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Effect of ternary deep eutectic solvents on delignification of stone pine cone

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Abstract: Due to their cost-effectiveness and environmentally friendly nature, deep eutectic solvents (DESs) hold great potential for applications in biomass conversion and the production of green chemicals. In this study, the delignification of the stone pine (*Pinus pinea* L.) cone was performed using seven different ternary deep eutectic solvents (TDESs). TDES treatments of stone pine cone samples were carried out in a microwave for 30 min. at 150 °C. The two-based components of TDESs were choline chloride (ChCl - 1 mol) and lactic acid (LA - 9 mol). The formic acid (FA – 2 mol), boric acid (BA – 1 mol), acetic acid (AA – 2 mol), sorbitol (S – 1 mol), triethylene glycol (TEG – 2 mol), ethylene glycol (EG – 2 mol), and glycerol (G – 2 mol) were used as third component of TDES. ChCl:LA:BA gave the lowest solid residue yield (57.90%) and highest lignin purity (86.89%). Klason lignin content of control was 35.08%. The lowest lignin content (19.42%) and highest delignification (68.89%) were obtained with ChCl:LA:FA treatment. The lowest and the highest L* values were obtained from ChCl:LA:BA and ChCl:LA:EG treatments with 21.76 and 37.36, respectively. This results showed that the third component of TDES affects the delignification efficiency of stone pine cone.
Keywords: Delignification, Lignin color, Lignin purity, Stone pine, TDES

Üçlü derin ötektik çözücülerin fıstık çamı kozalağının delignifikasyonuna etkisi

Özet: Uygun maliyetli olmaları ve çevre dostu doğaları nedeniyle derin ötektik çözücüler (DÖÇ), biyokütle dönüşümü ve yeşil kimyasalların üretimindeki uygulamalar için büyük potansiyele sahiptir. Bu çalışmada, Fıstık çamı (*Pinus pinea* L.) kozalaklarının delignifikasyonu yedi farklı üçlü derin ötektik çözücü (ÜDÖÇ) kullanılarak gerçekleştirildi. Fıstık çamı kozalağı örneklerinin ÜDÖÇ işlemleri mikrodalgada 150 °C'de 30 dakika süreyle gerçekleştirildi. ÜDÖÇ'lerin iki bazlı bileşenleri kolin klorür (ChCl - 1 mol) ve laktik asittir (LA - 9 mol). ÜDÖÇ'in üçüncü bileşeni olarak formik asit (FA – 2 mol), borik asit (BA – 1 mol), asetik asit (AA – 2 mol), sorbitol (S – 1 mol), trietilen glikol (TEG – 2 mol), etilen glikol (EG –2 mol) ve gliserol (G - 2 mol) kullanıldı. ChCl:LA:BA en düşük katı kalıntı verimini (%57,90) ve en yüksek lignin saflığını (%86,89) verdi. Kontrolün Klason lignin içeriği %35.08 idi. En düşük lignin içeriği (%19,42) ve en yüksek delignifikasyon (%68,89) ChCl:LA:FA işlemiyle elde edildi. En düşük ve en yüksek L* değerleri sırasıyla 21,76 ve 37,36 ile ChCl:LA:BA ve ChCl:LA:EG işlemlerinden elde edildi. Bu sonuçlar, ÜDÖÇ 'ün üçüncü bileşeninin fıstık çamı kozalağının delignifikasyon verimini etkilediğini göstermiştir.
Anahtar kelimeler: Delignifikasyon, Lignin rengi, Lignin saflığı, Fıstık çamı, ÜDÖÇ

1. Introduction

The stone pine (*Pinus pinea* L.) stands out as one of the distinctive tree species in the Mediterranean landscape due to its unique umbrella-like shape and the historical consumption of its sizable, nut-like seeds as food by humans (Mutke et al., 2012). The stone pine covers around one million hectares in countries surrounding the Mediterranean e.g. 193 000 ha in Portugal and 176 732 ha in Türkiye (Mutke et al., 2017; OGM, 2021; Costa et al., 2023). Spain accounts for around half of the spread of stone pine worldwide. It also spreads in Italy, France, Lebanon, Algeria, Tunisia, and Morocco (Mutke et al., 2019).

