a rotary evaporator (R-210, Buchi, Chadderton, England). To precipitate lignin, the liquid fraction was further mixed with distilled water as an antisolvent at a ratio of 1:6 (v/v) and the mixture was left for 24 hr. Lignin was separated by centrifugation using an ultracentrifuge (J2-MC, Beckman Coulter, Indianapolis, United States) at a speed of 30100 xg for 20 min. The lignin precipitate was washed with distilled water to remove residual hemicellulose and solvent impurities.

The lignin precipitate was dried in a hot air oven (DHG-9050A, Shanghai Bluepard Instruments Co., Ltd, Shanghai, China) at 80 °C for 24 hr[25,34]. Each sample was assigned the code XXX-LE-T, where XXX refers to the source of biomass (CPS or CBS), LE is lignin extract, and T is extraction temperature. The lignin extract was weighed, and the yield (%) was calculated using **Equation 1.**

$$\% \text{ Yield} = (\text{Mass of CPS or CBS lignin extract}) / (\text{Mass of raw material}) \times 100 \qquad (1)$$
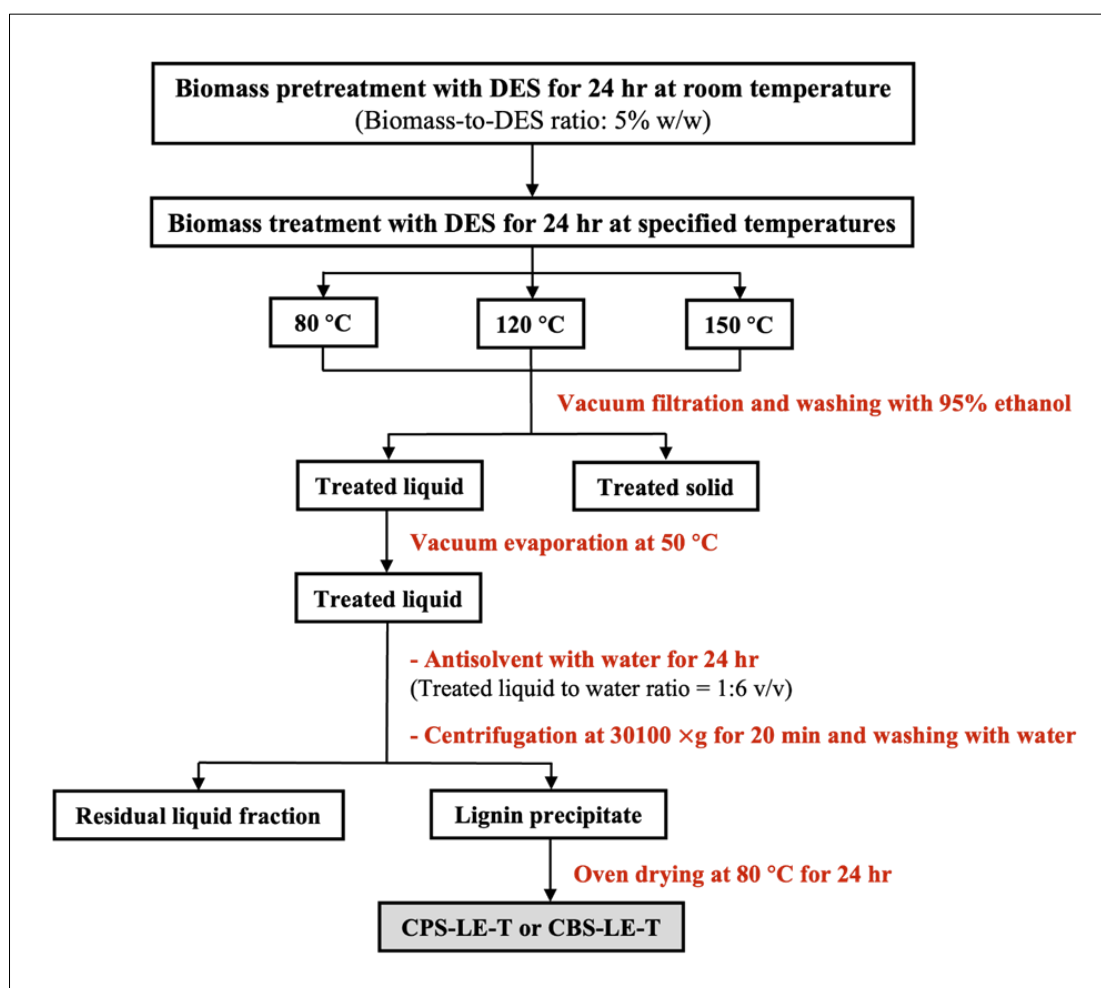

Figure 1. Flow chart summary of lignin extraction from cocoa pod shells (CPS) and cocoa bean shells (CBS) using deep eutectic solvent (DES).

