We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

5,000
Open access books available

125,000
International authors and editors

140M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK
CITATION
INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Use of Deep Eutectic Solvents in the Treatment of Agro-Industrial Lignocellulosic Wastes for Bioactive Compounds

Ayşe Ezgi Ünlü and Serpil Takaç

Abstract

Lignocellulose is the most abundant component in nature since it refers to plant material. Beyond the enormous utilization of lignocellulose by human being, unignorable amount of waste is also formed simultaneously. Agro-industrial lignocellulosic wastes can cause environmental pollutions if not processed before discharged. An innovative approach for lowering the detrimental influences of lignocellulosic wastes is to consider them as a source of useful products rather than a waste to be decontaminated. Beyond the conventional techniques for evaluation of the wastes, new emerging techniques and the use of new solvents have drawn attention recently. Among new generation solvents, deep eutectic solvents (DESs) have been increasingly used in the treatment of lignocellulosics to produce value-added products such as biofuels, chemicals, and solvents and also used for the recovery of bioactive phenolic compounds. DESs are used extensively for fractionation of lignocellulosic wastes, often in combination with enzymatic hydrolysis of the biomass. On the other hand, extraction and recovery of bioactive compounds are also under research using DESs. This mini review summarizes the very recent literature reports on the use of DESs in treating agro-industrial wastes within the concept of valorization of biomass.

Keywords: agro-industrial wastes, bioactive phenolic compounds, deep eutectic solvents, lignocellulosic biomass, pretreatment

1. Introduction

Along with the increase of the global consumption manner of the humanity, the general waste amount has been increasing significantly. Global municipal solid waste estimated to increase to 2.2 billion tons annually by the third decade of 2000 [1]. The accumulation of this huge amount of waste creates tedious environmental problems such as the generation of greenhouse gases along with the physical appearance. Despite the studies on the recycling and recovery processes, landfill is still commonly used procedure for the waste disposal in many countries [2].

The main constituent of the municipal solid waste is the lignocellulosic waste having a percentage of 29 [3]. The lignocellulosic waste consists of paper, garden

waste, wood, food, and also agricultural wastes. In this chapter, we will focus on the agricultural lignocellulosic waste. A general classification for lignocellulosic waste consists of three subclasses [4], namely, wood leftovers, farming crops, and secondary biomass. Logging leftovers, wastes from pulp, and paper industry are the subclasses of wood leftovers, whereas grasses, short rotation crops, as well as oil and grain crops belong to farming crops. On the other hand, secondary biomass has also two subclasses, namely, municipal solid wastes and food processing wastes.

Lignocellulose represents the matter of plants in general terms. It is the most abundant sustainable carbon source, and the main constituent is lignin that consists of complex organic polymers. Agricultural lignocellulosic biomass is composed of ~35–50% cellulose, 20–35% hemicellulose, and 10–25% lignin [5]. Lignin forms the plant cell walls providing the mechanical endurance to the plant (**Figure 1**). They are mainly composed of monolignols that are methoxylated derivatives of benzene.