Cones, the reproductive organs of coniferous species, are regarded as forest waste and, over time, get mixed with the soil on the forest floor (Gulsoy and Ozturk, 2015). The average cone production in stone pine stands is between 200 and 1000 kg/ha, which equates to 36 to 180 kg/ha of kernels and 8 to 40 kg/ha of pine nuts per year (Sbay and Hajib, 2016). The most significant edible seed and one of the most important non-wood products acquired from the Mediterranean woodlands are pine nuts from stone pine. Pine nuts are a highly expensive crop, with current retail values surpassing 100 €/kg and a growing market outside of the region (Calama et al., 2016). Pine nuts are extracted from cones and sold due to their economic importance. The remaining woody part of the cone is often burnt to generate heat and energy. The use of these parts in the manufacturing of wood-based composites was examined (Ayrilmis et al., 2009; Buyuksari et al., 2010). Also, the suitability of cone fibers for pulp and paper production was evaluated by Gülsoy et al., 2017.

Lignin is a complex polymer found in the cell walls of plants, particularly in woody tissues. It acts as a binding agent, providing structural support and rigidity to the plant. After cellulose, lignin ranks as the second most prevalent natural polymer on Earth. Lignin is an important component in several industrial processes. It is a major byproduct of the pulp and paper industry, where lignin is separated from cellulose fibers during the production of paper (Fengel and

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Wegener, 1989). Lignin can be used in binders and adhesives, concrete additives, dispersants and emulsifiers, carbon fiber production, fuel and energy, specialty chemicals (vanillin), agricultural applications (biostimulants), water treatment (flocculants). Cones contain more lignin than many wood species (Dönmez et al., 2012; Gonultas and Balaban Ucar, 2013; Gulsoy and Ozturk, 2015).

DESs were discovered at the beginning of the century by combining two or more components. DESs with two components are referred to as binary DES (BDES), while those with three components are referred to as ternary DES (TDES). DESs are low-cost, non-toxic, ecologically friendly, and biodegradable (Zhang et al., 2012). These benefits make them potential solvents for biomass pretreatment or extraction (Sumer and Van Lehn, 2022). Therefore, DES is a growing significant interest. The rising importance of DES can be observed from the increasing publication number in the last decade from the Web of Science (2023) (Figure 1). During the last decade, DESs have been extensively studied in biomass fractionation (Li et al., 2017; Pan et al., 2017; Xu et al., 2018; Lyu et al., 2018; Kwon et al., 2020; Yan et al., 2023) and pulping (Choi et al., 2016a; 2016b; Smink et al., 2019; Gülsoy et al., 2022a; 2022b; Gülsoy, 2023).

There are several factors in biomass DES treatment, such as treatment temperature, treatment time, solid/liquid ratio, DES components and their mole ratios, etc. DES components are a key factor that influences DES efficiency in biomass pretreatment. ChCl is extensively studied HBA (Hydrogen Bond Acceptor) in DES treatments. Alanine, betaine, proline, histidine, and ethyl ammonium chloride are other HBAs used compound to prepare DES. The most commonly used HBDs (Hydrogen Bond Donor) in DES preparation are amides (urea, acetamide, etc.), acids (lactic acid, acetic acid, etc.), alcohols (ethylene glycol, glycerol, etc.), carbohydrates (glucose, xylose, etc.).

There have been limited studies on the use of TDESs in biomass treatment, despite the fact that BDESs have received a lot of attention. Yan et al. (2023) synthesized lignin nanoparticles from rice straw using microwave-assisted lignocellulose fractionation with TDESs (ChCl, OA, and LA in a 1:0.5:1 ratio). The TDES efficiently fractionated rice straw under microwave irradiation (680 W) in about 4 minutes, and 63.4% of lignin was removed from the rice straw with 86.8% purity. Ee et al. (2023) investigated the effect of two different TDESs on delignification of rice husk. They discovered that at 100 °C and 3 hours, the delignification rate of alanine/lactic acid/ethylene glycol was 79.3%, whereas the delignification rate of lactic acid/tartaric acid/choline chloride was 71.6%. In their study, Ji et al. (2020) subjected garlic skin and green onion root to TDES treatment for the purpose of lignocellulose fractionation and cellulose enzymatic hydrolysis.

To the best of our knowledge, the effect of DES components on Scots pine cone TDES treatment efficiency has not previously been evaluated. Scots pine cone was treated with seven TDESs (ChCl:LA:FA, ChCl:LA:AA, ChCl:LA:BA, ChCl:LA:S, ChCl:LA:TEG, ChCl:LA:EG, and ChCl:LA:G) at 150 °C for 30 min. The effects of the third component of the TDES on solid residue yield, delignification, lignin yield, lignin purity, and lignin color were studied.