2.4 Determination of lignocellulosic composition in prepared extracts

To evaluate the lignocellulosic composition of the prepared extracts, the lignin content was determined using the TAPPI official method T222-om-88[35]. Before the measurement of cellulose and hemicellulose content, the holocellulose (i.e., cellulose and hemicellulose) content was analyzed using the acid chlorite method. The cellulose and hemicellulose contents were determined using the TAPPI official method T203 om-88[36]. The cellulose and hemicellulose contents were calculated based on the weight of the lignin extracts.

2.5 Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FTIR spectra were obtained by a Fourier transform infrared spectrometer (Frontier, Perkin Elmer, Massachusetts, USA). The assessment was carried out using an attenuated total reflectance (ATR) technique.

All spectra were recorded at a spectral resolution of 4 cm^{-1}, and 16 scans were performed per sample in the 4000–400 cm^{-1} range.

2.6 Thermal stability and photostability of the lignin extracts

The evaluation of thermal stability and photostability was performed based on the method described by Kim, N.H. et. al.[37], with slight modifications. For thermal stability, the color of the lignin extracts was visually observed after storing the extracts in the hot air oven (Model 600, Memmert, Schwabach, Germany) at temperatures of 25 °C, 40 °C, 60 °C, and 80 °C for 2 hr. Additionally, the photostability of the extracts was examined using a UV test chamber (BS-04, Opsytec Dr.Gröbel, Ettlingen, Germany) and 400 W of UV radiation for 0, 2 hr, 4 hr, 6 hr, and 8 hr. The color of the extract was also observed. The digital photographs were taken in an identical environment and were used to determine the visually perceptible color variation of the extracts.

2.7 Elemental analysis of the lignin extracts

The elemental analysis of carbon (C), oxygen (O), nitrogen (N), and chlorine (Cl) in the CBS material and CBS-LE-T extracts were determined by an Energy Dispersive X-ray detector (Ultim® Max, Oxford Instruments, UK)[38].

2.8 Statistical analysis

All the experiments were done in triplicate. The data analyses were evaluated using the R Project for Statistical Computing (R-4.4.1 version) (Auckland, New Zealand). Analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons with a confidence level of $p<0.05$.

3. RESULTS AND DISCUSSION

Based on literature reviews, the comparative advantages of using DES for lignin extraction, over other methods, are: (i) The DES extraction process is conducted at a lower temperature range (80–150 °C) than other techniques, (e.g, hot water pretreatment, steam exploding pretreatment), which require higher temperatures (160–240 °C)[31,39]. (ii) The extracts obtained from the DES process in this study yielded a lignin content (>75%) higher than the mechanical milling pretreatment method (<40%), which uses ball milling followed by the diluted natural solvents such as dioxane to extract lignin[40,41]. (iii) The use of organic solvents (organosolv process) under harsh conditions (e.g., high temperature, strong acids, and alkalis) can generate undesired condensed lignin[42]. Also, the process can produce pseudo-lignin[43,44]. (iv) The use of alkali solvents (kraft and sulfite processes) results in lignin that has sulfur content in its structure. This affects the cosmetic safety and environmentally friendly perspective of the cosmetic product[45]. (v) The chemical substances and organic solvents are known to be corrosive, volatile, and highly toxic, which makes them prohibitive for commercial use[32]. (vi) Ionic liquids are one of the solvents using lignin extraction. They are expensive, present hazardous toxicity, require complex synthesis procedures, and have poor recoverability, all of which limit the practical usage of ionic liquids in sustainable processes[46]. Therefore, to offer alternative options to prevent previous limitations, the use of new green solvents to obtain proper lignin for application as a UV-protecting agent is an important issue. This solvent has drawn attention in this study due to its low cost, biodegradability, recyclable nature, low vapor pressure, and low flammability[47].