The carbohydrates found in the lignin structure are cellulose (**Figure 2**) and hemicellulose (**Figure 3**) that are covalently and hydrogenically bonded to lignin molecules. As a linear-chain polysaccharide, cellulose is made up of D-glucose monomers that are linked with ß-1-4 glycosidic bonds [6]. Hydrogen bonding interactions are present between linear chains that are found in microfibrils [7], and cellulose has several types of crystalline structure. This complex structure provides the rigid and recalcitrance to dissolution of cellulose. Hemicellulose is structurally similar to cellulose as it also consists of polysaccharides, but it has a lower chain amount. On the other hand, hemicellulose contains branched heteropolymer consisting of pentoses – mostly D-xylose and D-arabinose; hexoses – mostly D-mannose, D-glucose, and D-galactose; and sugar acids – mostly 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and D-glucuronic acid. Lignocellulosic biomass also contains pectins, proteins, extractives, and ash in low amounts [8]. Lignin has a three-dimensional structure holding the lignocellulosic structure together, and it is water insoluble [9, 10]. Beyond the massive
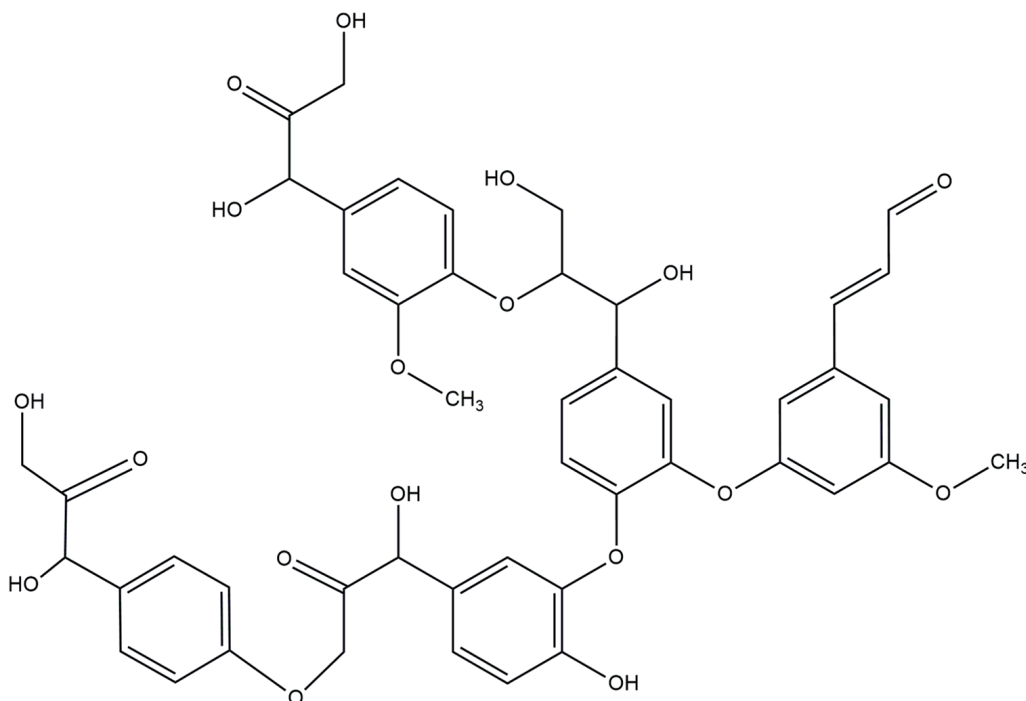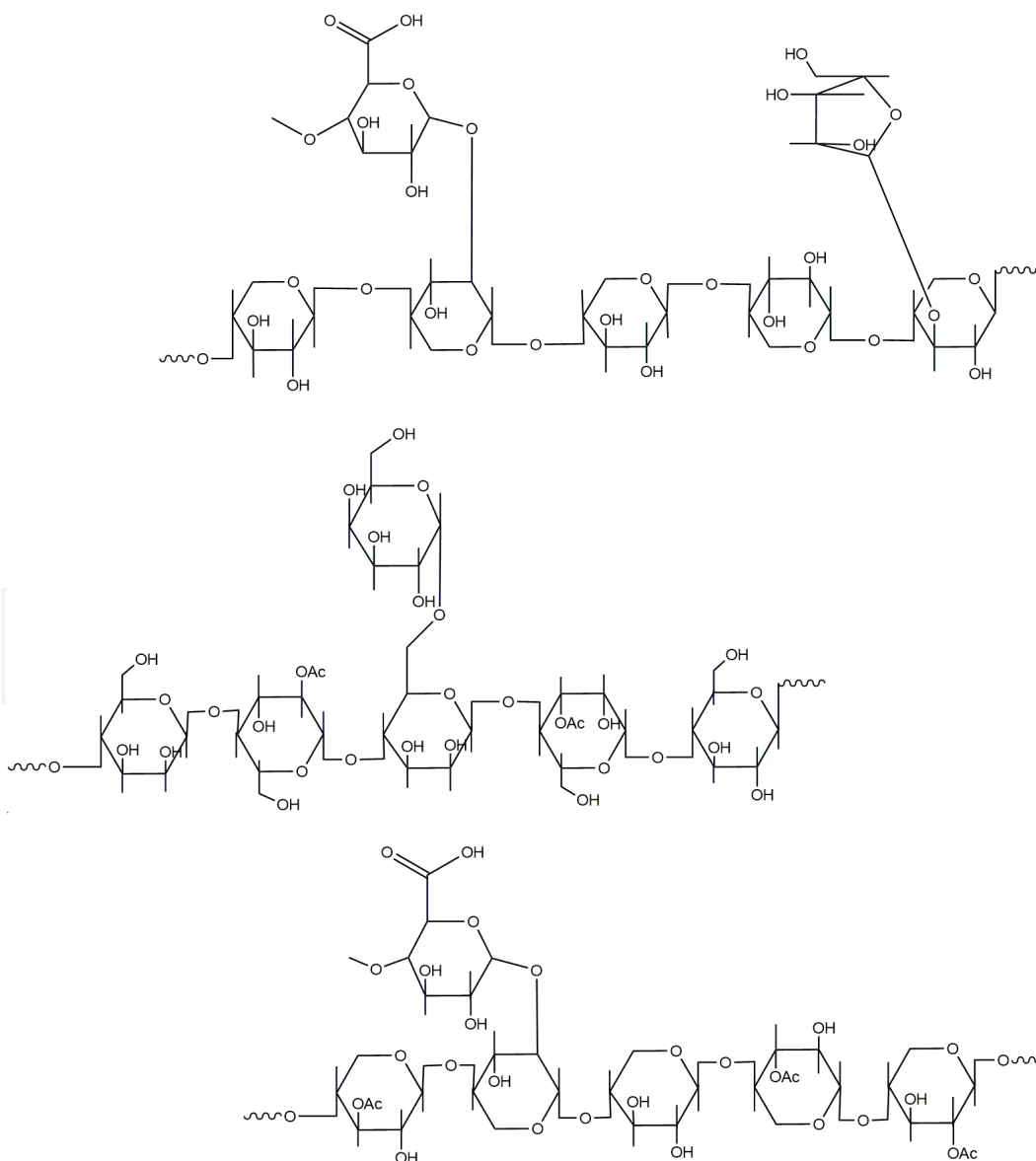

Figure 1.
Chemical structure of lignin.

common information we know about lignin structure, new articles show us [11] that there are still many things to be clarified [12].
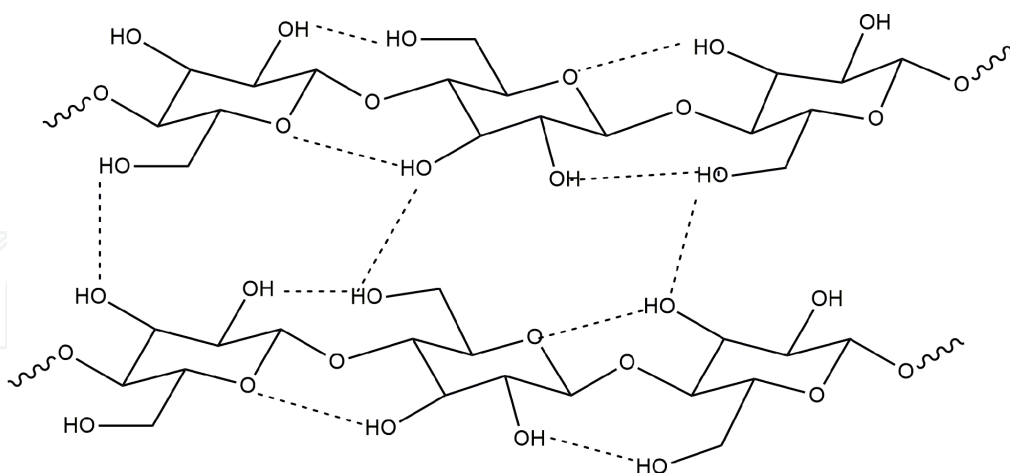
Figure 2.
Chemical structure of cellulose with the schematic illustration of the hydrogen bonding between monomers.