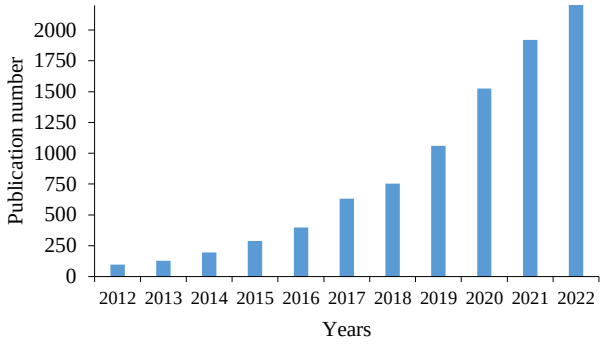

Figure 1. The number of research using DES published from 2012 to 2022 according to the Web of Science Database Platform (Web of science, 2023)

2. Materials and methods

2.1. Materials

Stone pine cones were obtained from İzmir Province, Türkiye. Nuts were removed before it was grounded. A Wiley mill was used to grind the cone samples, and a Retsch AS 200 sieve shaker was used to screen it. Samples remaining on a 60 mesh sieve were used. ChCl (CAS: 67-48-1, Sigma Aldrich), LA (CAS: 79-33-4, Sigma Aldrich), FA (CAS: 64-18-6, Merck), AA (CAS: 64-19-7, Merck), G (CAS: 56-81-5, Merck), EG (CAS: 107-21-1, Merck), TEG (CAS: 112-27-6, Merck), S (CAS: 50-70-4, ZAG Chemistry, Türkiye), and BA (CAS: 10043-35-3, Eti Mine, Türkiye) were purchased from relevant suppliers.

2.2. Preparation of TDES

The two components of TDES mixture were prepared with a molar ratio of 1:9 of ChCl and LA. The third components and their molar ratios of TDESs are given in Table 1. The mixture was heated at 70 °C with continuous stirring for 30 minutes until a clear solution formed. To minimize moisture absorption, it was then cooled to room temperature in a glass desiccator.

2.3. Characterization of TDESs

The viscosity of TDESs at 25 °C was measured with a AND SV-10 viscometer. Milwaukee MW805 was used to measure the electrical conductivity of TDESs at 25 °C. The density of TDESs at 25 °C was determined via a pycnometer.

Table 1. TDES components and their molar ratios

HBA	HBD1	HBD2	Molar ratios
ChCl	LA	FA	1:9:2
ChCl	LA	BA	1:9:1
ChCl	LA	AA	1:9:2
ChCl	LA	S	1:9:1
ChCl	LA	TEG	1:9:2
ChCl	LA	EG	1:9:2
ChCl	LA	G	1:9:2

2.4. TDES treatment of stone pine cone

In all TDES treatments of stone pine cone sample, a microwave (Milestone Start D, Italy) was used. The TDES treatments were carried out using a mixing ratio of 1:15 (3.0 g of dry biomass and 45.0 g of TDES) in a Teflon® sample holder of 100 mL capacity. To reduce temperature variations, the sample holders were rotated around a central axis within a carousel. Additionally, the microwave utilized a fiber optic temperature sensor to ensure precise temperature control. The pretreatment temperature was raised from room temperature to 150 °C over 10 min and held for 30 min. After the TDES pretreatment, the sample holder was filled with 50 mL of ethanol. The solid residue was then filtered through a medium porosity (2 number) Gooch crucible and washed twice with ethanol. The solid residue was then dried in a 103 °C oven. To recycle ethanol, the filtrate was evaporated in a rotatory evaporator at 45 °C. Following that, the remaining solution was supplemented with 500 mL of deionized water to facilitate the separation of lignin. The lignin samples were filtered using a Whatman No. 42 filter paper and washed with a 1/9 ethanol/distilled water (v/v) solution. Finally, the lignin samples were dried in a freeze-dryer.

2.5. Determination of Klason lignin and solid residue yield

The Klason lignin content in both the control and TDES-treated samples was determined using the TAPPI T 222 standard method. After TDES treatment, the solid residue yield was calculated as a percentage of the original weight.

2.6. Determination of lignin purity and delignification ratio

Lignin purity was calculated as a percentage of the mass of the extracted lignin sample using Klason lignin. The delignification ratio was calculated according to the following formula 1 (Liu et al., 2019).

$$Delignification\ (\%) = \left[1 - \frac{m2*c2}{m1*c1}\right] * 100 \quad (1)$$

where m1 denotes the dry weight of the sample used in the DES treatment (g), c1 denotes the Klason lignin ratio of the control sample (%), m2 denotes the dry weight (g) of the remaining sample after DES treatment (g), and c2 denotes the Klason lignin ratio of DES-treated sample (%).