The appearance, extraction yield, purity, and chemical characteristics of lignin have been reported to be dependent on the biomass source, the molar ratio of hydrogen bond donor and acceptor[24,31], and the reaction temperature[25,27,48]. In this study, cocoa pod shells (CPS) and cocoa bean shells (CBS) are major industrial wastes from cocoa industries. The International Cocoa Organization (ICCO) reported that over 4,000 tons of CPS and CBS are used per year around the World[49]. Also, there are plenty of cocoa plantations in the Phitsanulok province, The remainder of CPS and CBS materials were used as biomass sources for lignin extraction. DES was prepared by mixing two components of hydrogen bond acceptor (choline chloride: ChCl) and hydrogen bond donor (lactic acid) at a molar ratio of 1:10. DES was named because the freezing point of the mixture drops below that of the individual component. The biomass-to-DES ratio refers to the proportion of biomass and DES. It was fixed at 1:20 because the decreasing ratio of biomass to DES, particularly lower than 1:9, reduces the viscosity of the system by boosting DES capability on lignin extraction[31-33]. The treatment duration is one of the factors that affect the yield of lignin extracted. The increasing duration significantly promotes DES performance on lignin extraction. Zhang et al.[34] performed the effect of pretreatment time on the corncob by ChCl:lactic acid (1:2 molar ratio). The subsequent lignin yields greatly increased from 8.6% to 48.5%, obtained by a prolonged 1.5-36 hr. However, the pretreatment time of 24 hr was determined to be moderate. Therefore, the treatment duration in the pretreatment step and extraction process was fixed at 24 hr.

3.1 Effect of temperature on the lignin extraction from CPS and CBS

In the preliminary study, the sources of biomass investigated were CPS and CBS. The extraction of lignin from both biomasses was carried out for 24 hr at three different temperatures (80 °C, 120 °C, and 150 °C) to determine the effect of temperature on this process. Each sample was assigned the code XXX-LE-T, where XXX refers to the source of biomass (CPS or CBS), LE is lignin extract, and T is extraction temperature.

The appearance of the lignin extracts derived from both biomasses and their raw materials is displayed in **Figure 2**. The CPS and CBS powders were light brown, while their prepared extracts were dark brown to black powder at intensified temperatures. The extracts prepared at 80 °C were lighter in color than those prepared at 120–150 °C, whereas those prepared at 120 °C had a dark brown color like those prepared at 150 °C. Moreover, the color trend of CPS-LE-T extracts was the same as that of CBS-LE-T extracts. The deep color of the extracts is due to several possible reasons, as follows: (i) residual lignocellulosic matrix structure can form conjugated systems with lignin[50]; (ii) the conjugated structures were formed by the side chains (e.g., carbonyl group, carboxyl group) and aromatic ring

or the carbon-carbon double bond[50]; (iii) the formation of colored quinone in lignin structure[51]; (iv) the condensation reactions between lignin macromolecules under severe conditions (e.g., high reaction temperature)[38,52].
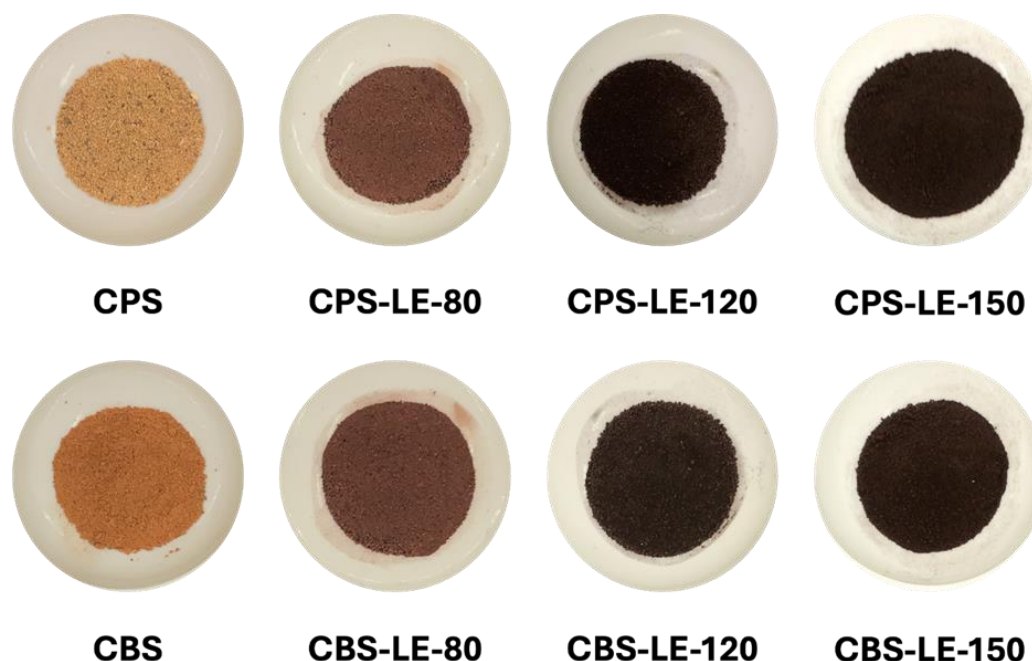