Figure 3.
Three common structures of hemicellulose.

Since lignocellulosic biomass is the most abundant natural source in the world, it may be evaluated as an alternative to unsustainable sources in many aspects. On the other hand, the wastes formed by lignocellulosic materials cause environmental problems arising from organic constituents with high COD and BOD degrees. Most of the lignocellulosic wastes contain phenolic compounds that may cause damage to the environment when discharged without any treatment [13]. These wastes may produce odor, soil pollution, and harborage for insects, if not processed further [14]. A promising approach to reduce the pollution problem of the lignocellulosic biomass is to use them as raw materials as a resource of value-added products such as biofuels (bioethanol, biogas, and biohydrogen), chemicals, and solvents [15] and also to use them for the recovery of bioactive phenolic compounds (flavonoids, phenolic acids, stilbenes, and tannins) [16]. According to the recent literature, the use of lignocellulosic biomass is encouraged as a natural source to be used in biotechnological process that will spontaneously lead to a decrease in the pollution effects of the waste. There are various methods to evaluate the lignocellulosic biomass such as fractionation or recovery of the valuable compounds. Besides conventional methods, the use of green techniques has gained a considerable attention due to environmentally friendly characteristics. In this mini review, the very recent literature on the use of deep eutectic solvents (DESs) in treating agro-industrial wastes, within the concept of valorization of biomass, is summarized.

2. Deep eutectic solvents

For both chemical and pharmaceutical processes, solvents are essential constituents. They are utilized in a broad range of fields including bulk chemicals, medicines, cleaning agents, dyes, and so on. The solvents used in such processes are mainly petroleum-based organic solvents, as well as ammonia and water. However, along with the increasing consciousness related to the environment, the solvents that are regarded as eco-friendly have been the focus for many researchers and are regarded as green solvents. Besides the formerly known green solvents such as bio-solvents and supercritical fluids, ionic liquids and lately deep eutectic solvents have been extensively utilized in various areas with an increasing trend. Additionally, green solvents are encouraged in many fields of research to promote sustainable processes [17].

DESs are one of the most popular green solvents that are mostly known as nontoxic, recyclable, and nonflammable, and they have low vapor pressures [18–20]. They can be easily prepared in the laboratory using numerous substances in different molar ratios that result in diverse properties of DES such as polar, non-polar, acidic, and basic. The most common method to prepare a DES is to mix the constituents in a certain molar ratio at a certain temperature until a homogeneous liquid form is obtained [18, 21] (**Figure 4**). In another method, the constituents (mostly solid) are mixed together with water, and subsequent evaporation of the excess water under vacuum is performed, which is called evaporation method [22]. Similar steps are followed for the freeze-drying method; indeed, water is removed by freeze-drying [23]. In grinding method, a glovebox in nitrogen atmosphere is used to grind the solid in a mortar till clear liquid is obtained [24]. If a novel DES to be formed for the first time, many tests should be performed to prove the "deep eutectic" property of the solvent. Otherwise, published articles' protocols should be followed exactly to synthesize DES in the correct form.

The very first description of DES was made by Abbot et al. as the liquid formed between a variety of quaternary ammonium salts and carboxylic acid. Later on,

Figure 4.
Choline chloride, urea, and clear liquid DES of ChCl-urea (1:2).

DESs were classified into four groups [25]: Type I: Organic salt + Metal salt; Type II: Organic salt + Metal salt hydrate; Type III: Organic salt + hydrogen bond donor (HBD); and Type IV: Metal salt + HBD. Lately, many researchers presented so many different DESs from so many different types of molecules that the definition of DES converged to a simple form: DESs are composed of two or more components, which in minimum two of them have a hydrogen bonding interaction ability: one as a HBD and one as a hydrogen bond acceptor (HBA) [26]. On the other hand, DESs that are formed by natural compounds such as organic acids, sugars, and choline chloride are called natural deep eutectic solvents (NADESs) [21]. NADESs may be classified as sugar based (glucose-fructose-water, glucose fructose-sucrose-water), polyol based, acid based, and so on. Since DESs can be formed by a number of components, physicochemical properties vary from type to type. Therefore, one can tune the physicochemical property by changing the type and the molar ratio of the constituents. Depending on the type of the constituents, viscosity of DESs may be low or high. High viscosity DESs are hard to be handled, but in some cases, they are preferred to be used as a mixture of alcohol and water to decrease the viscosity. DESs generally have low melting points. This is related to the hydrogen bond interaction between the constituents. Some DESs were reported to have a glass transition temperature [22, 24, 27]. Density ranges of 800–1600 kg/m^3 are presented in the literature, but in general, they have higher density than water [28–30]. On the other hand, hydrophobic DESs are reported to have lower density than hydrophilic DESs [31–33].

The use of DESs in different fields such as biochemistry, electrochemistry, synthesis, nanomaterials, separation, and metal processing [25, 34–38] has been increasing since 2003, when it was first described. Recently, they were shown to be used as solvents in many types of enzyme-catalyzed reactions such as esterification, transesterification, polymerization, and hydrolysis [39–43]. On the other hand, their catalytic effects in several different types of reactions have also been reported [39–41, 44–48]. In detail, the number of DES-related publications was more than 300 between 2009 and 2013, while it was only 29 until 2008 [49]. In 2017, the number of publications on DESs reached up to almost 750 [50].