2.7. Color of lignin

The color of lignin samples was evaluated using a Konica Minolta CM-700d spectrophotometer and three replicates according to the CIE Lab system. The color coordinates include the lightness L* (ranging from 0 for black to 100 for white), redness a* (with negative values for green and positive values for red on the green-red axis), and yellowness b* (with negative values for blue and positive values for yellow on the blue-yellow axis).

3. Results and Discussion

3.1. Physical properties of TDES

The viscosity, density, and electrical conductivity values of prepared TDES are listed in Table 2. Lower viscosity and density of DES allow better penetration into the lignocellulosic material during DES treatment (Jablonsky et al., 2019). Thus, the delignification efficiency of DES increases. On the other hand, DESs have poor conductivity (lower than 2 mS/cm) (Zhang et al., 2021). Microwave-assisted DES treatment is generally used to increase delignification. In microwave heating, DESs with high electrical conductivity cause the effective delignification of lignocellulosic material (Muley et al., 2019; Kohli et al., 2020). ChCl:LA:S had the highest viscosity (151.0 cp) and density (1.2338 g/cm^3), and the lowest ionic conductivity (565 µS/cm). ChCl:LA.TEG had lowest viscosity (27.0 cp) and density (1.1757 g/cm^3). The highest ionic conductivity determined in ChCl:LA.AA with 2 955 µS/cm.

3.2. Klason lignin and solid residue yield

Figure 2 depicts the influence of TDES treatment time on the Klason lignin content of a cone sample. It was observed that the third component used in TDES treatment had a significant effect on the Klason lignin values of the cone sample. The Klason lignin content was reduced after all TDES treatments. The lowest Klason lignin content observed in ChCl:LA:FA treatment with 19.42%. ChCl:LA:EG treatment resulted in the highest Klason lignin content (27.83%). Acids (FA, AA, and BA) delignified better than polyols (S, TEG, EG, and G) when utilized as the third component in TDES (Figure 2). Klason lignin content of stone pine cone (control) determined as 35.08%. This value was agree with literature. Dönmez et al. (2012) noted that Klason lignin content of stone pine cones was 39.29%. Gonultas and Ucar, (2013) reported that Klason lignin contents of stone pine cones grown in different regions are between 33.47% and 37.23%.

Table 2. Physical properties of TDESs (at 25 °C)

TDES	Molar ratios	Viscosity (cp)	Density (g/cm^3)	Ionic Conductivity (µS/cm)
ChCl:LA:FA	1:9:2	28.0	1.1990	1915
ChCl:LA:BA	1:9:1	45.2	1.1872	1079
ChCl:LA:AA	1:9:2	59.6	1.2105	2955
ChCl:LA:S	1:9:1	151.0	1.2338	565
ChCl:LA:TEG	1:9:2	27.0	1.1757	1590
ChCl:LA:EG	1:9:2	34.7	1.1902	1574
ChCl:LA:G	1:9:2	47.3	1.2063	1230

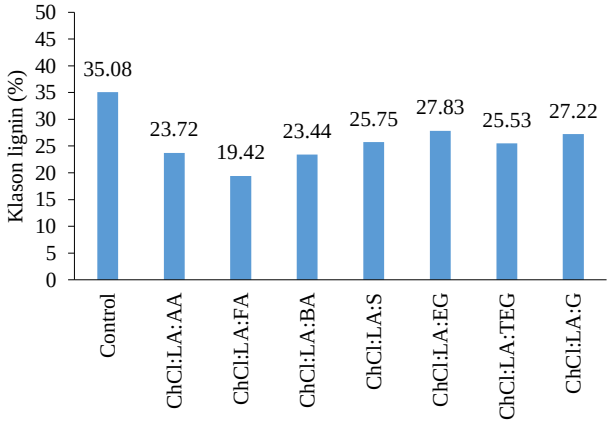

Figure 2. Effect of TDES type on Klason lignin content

Figure 3 depicts the influence of TDES treatment on solid residue yield in cone sample. It was observed that the third component used in TDES treatment had a significant effect on the solid residue yield of TDES treatment. The lowest and highest solid residue yield values were observed in ChCl:LA:BA and ChCl:LA:TEG with 57.90% and 74.50%, respectively. Solid residue yield shows the effectiveness of TDES treatment. High value means low efficiency, low value means high efficiency. In this context, ChCl:LA:BA is the most effective TDES treatment, while ChCl:LA:TEG is the least efficient TDES treatment. According to Lu et al. (2022), the residual solid yield of corn stover treated with TDES (ChCl: FA, and maleic acid) was reduced with longer treatment time (1, 1.5, 2, 2.5, and 3 h) and higher temperature (90, 100, 110, 120, and 130 °C).