Figure 2 Appearance of cocoa pod shells (CPS), cocoa bean shells (CBS), and their lignin extracts (LE) prepared at 80 °C (CPS-LE-80 and CBS-LE-80), 120 °C (CPS-LE-120 and CBS-LE-120), and 150 °C (CPS-LE-150 and CBS-LE-150).

The lignin extraction yield from both biomasses was enhanced with an increase in extraction temperatures (from 80 °C to 150 °C). Comparing two sources of biomass, the lignin content of the extracts derived from CBS (around 76.4±2.1% to 79.7±3.3%) was significantly higher than that of the extracts derived from CPS (around 54.7±0.8% to 59.5±3.1%) **Table 1** For each source of biomass, the extraction temperature from 80 °C to 150 °C did not affect the lignin content. Therefore, the lignin extracts obtained from CBS were chosen for further studies.

Table 1. Yield and lignocellulosic composition of cocoa pod shells (CPS), cocoa bean shells (CBS), and their lignin extracts prepared at 80 °C (CPS-LE-80 and CBS-LE-80), 120 °C (CPS-LE-120 and CBS-LE-120), and 150 °C (CPS-LE-150 and CBS-LE-150).

Samples	Contents (%w/w)			
	Yield	Lignin*	Cellulose*	Hemicellulose*
CPS	-	36.0±0.9[c]	-	-
CPS-LE-80	4.5	59.5±3.1[b]	-	-
CPS-LE-120	19.8	56.6±2.1[b]	-	-
CPS-LE-150	22.8	54.7±0.8[b]	-	-
CBS	-	32.0±1.9[d]	5.6±0.7[a]	24.4±0.7[a]
CBS-LE-80	5.8±1.3[b]	77.9±2.1[a]	0.0±0.0[b]	5.6±3.0[b]
CBS-LE-120	17.4±3.9[a]	79.7±3.3[a]	1.0±0.9[b]	6.3±2.5[b]
CBS-LE-150	19.7±3.7[a]	76.4±2.1[a]	0.6±0.1[b]	4.3±2.3[b]

Note: Different superscripts of each content indicate statistical differences (p≤0.05).
* The lignocellulosic contents in the samples were based on the weight of the lignin extract.

The cellulose and hemicellulose contents of the CBS-derived lignin extracts were quantified to determine the ability of the DES extraction procedure to separate this lignocellulose from lignin. The results indicated that the CBS-derived lignin extracts had a cellulose content of 0% to 1.0±0.9 % and a hemicellulose content of 4.3±2.3% to 6.3±2.5%. The cellulose and hemicellulose contents of the CBS-derived lignin extracts were substantially lower than the raw material, and this reduction did not depend on extraction temperature. It can be concluded that the DES performs efficiently on lignin extraction from CBS.

CBS-LE-80 had a yield of 5.8±1.3%, CBS-LE-120 yielded 17.4±3.9%, and CBS-LE-150 had a yield of 19.7±3.7%. These values increased with higher temperatures. Representative information on the effect of temperature on lignin extraction is: (i) The increasing temperature affects the reducing viscosity of DES, resulting in greater heat and mass transfer. (ii) The high temperature activates the degradation of the β-O-4 and aryl ether linkages in lignin derivative molecules. (iii) The enhanced temperature induces the condensation reaction in lignin macromolecules containing aromatic rings through carbon-carbon double bonds[38,50,52]. As previously described, this suggests that the reaction temperature has an impact on the DES capacity to extract lignin from CBS. Furthermore, the extraction temperature of 120 °C was enough to provide lignin with a high yield and purity from CBS.