3. Fractionation of agro-industrial wastes with deep eutectic solvents and recovery of lignin

In the studies carried out within the scope of sustainability, agricultural lignocellulosic wastes such as corn straw, rice straw, wheat straw, fruit wastes, and sunflower stalk have been subjected to various treatments prior to conversion processes. The most challenging step in such a process is the resistance of lignocellulosic material to degradation; therefore, a treatment method is required prior to

utilization. These methods can be chemical, physical, mechanical, physicochemical, or biological. In some cases, a combination of these methods is also preferred since each one has different advantages and disadvantages [5]. The most commonly used method is the chemical pretreatment; however, it has undesired environmental impacts. On the other hand, physical pretreatments require high energy, whereas biological pretreatments progress relatively slowly. Therefore, in addition to efficiency, cost, environmental impacts, and ease of use should be taken into consideration for the selection of the pretreatment method.

Ionic liquids as green solvents are effective and promising solvents in the pretreatment of lignocellulosic biomass [31, 51]. However, high prices and toxic properties limit their utilization in industrial applications [31]. Recently, DESs that have superiority to ionic liquids due to their low cost, low volatility, biodegradability, easy preparation techniques, and environmental friendliness have been successfully used in the pretreatment of lignocellulosic materials [50].

Casal et al. [52] were among the first researchers to report that the solubility of wheat stalk in DES was promising. Later on, Francisco et al. [53] studied the solubility of alkali lignin, cellulose, and starch in DESs prepared with choline chloride and carboxylic acid. They reported that lignin was soluble in DESs, whereas cellulose was nearly insoluble, which was a promising result. Among the tested eutectic solvents, the best result was obtained with ChCl-lactic acid (LA) (1:9). Afterward, several researchers treated agro-industrial lignocellulosics with DESs and reported satisfactory results. Procentese et al. [54] pretreated corncob with different choline chloride-based DESs and achieved a total of 41 g fermentable sugars from 100 g corncob after a subsequent enzymatic saccharification. The concentrations of inhibitory agents, that is, acetic acid and furfural were low following the pretreatment with DESs. The authors also reported that the decrease in lignin and hemicellulose contents increased the crystallinity index (CrI) of the pretreated biomass. Zhang et al. [55] pretreated corncob with DESs consisting of choline chloride as HBA and monocarboxylic acid, dicarboxylic acid, or polyalcohol as HBDs. SEM, XRD, and FTIR analyses of treated corncob showed that pretreatment with DESs disrupted the structure of biomass. Polyalcohol-ChCl was found to be more effective in lignin extraction than others. Kumar et al. [56] treated rice straw with lactic acid-betaine and lactic acid-ChCl NADESs and could extract high purity of lignin (>90%). They also reported that approximately 60% of lignin could be separated from the lignocellulosic material. Additionally, higher lignin solubility was achieved when lactic acid-ChCl was used in the treatment. The addition of water (5%) during pretreatment caused a further increase (about 22%) in the extracted amount of lignin. The authors also reported that the CrI of biomass decreased after pretreatment and that subtle structural differences were detected in the crystalline and also amorphous zones of the cellulosic portions. Procentese et al. [57] treated waste lettuce leaves with ChCl-glycerol and used the pretreated biomass sequentially in the enzymatic hydrolysis and acetone-butanol-ethanol fermentation. The authors reported that less energy was consumed with the use of DES than both NaOH and steam explosion pretreatment techniques for the same degree of fragmentation. In the study of rice straw pretreatment using DES, Hou et al. [58] reported that two-step pretreatment increased the yield of sugar by creating a synergism. The researchers found that the yield of glucose was 90.2% as a result of sequential ChCl-oxalic acid and ChCl-urea pretreatments, and also the addition of water during the process increased the yield. Procentese et al. [59] investigated the production of fermentable sugars from biomass by pretreating apple residues, potato peels, coffee silverskin, and brewer's spent grains with ChCl-glycerol and ChCl-ethylene glycol. The highest glucose yield was 0.20 with ChCl-glycerol and 0.19 with ChCl-ethylene glycol. Liu et al. [60] treated wheat