3.3. Lignin purity and delignification ratio

Figure 4 depicts the influence of TDES treatment on lignin purity. It was observed that the third component used in TDES treatment had a significant effect on the purity of lignin. The purity of lignin obtained from different TDESs is between 77.63% (ChCl:LA:TEG) and 86.89% (ChCl:LA:BA). Lu et al. (2022) reported that lignin purity of corn stover treated with TDES (ChCl: FA, and maleic acid) was increased with longer treatment time and higher temperature.

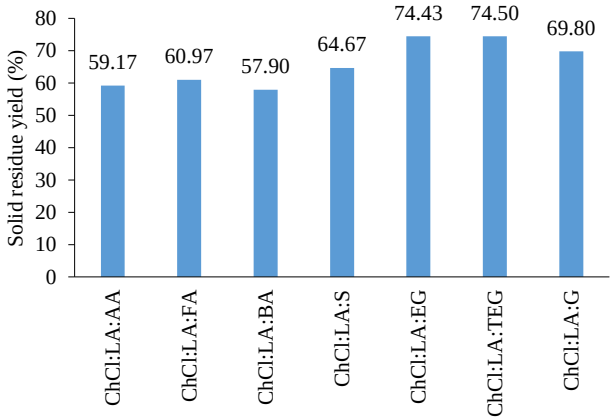

Figure 3. Effect of TDES type on solid residue yield

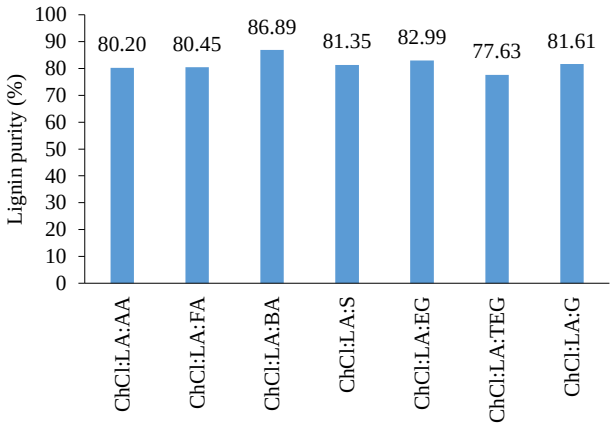

Figure 4. Effect of TDES type on lignin purity

The effect of TDES treatment on the delignification ratio was shown in Figure 5. It was observed that the third component used in TDES treatment had a significant effect on the delignification. The highest delignification ratio was obtained in ChCl:LA:FA treatment with 68.89%, while the lowest delignification ratio was determined in ChCl:LA:TEG treatment with 45.55%. When acids (FA, AA, and BA) were used as the third component in TDES, they delignified better than polyols (S, TEG, EG, and G) (Figure 5). Lu et al. (2022) noted that the highest delignification ratio of corn stover TDES (ChCl: FA, and maleic acid) treatment was obtained in 130 °C for 3 h (79.4%).

3.4. Color of lignin

Figure 6 indicates lignin samples obtained from TDES-treated stone pine cone. Figure 7 depicts the L*, a*, and b* values of lignin samples. The L*, a*, and b* values of lignin samples affected from third component used in TDES treatment. The lowest and the highest L*, a*, and b* values were 21.76 (ChCl:LA:BA) and 37.36 (ChCl:LA:EG), 5.26 (ChCl:LA:BA) and 11.81 (ChCl:LA:S), and 1.59 (ChCl:LA:G) and 11.85 (ChCl:LA:EG), respectively. Lu et al. (2022) noted that the L* values of lignin samples obtained from TDES (ChCl: FA, and maleic acid) treatment at 110 °C of corn stover samples were 62.96, 57.66, and 52.14 for 1 hour, 2 hours, and 3 hours TDES treatment times, respectively. Wu et al. (2023) have recently examined how different organic solvents (methanol, ethanol, and acetone) affect the separation of alkali lignin. Their findings revealed that the alkali lignin (control) has L*, a*, and b* values of 46.09, 10.27, and 17.84, respectively. Moreover, the most lightly colored lignin (L*: 59.27) was obtained from the lignin fractions extracted using acetone.