3.2 Chemical structure of CBS-derived lignin extracts on FT-IR spectra

The FT-IR analysis was conducted to identify the chemical structure and the purity of lignin in CBS-derived extracts. The signal assignments as listed in **Table 2**. are based on Li, T. et al.[53]. The bands at 1605 and 1517 cm^{-1} represent the lignin aromatic ring skeleton, while the bands at 865, 820, and 717 cm^{-1} represent the aromatic C-H bending. The bands at 1369 and 1196 cm^{-1} were related to C-O stretching of the S unit, whereas the band at 1121 cm^{-1} corresponded to C-H stretching. C-O and C-H stretching of the G unit appeared at 1270 and 1040 cm^{-1}, respectively. Moreover, the bands at 3274, 2915, and 2849, as well as 1708 cm^{-1} represented the hydroxyl and carboxyl groups in the lignin structure. As shown in **Figure. 3**, the intensity of the specific bands (1605, 1517, 1270, and 865 cm^{-1}) of the lignin aromatic skeleton in the CBS-LE-T extracts was remarkably increased compared to the initial CBS material, indicating that the lignin was fractionated after DES treatment. Furthermore, the absence of 1153 and 896 cm^{-1} in the CBS-LE-T extracts represented C-O-C symmetrical stretching in carbohydrates. The band of glucose ring stretching in cellulose at 1110 cm^{-1} appeared in the CBS material only, suggesting that most cellulose and hemicellulose were absent in the CBS-LE-T extracts that were obtained. Moreover, increasing extraction temperatures did not affect the lignin, cellulose, or hemicellulose contents of the CBS-LE-T extracts. This indicates that the main composition in CBS-LE-T extracts is lignin.

Table 2 List of band assignments for Fourier-transform infrared (FT-IR) spectra from the cocoa bean shells (CBS) and its lignin extracts prepared at 80 °C (CBS-LE-80), 120 °C (CBS-LE-120), and 150 °C (CBS-LE-150).

| Wavenumber (cm^{-1})	Assignment (bond)	Samples			
		CBS	CBS-LE-80	CBS-LE-120	CBS-LE-150
3274	O-H stretching	3274	3274	3274	3274
2915 and 2849	C-H stretching in CH$_3$ and CH$_2$	2915 and 2849	2915 and 2849	2915 and 2849	2915 and 2849
1708	C=O stretching	1708	1708	1708	1708
1605 and 1517	Aromatic ring skeleton	1605 and 1517	1605 and 1517	1605 and 1517	1605 and 1517
1444	C-H deformation in -CH$_2$-	1444	1444	1444	1444
1369 and 1196	C-O stretching in S unit	1369	1369 and 1196	1369 and 1196	1369 and 1196
1270	C-O stretching in G unit	1270	1270	1270	1270
1153	C-O-C symmetrical stretching in carbohydrate	1153	-	-	-
1121 and 865	C-H stretching in S unit	-	1121 and 865	1121 and 865	1121 and 865
1040	C-H stretching in G unit	-	1040	1040	1040
820 and 717	Aromatic C-H bending	820 and 717	820 and 717	820 and 717	820 and 717
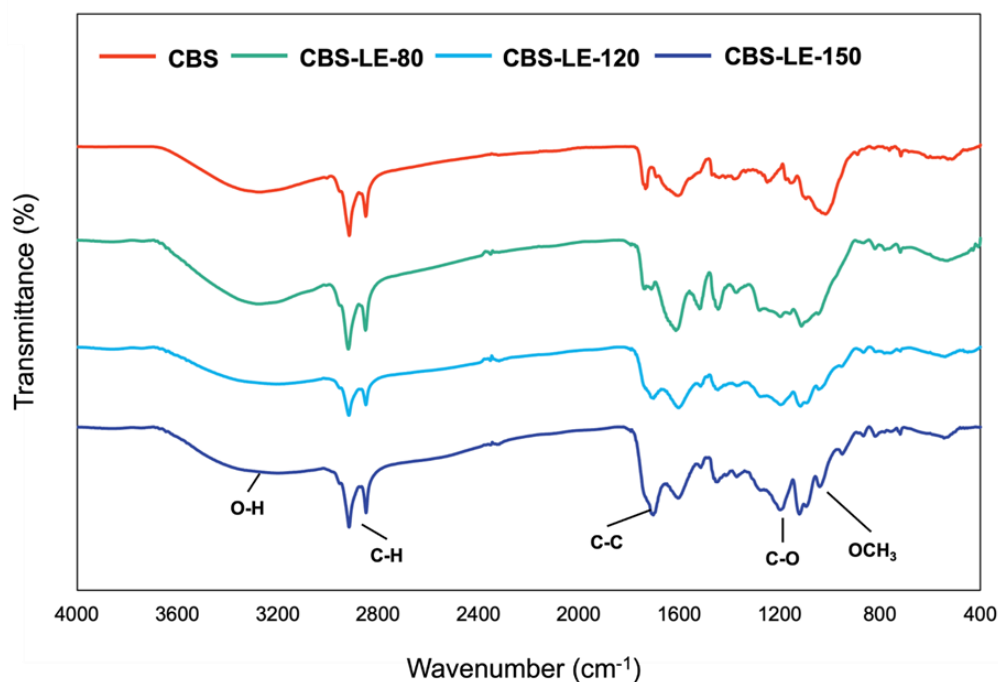
Figure 3 Fourier-transform infrared (FTIR) spectra of cocoa bean shells (CBS) and its lignin extracts (LE) prepared at 80 °C (CBS-LE-80), 120 °C (CBS-LE-120), and 150 °C (CBS-LE-150).