straw with triethylbenzyl ammonium chloride/lactic acid (TEBAC/LA)-based deep eutectic solvents under different conditions. The authors reported that the use of TEBAC/LA (1:9) at 373 K for 10 h provided the highest subsequent enzymatic hydrolyses of cellulose and xylan. About 80% removal of lignin was achieved using TEBAC/LA DES in the pretreatment. New et al. [61] investigated the effect of water content of ChCl-urea (1:2) on delignification of oil palm fronds and showed that aqueous DES provided more lignin removal than pure DES. The presence of 30% (v/v) water in DES was reported as the best amount for optimal delignification (16.31%). Ong et al. [62] used two-pot sequential pretreatment for oil palm fronds. They ultrasonicated the palm fronds in water and subsequently pretreated with ChCl-urea. The authors reported that the ultrasound pretreatment facilitated the degradation of lignin matrix by DES. The hydrogen bonding between the halogen component of ChCl and the hydroxyl groups of lignin was proposed to be a facilitation in the cleavage of ether or ester bonds among hemicellulose and lignin. At the optimum conditions (70% amplitude and 30 min), 36.42% of lignin removal and 58% of xylose recovery were achieved. Tan et al. [63] synthesized several DESs using ChCl and organic carboxylic acids and used them in the pretreatment of oil palm empty fruit bunch. It was reported that the presence of hydroxyl moiety and short alkyl chain enhanced the biomass fractionation and lignin extraction. ChCl-LA (1:15) and ChCl-formic acid (1:2) extracted more than 60 wt% of lignin. Fang et al. [64] proposed that a hydrothermal pretreatment could reduce the recalcitrance of lignocellulosic biomass if applied before a DES treatment. The hydrothermal pretreatment was performed at 200°C for 10 min with 10% dry matter loading. The results showed a consistency with the initial proposal. Both xylan and lignin removals were successfully enhanced around 25% during the treatment using ChCl-glycerol (1:2). Similar liquid hot water pretreatment was studied by Tian et al. [65] for the delignification of poplar wood shavings. To provide a mutual agreement for both hemicellulose recovery and solid yield, 170°C was preferred as temperature for the hot water extraction for 40 min. For the subsequent DES treatment step, acidic eutectics were prepared by using ChCl as HBA and formic acid, acetic acid, or lactic acid as HBDs in a molar ratio of 1:2. The hydrothermal processing together with DES treatment increased the lignin selectivity and also the porosity of the resulting cellulose. The ionic properties of the DESs were proposed to provide the selective lignin removal and cellulose deconstruction, thereby increasing cellulose chemical reactivity. A 79.8% of solid yield and 54.4% of hemicellulose removal were reported in the study. Chen et al. [66] aimed to obtain platform chemicals such as furfural, 2,3-butanediol by the pretreatment of switchgrass with ChCl-ethylene glycol. They reported that neat ChCl-ethylene glycol provided a removal of only 24% of lignin, while acidified form provided 87% removal. They also could enrich cellulose up to 72.6% in pretreated switchgrass with the solid loading levels of between 20 and 27%. At this high level of solid-loading efficient, removal of lignin and xylan was achieved. Lim et al. [67] synthesized new DESs using potassium carbonate and glycerol in different molar ratios. The most appropriate molar ratio was reported as 1:7 in terms of pH, viscosity, and thermal stability. They tested different parameters such as temperature (110–150°C), reaction time (40–120 min), and solid-to-liquid ratio (1:8–1:12) on the treatment of rice straw. They could achieve 73.8% cellulose under the optimum conditions that were a temperature of 140°C, a reaction time of 100 min, and a solid-to-liquid mass ratio of 1:10. CrI was reported to increase to 60% from 52.8% after the treatment. Wan and Mun [68] tested the use of different DESs [ChCl-urea (1:2), ChCl-citric acid (1:2), and ChCl-glycerol (1:1)] for the treatment of sago waste. The optimum pretreatment conditions were reported as 110°C and 3 h at 5% solid loading. According to the apparent structural disruption created by

ChCl-urea, it was selected as the DES to give the best result. The authors subsequently performed enzymatic hydrolysis to be mentioned in the next part. A distinct study presented the *in situ* synthesis of DES for the delignification of *Roystonea regia* leaves and leaf sheaths [69]. They claimed that DES could be formed when ChCl was added into water during hydrothermal processing by *in situ* polyhydrogen bonding. The deep eutectic structure was proposed to occur between ChCl as HBA and hemicellulose-derived acids (including formic, acetic, and glucuronic acids) and hydronium ions as HBDs. According to the results, 53.6% lignin removal was obtained for leaf sheaths, while 44.6% was obtained for *Roystonea regia* leaves. They also reported nearly a threefold increase in biomethane yield when *in situ* DES treatment was performed in comparison to hydrothermal processing. Shen et al. [70] reported a reduction in the *Eucalyptus camaldulensis* recalcitrance using ChCl-lactic acid DES. They also declared the preserved structure of important linkages and noncontaminated carbohydrates under the optimum conditions (110°C, 6 h, and 10% solid loading). On the other hand, the increase of lactic acid mole in the DES was reported to cause a relatively lower molecular weight of lignin. In another study, the authors presented the use of diluted alkaline hydrogen peroxide together with DESs in the delignification of oil palm fronds [71]. In the optimum sequential treatment procedure, oil palm fronds were added to 0.25% of alkaline hydrogen peroxide solution at 5% solid loading for 90 min followed by a ChCl-urea (1:2) treatment at 120°C, 4 h, and 10% loading. The authors reported 18.99% of delignification under the optimum conditions with sequential treatment, whereas only 12.16% could be achieved with sole DES treatment. Liu et al. [72] treated moso bamboo using ChCl (1:9) and investigated the effect of temperature (100–120°C), time (2–4 h), and solid-to-liquid mass ratio (1:15–1:25) as parameters in the experimental design. The optimal reaction conditions were determined as a temperature of 120°C, a time of 3 h, and a solid-to-liquid ratio of 1:25. The chemical composition of fibers was reported as 81.4% cellulose, 14.8% hemicellulose, and 3.0% lignin at the optimum conditions. Tan et al. [73] investigated the effect of six different DESs in the single-step fractionation and delignification process of oil palm empty fruit bunch. With this aim, they prepared ChCl-lactic acid (1:5), D-glucose-lactic acid (1:5), ChCl-D-glucose (1:1), ChCl-glycerol (1:2), ChCl-urea (1:2), and potassium carbonate-glycerol (1:6). According to the results, the pH of DESs was reported to have an important effect on the fractionation efficiency. ChCl-lactic acid provided 100% hemicellulose extraction, 88% delignification, and 50% lignin pellet extraction from oil palm empty fruit bunch. Kandanelli et al. [74] presented the utilization of DES together with different cosolvents, such as phloroglucinol, HCl, *n*-butanol, and ethyl acetate, for the efficient delignification of rice husk, rice straw, and wheat straw. They studied the effect of the type of cosolvent, DES-cosolvent ratio (2:1, 1:1, and 1:2), and temperature (50, 80, and 120°C) on the delignification process. The best cosolvent (*n*-butanol) was selected according to the miscibility with DES and also to lignin solubility. The highest delignification was achieved around ~50% using *n*-butanol-assisted DES [ChCl-oxalic acid (1:1) at a ratio of 2:1, with high solid loading of 15% (w/v) at 120°C (~1.2 bar) after 60 min]. They reported about a 2.3-fold increase in the delignification when the temperature increased from 80 to 120°C. A similar usage of DES together with alcohol was also studied by Jablonsky et al. [75] for the delignification of unbleached pulp. Additionally, they synthesized 23 new component DESs and screened for the utilization in the lignin extraction. The authors reported that the addition of alcohol was an advantage in the control of the density and viscosity of DESs. The structure and composition of lignin depended on the pH of the DES used. The best DES for the delignification was reported as malonic acid-ChCl-propanediol (1:1:3), providing 39.80% delignification. On the other hand, all DESs