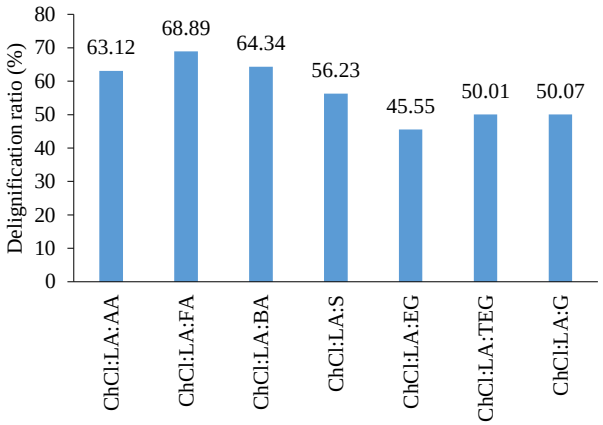

Figure 5. Effect of TDES type on delignification ratio

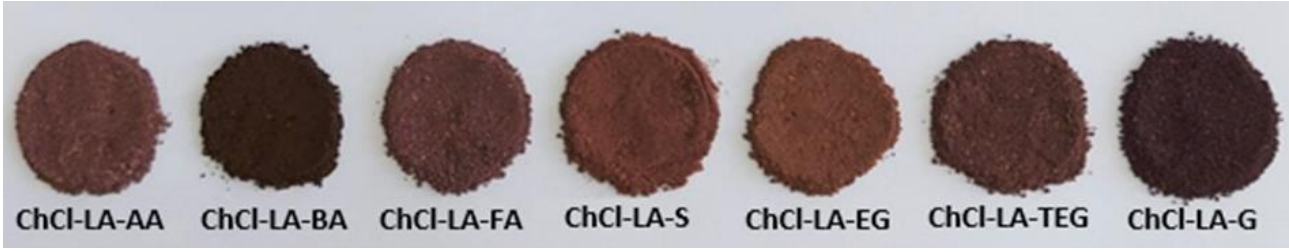

Figure 6. Lignin samples obtained from TDES-treatment of stone pine cone

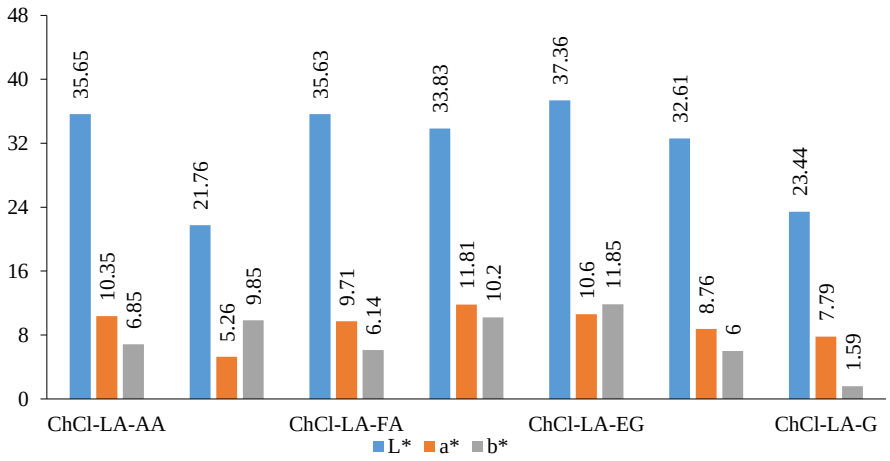

Figure 7. Optical properties of lignin samples

4. Conclusions

The results demonstrated that the third component used in TDES treatment of stone pine cone had a significant effect on solid residue yield, delignification, lignin yield, lignin purity, and lignin color. ChCl:LA:BA treatment exhibited the lowest solid residue yield (57.90%) and the highest lignin purity (86.89%). The control sample had a Klason lignin content of 35.08%. The ChCl:LA:FA treatment yielded the lowest lignin content (19.42%) and the highest delignification efficiency (68.89%). The ChCl:LA:BA and ChCl:LA:EG treatments produced the lowest (L*: 21.76) and highest (L*: 37.36) L* values, respectively. Obtaining light-colored lignin in less time is critical, particularly for lignin uses in the cosmetic sector. These results indicate that the third component of the TDES has an impact on the delignification efficiency of stone pine cone.

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