3.3 Thermal- and photostability of CBS-derived lignin extracts

The appearance of CBS-LE-T extracts left for 2 hr at various temperatures (i.e., 25 °C, 40 °C, 60 °C, and 80 °C) is shown in **Figure 4**. The investigations found that the colors of the CBS-LE-T extracts were not different at all temperatures. **Figure 5** displays the appearance of CBS-LE-T extracts left under 400W UV irradiation for 0 hr, 2 hr, 4 hr, 6 hr, and 8 hr. The UV light of 280-400 nm was used to study photostability.

According to the findings, the color of the partial CBS-LE-T extracts was lighter when the extracts were irradiated for 2 hr. It was expected that the discoloration of the CBS-LE-T extracts containing lignin. The lignin structure has conjugated phenolic groups, which cause a photo-oxidation reaction when they absorb UV light directly, resulting in the generation of phenoxy radicals through the decomposition of lignin[54]. The World Meteorological Organization (WMO) reported that one UV index unit is 0.0025 mW/cm^2 [55]. Currently, the summer in Bangkok has the highest UV index, up to 12 units[56]. Therefore, the total UV index is 0.03 mW/cm^2, which is obtained from the one UV index unit value multiplied by the unit contents. In this work, using UV light had a UV index of 15 mW/cm^2, which was much greater than UV light in nature six thousand folds. Thus, the extracts were irradiated under UV light for 2 hr, which referred to the extracts being exposed to sunlight for 1000 hr.