were determined to be selective for cellulose. In another study, fractionation of beech wood polymers was investigated using ChCl-oxalic acid (1:1), ChCl-oxalic acid (1:2), ChCl-potassium hydroxide (1:4), ChCl-lactic acid (1:2), and ChCl-urea (1:2) [76]. The process parameters were investigated in the ranges of 2–24 h time, 60–100°C temperature, and 1:100–1:10 solid-to-liquid mass ratio. The effective DESs on the fractionation were determined as ChCl-oxalic acid (1:1), ChCl-oxalic acid (1:2), and ChCl-potassium hydroxide (1:4). The optimum values for the parameters were reported as 2.5% biomass loading, 6 h time, and 100°C temperature. Muley et al. [77] used microwave-assisted delignification for the treatment of pinewood sawdust. Three different DESs, namely, ChCl-oxalic acid (1:1), ChCl-lactic acid (1:1), and ChCl-formic acid (2:1), were used to test the effect of temperature (110, 130, and 150°C) and time (1, 5, 10, and 15 min) at 2450 MHz. ChCl-oxalic acid and ChCl-formic acid were reported to provide the highest lignin yield. The advantages of the microwave heating were reported as the diminished reaction time in addition to the promotion of selective bond cleavage during lignin depolymerization and a narrow molecular weight distribution. *Eucalyptus globulus* chips were used in the delignification process in which the effect of ChCl in the DES was investigated [78]. Experimental conditions used were a temperature of 120°C, a time of 8 h, and a liquid-to-solid ratio of 20:1. According to the results, chloride anion was determined as the active component of ChCl providing the increase in the cleavage rate of β-O-4 bonds and consequently increasing the delignification rate of biomass. Quek et al. [79] used ChCl-lactic acid, ChCl-glycerol, and ChCl-urea in the ultrasound-assisted pretreatment for the delignification of oil palm empty fruit bunch. The treatment was conducted at 50°C, 10% solid loading, and 240 W for 30 min. The lowest lignin content was obtained using ChCl-lactic acid as 18.8% followed by ChCl-glycerol and ChCl-urea as 19.4 and 21.2%, respectively.

The main idea of the utilization of DES in the pretreatment is the possibility of the strong intramolecular hydrogen bonds in DES to promote the breakage of the hydrogen bonds in the lignocellulosic structure [12, 80–82]. There are also some reports on the mechanism of the interaction between DES and lignocellulosic components. In general aspects, to increase the solubility of a hydrophobic compound in an aqueous solvent, the following well-known methods are utilized, that is, cosolvency, hydrotropy, complexation ionization, and the use of surface-active components [83]. Therefore, the mechanism of the enhanced solubility of lignin in DES-water mixtures is investigated in terms of hydrotropic effect. Such a study was conducted by Soarez et al. [84]. The authors reported that syringic acid solubility was increased by decreasing the polarity of the carboxylic acids in DESs. Apart from the hydrogen bond interactions and pi-pi interactions, the main reason for high solubility was reported as the dispersive interactions between organic acid alkyl chain and syringic acid. Furthermore, when urea was used instead of choline chloride, a fourfold increase in the solubility of lignin was reported. Nearly, 50% of DES-water mixtures provided the best solubility of the monomer. This was explained by the hydrotropic mechanism. They obtained the same result when they used organosolv and kraft lignin and proved the mechanism by dynamic light scattering. Xia et al. [85] searched for the weak fractionation efficiency of ChCl-glycerol using different techniques such as quantum mechanics calculations and solvatochromic parameters. The intramolecular interactions of lignin-carbohydrate complexes were found to be stronger than the interactions with DES and lignin-carbohydrate complexes. Interestingly, chloride ion in DES was reported to be surrounded by mutually anionic hydrogen bonds and cationic hydrogen bonds. This case resulted in a lowered ability of occupied-site anions and insufficient protons, which meant inactive acidic sites. To overcome this, a ternary DES was formed by adding the aluminum chloride into DES. The resulting supramolecular complexes

of chlorine ion-metal cation-hydrogen bond acceptor showed deep eutectic characteristics and resulted in a significant enhancement of the efficiency of the lignin extraction. On the other hand, Alvarez-Vasco et al. [86] found that DESs have the ability to cleavage ether bonds without affecting C-C linkages. Considering the ability of the solvents with high ß and π* to provide solubility of lignin, such as dimethylsulfoxide and pyridine, DESs can be possibly declared as new green candidates in lignin solubility.

Aforementioned studies clearly show that the use of DES is a good alternative for the removal of lignin from agro-industrial wastes to conventional pretreatment methods. Subsequent use of these pretreatment products allows the lignocellulosics to be valorized for several industries (**Figure 5**).