It is well known that lignin has been promoted as a safer and biodegradable alternative to synthetic sunscreen. The UV performance of lignin in different structures with various functional chromophore groups and interunit linkages is responsible for UV absorption. Lignin contains UV-absorbing chromophore groups such as carbon-carbon double bond, aromatic ring, carbonyl, and other chromophores[13, 14]. Hence, it can be indicated that the CBS-LE-T extracts possess good thermal stability and adequate photostability for application in sunscreen cosmetic fields.
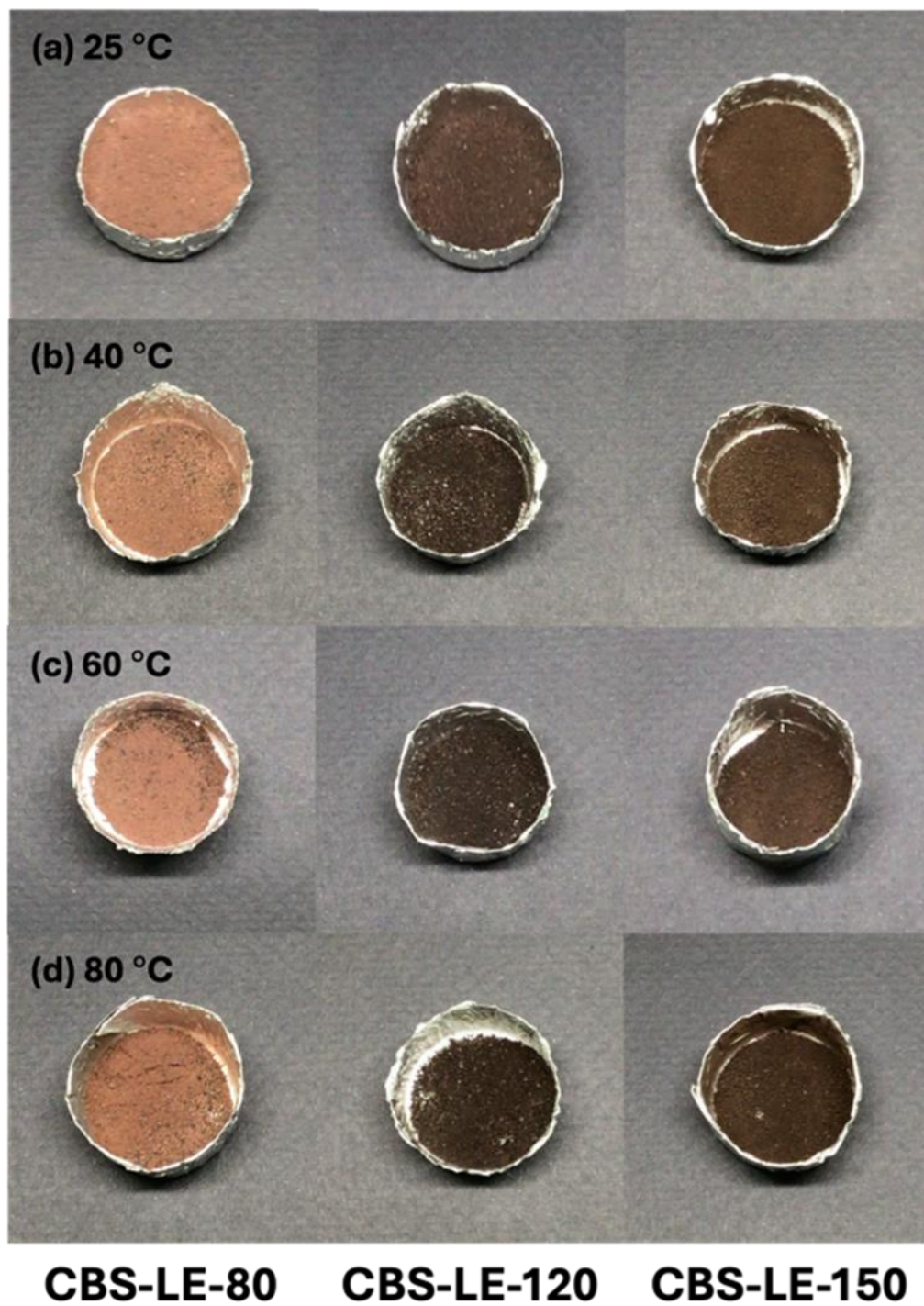
Figure 4 The color of lignin extracts prepared from cocoa bean shells at temperatures of 80 °C (CBS-LE-80), 120 °C (CBS-LE-120), and 150 °C (CBS-LE-150) was observed following storage at 25 °C, 40 °C, 60 °C, and 80 °C for 2 hr.

Figure 5 The color of lignin extracts prepared from cocoa bean shells (CBS) at temperatures of 80 °C (CBS-LE-80), 120 °C (CBS-LE-120), and 150 °C (CBS-LE-150)