3.1 Enzymatic hydrolysis of biomass components

Apart from the treatment studies on the lignocellulosic waste by DESs, additional enzymatic hydrolysis is performed in many studies to remove lignin. Some of the above-mentioned literature contains subsequent enzymatic hydrolysis of the treated biomass as summarized. Procentese et al. [54] performed hydrolysis using Cellic CTec 2 enzymes (Novozyme) after increasing the digestibility of corncob by DES pretreatment. The hydrolysis conditions were 50°C, 180 rpm, and up to 80 h in a rotary shaker. The saccharification rate was found to be the highest at 80°C for the ChCl-imidazole pretreated sample from which 55% lignin was successfully removed. The enzymatic glucose and xylose yields increased with the increasing pretreatment temperature. The highest recovery of the initial carbohydrates was reported as 76%. In their following research, Procentese et al. [57] studied the enzymatic hydrolysis with the same commercial enzyme after the treatment of waste lettuce leaves with DESs. The completion time for the hydrolysis of the pretreated biomass was reported as 9 h. On the other hand, the higher the pretreatment temperature was the higher fraction of monomers was obtained during enzymatic hydrolysis. The authors also used the enzymatic hydrolysate of the pretreated lettuce in the batch culture of *Clostridium acetobutylicum* DSMZ 792 and reported full consumption of the sugars after 60 h. On the other hand, Kumar et al. [56] investigated the saccharification of pretreated rice straw using cellulose. Saccharification efficiency was reported as 36.0 ± 3.2% in 24 h at 10% solid loading. Liu et al. [60] used cellulase and ß-glucosidase for the hydrolysis of DES-pretreated wheat straw. According to the results, 89.06% of cellulose and 71.00% of xylan could be hydrolyzed. Fang et al. [64] proposed a prior treatment using hydrothermal processing for the date palm residues before DES treatment. They reported that the presence of hydrothermal pretreatment provided more efficient hydrolysis using Cellic CTec2. The conversion of glucan to glucose increased by 1.7-fold as a result of the pretreatment. However, cellulose crystallinity was not affected by the pretreatment. Apart from the advantages that hydrothermal processing provided to the process, the
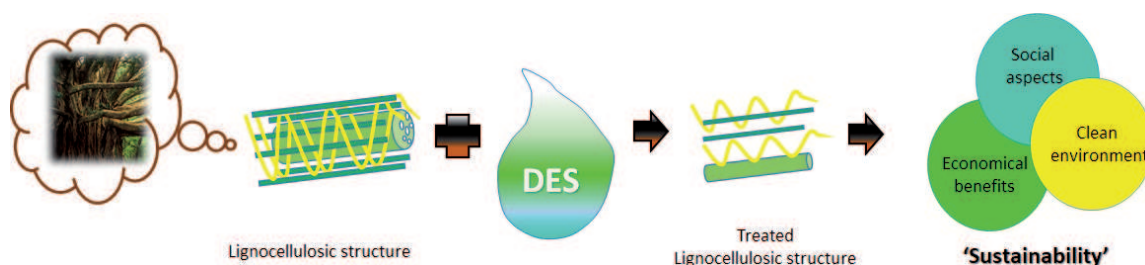
Figure 5.
Schematic illustration of the effect of DES on the lignocellulosic structure to yield sustainable processes.

operating and the investment cost remain a challenge for the process. Chen et al. [66] enzymatically hydrolyzed the pretreated switchgrass with CTec2 and HTec2. They could obtain 206.5 g/L glucose and 34.7 g/L xylose with 86.2% glucose yield within 48 h and reported 90.2 g/L 2,3-butanediol concentration without extra sugar addition for the first time. On the other hand, Wan and Mun [68] performed a subsequent hydrolysis step after the treatment of the sago waste with DESs. The hydrolysis was conducted at 50°C and 100 rpm for 48 h. The highest amount of glucose yield was obtained using ChCl-urea as 5.2 mg/mL. Acidic or alkaline pH DES increased the glucose yield.

4. Extraction and recovery of flavonoids from agro-industrial lignocellulosic wastes

Agro-industrial wastes represent sources of phenolic compounds that have beneficial effects to health due to their antioxidant, antimicrobial, anti-inflammatory, and immune-stimulant properties [87–89]. The prevention of cancer and cardiovascular diseases by phenolic compounds is attributed to their antioxidant and scavenging properties against reactive oxygen species. Apart from their use in biomedical applications, phenolic compounds can also be used in food industry as nutraceuticals. More than 8000 phenolic structures are identified in the structure of plants [90]. The extraction of biophenols from plants is always attractive; moreover, during the last decade, the recovery of phenolic compounds from agro-industrials has gained enormous attention. In spite of their distinct health beneficial properties, the massive phenolic compounds in lignocellulosic wastes have detrimental effects on the environment. The removal/recovery of phenolics from biomass has been conventionally performed with organic solvent extraction; however, recent studies show that DESs can be successfully used in the extraction [91–94]. On the other hand, the use of DESs in biomass processing is less studied than other applications of DESs and needs to be improved [80]. Below, the very recent studies dedicated to the DES selection and condition development for the extraction of polyphenolic bioactive compounds, especially flavonoids, from most abounded agro-industrial wastes are briefly summarized.

Jeong et al. [95] tested several DESs for the recovery of anthocyanin from grape skin and reported that ChCl combined with citric acid, D-(+)-maltose, and fructose was the most effective ChCl-based DESs. In addition, a newly designed DES – citric acid-D-(+)-maltose (4:1) – provided considerably high level extraction yield of anthocyanin. Under the optimized conditions identified by the response surface methodology, total anthocyanin content was found to be 63.36 mg g^{-1} using the new DES. Radosevic et al. [96] used ChCl-based DESs containing glucose, fructose, xylose, glycerol, and malic acid for the recovery of phenolics from grape skin and tested the biological activity of extracts *in vitro* using HeLa and MCF-7 human tumor cell lines. Decreased amount of cytotoxicity of DESs was observed against HeLa and MCF-7 cells. ChCl-malic acid (1:1) provided the highest extraction capability of total phenolic and anthocyanin contents as 91 and 24 mg g^{-1} dw, respectively. Antioxidant activity, in terms of ORAC value, was obtained as 371 mmol TE g^{-1} dw, while antiproliferative activity was around 20%. Recently, Panic et al. [97] reported a scale-up of the extraction process of grape pomace in which anthocyanins were extracted using NADESs. They also studied on the separation of the valuable bioactive compounds from the extracts. ChCl-citric acid (2:1) was successfully used in multimode-microwave and low-frequency-ultrasound irradiation extractions, and it was found that a simultaneous ultrasound/microwave-assisted extraction provided 1.77 mg g^{-1} dw of anthocyanins. Anthocyanins were successfully