3.4 Elemental analysis

The CBS material and CBS-LE-T extracts were analyzed for their elemental contents using Energy Dispersive X-ray Spectroscopy (EDS). The elemental contents including carbon, oxygen, nitrogen, and chlorine, are shown in **Table 3**. The CBS-LE-T extracts had higher carbon, lower oxygen, and lower nitrogen contents than the CBS material. The initial nitrogen in the CBS material is from cell proteins[57] and greatly declined in CBS-LE-T extracts at increasing extraction temperatures. The small remaining chlorine content in the CBS-LE-T extracts was due to using ChCl in the DES treatment. The results indicate that the cleavage of ether bond linkages during the DES treatment at different temperatures led to increased carbon and decreased oxygen and nitrogen contents in CBS-LE-T extracts[38]. This indicates that DES was efficient for lignin extraction and provided low-nitrogen and low-chlorine CBS-LE-T extracts, which were low toxicity and made them suitable for cosmetic applications, including sunscreen products[58]. Moreover, The safety of different lignins was demonstrated through cytotoxicity tests using two cell lines including HaCaT human keratinocyte and 3T3 murine fibroblast cell lines, which proved lignin is safe and able to be used in cosmetic and pharmaceutical fields[59].

Table 3 Elemental analysis of the cocoa bean shells (CBS) and its lignin extracts prepared at 80 °C (CBS-LE-80), 120 °C (CBS-LE-120), and 150 °C (CBS-LE-150).

Samples	Elemental composition* (%w/w)			
	C	O	N	Cl
CBS	59.8 ± 1.3^c	35.2 ± 2.5^a	5.1 ± 1.2^a	0.1 ± 0.1^b
CBS-LE-80	70.1 ± 1.3^b	24.4 ± 1.1^b	5.2 ± 0.1^a	0.4 ± 0.1^a
CBS-LE-120	72.4 ± 1.1^{ab}	24.1 ± 0.8^b	3.0 ± 0.4^b	0.5 ± 0.1^a
CBS-LE-150	75.3 ± 1.5^a	21.9 ± 1.7^b	2.5 ± 0.4^b	0.4 ± 0.1^a

Note: Different superscripts of each content indicate statistical differences ($p \leq 0.05$).
* The elemental contents in the samples were based on their weight

Apart from several advantages, DES has some disadvantages including high viscosity[60] which can be achieved by operating at suitable temperatures. From the above results, extraction yield increased with higher temperatures. Representative information on the effect of temperature on lignin extraction is: (i) The increasing temperature affects the reducing viscosity of DES, resulting in greater heat and mass transfer. (ii) The high temperature activates the degradation of the β-O-4 and aryl ether linkages in lignin derivative molecules. (iii) The enhanced temperature induces the condensation reaction in lignin macromolecules containing aromatic rings through carbon-carbon double bonds[38,50,52]. As previously described, this suggests that the reaction temperature has an impact on the DES capacity to extract lignin from CBS. Furthermore, the extraction temperature of 120 °C was enough to provide lignin with a high yield and purity from CBS.

Additionally, these rising temperature conditions led to the dark color of the extracts. Optimal extraction conditions at 120 °C provided a 17.4% yield with a lignin content exceeding 75% from CBS. Fourier Transform Infrared (FT-IR) spectroscopy confirmed the unique lignin structure, characterized by benzene rings, hydroxyl, and carboxyl groups. Additionally, the extracts from CBS exhibited robust thermal stability and adequate photostability. Future investigations will explore specific applications of these CBS-LE-T extracts, particularly in industries necessitating natural UV filter utilization, such as the sunscreen cosmetic field. The CBS-LE-T extracts will be evaluated in terms of UV-to-blue-light protection as an alternative option to synthetic UV filters and might be investigated for synergism with conventional UV filters.

4. CONCLUSIONS

The study demonstrates the efficacy of using a deep eutectic solvent (DES) composed of choline Chloride (ChCl) and lactic acid for lignin extraction from *Theobroma cacao* L. c.v. I.M.1. The increased temperatures did not affect the lignin content of either the extracts from cocoa pod shells (CPS) (54.7-59.5%) or the extracts from cocoa bean shells (CBS) (76.4-79.7%) but they greatly enhanced their yield.

5. ACKNOWLEDGEMENTS

Author contribution

Assoc. Prof. Tasana Pitaksuteepong was the Principal Investigator of the project, supervised Miss Supika Arkhasuwan, and critically reviewed and edited the manuscript. Miss Arkhasuwan was the graduate student who conducted the experiments, analyzed data, and prepared the manuscript in consultation with Assoc. Prof. Pitaksuteepong. All authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declare that they hold no competing interests.

Funding

None to declare.

Ethics approval

None to declare.

Article info:

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