recovered from NADES and recycled. Chanioti and Tzia [98] used ChCl-citric acid, ChCl-lactic acid, ChCl-maltose, and ChCl-glycerol NADESs to recover bioactive compounds from olive pomace. They added 20% v/v water to the homogenate and tested the effect of different techniques on the extraction process such as high hydrostatic pressure, microwave, and ultrasonication. The NADESs prepared using citric acid and lactic acid were more efficient in the extraction of phenolic compounds of olive pomace than conventional solvent. Ozturk et al. [99] studied the extraction of flavonoids from orange peel using ChCl-glycerol and ChCl-ethylene glycol and reported that ChCl-ethylene glycol (1:4) provided the highest total phenolic compounds (3.61 mg GAE g^{-1}) and also the highest antioxidant activity based on DPPH radical scavenging method (30.6 µg mL^{-1}). The phenolic acids extracted were identified as gallic, *p*-coumaric, ferulic, caffeic, and *trans*-cinnamic acids, as well as flavone and thymol. Ferulic acid was found to be the most abundant phenolic compound, while *p*-coumaric and gallic acids were on the second order. Pal and Jadeja [100] reported the microwave-assisted extraction of polyphenolic compounds from ripe mango (*Mangifera indica* L.) peels with DES consisting of lactic acid-sodium acetate-water (3:1:4). The highest values for the recovery of total phenolic content, ferric reducing antioxidant power, and DPPH scavenging activity were found to be 56.17 mg GAE g^{-1} dw, 683.27 µmol ascorbic acid equivalent g^{-1} dw, and 82.64%, respectively. Mangiferin was detected as the main flavonoid of the extracts. On the other hand, Fernandez et al. [101] designed a new NADES using lactic acid, glucose, and 15% water (LGH-15) and used it in the extraction of phenolic compounds from onion, olive, tomato, and pear industrial byproducts. The results showed that LGH-15 had high extractability characteristic for both high and low polar compounds in comparison to conventional solvents. The stabilizing ability of LGH-15 was found to be quite good since the phenolic compounds could remain stable over 2 months in it. In another study [102], various agro-industrial wastes such as lemon peels, olive leaves, onion solid wastes, red grape pomace, spent filter coffee, and wheat bran were used to test the performance of novel glycerol-based eutectic solvents in the recovery of polyphenolic compounds. Glycerol-ChCl and glycerol-sodium acetate provided high extraction efficiency comparable with aqueous ethanol, while glycerol-sodium-potassium tartrate-water showed lower efficiency in the extraction. The extracts with high polyphenol content also possessed higher reducing power and antiradical activity. Stefou et al. [103] screened sodium propionate-based DESs for the extraction of onion solid wastes, which are rich in quercetin and quercetin conjugates. It was suggested that the use of glycerol-sodium propionate (8:1) could result in high flavonoid content and high antioxidant power in comparison with aqueous glycerol, aqueous ethanol containing citric acid, and aqueous glycerol containing 2-hydroxypropyl β-cyclodextrin. Pal and Jadeja [104] studied the extraction of phenolic antioxidants from onion peel using DESs consisting of ChCl as HBA and sucrose, urea, and sorbitol as HBDs. The optimal conditions for the extraction found by Taguchi's method were reported as a temperature of 60°C, a time of 120 min, and a liquid-to-solid ratio of 50:1 using ChCl-urea (1:2). Under these conditions, total phenolic content was reported as 222.97 mg GAE g^{-1} dw. On the other hand, the extracts obtained using ChCl-sorbitol exhibited comparable DPPH radical scavenging activity to the extracts of aqueous methanol (82.40%). Major flavonoids were identified as quercetin, kaempferol, and myricetin. Very recently, Ruesgas-Ramón et al. [105] used agro-residues from coffee and cocoa industries to recover biomolecules using DESs. The authors reported that the use of lactic acid-ChCl (2:1) provided higher phenolics extraction with ultrasound-probe-assisted extraction than heat stirring-assisted extraction. The main compounds extracted were identified as chlorogenic acid, caffeine, and theobromine. The extraction with DESs from other types of agri-food wastes such

as saffron processing wastes [106], olive leaves [102, 107–109], and pigeon pea leaves [110, 111] has also appeared in the recent literature.

The number of studies in open literature on the extraction of bioactive compounds with DESs has been rapidly increasing. Therefore, DESs are easily expected to be used more for the extraction of bioactive phenolic compounds from various sources in the near future. On the other hand, the following issues should be more extensively studied; recovery of phenolics from DESs, stability of phenolics in DESs, reusability of DESs and the scale-up of the extraction processes. Additionally, the determination of biological activities of the phenolic compounds in DES extracts appears to be another field to be focused on in the near feature.

5. Instruments used for the extraction and recovery from lignocellulosic wastes

For the extraction and recovery of lignin or flavonoids from lignocellulosic waste, mostly preferred method is the heating and stirring method [54–61, 63, 65, 76, 78, 99, 103, 105–107, 112] in which oil bath is used to achieve relatively high temperatures. Apart from this conventional method, advanced techniques including the use of ultrasound and microwave irradiations are rarely studied for the extraction and recovery from lignocellulosic wastes. The yield of an extraction from a lignocellulosic material is related to the isolation of the target molecule from the matrix [113]. Ultrasound- and microwave-assisted procedures facilitate the isolation of the target molecules. Microwave heating process was reported to be a very efficient method as it promotes the selective bond cleavage and the stretching of certain bonds at a higher level due to the microwave irradiation [113]. Indeed, in the case of microwave, the disruption of the hydrogen bonds occurs by the dipole rotation of the molecules, and subsequently, dissolved ions migrate that lead to an increase in the penetration of the solvents into the matrix. This procedure results in an easier recovery of the target molecules. Beyond this, the pressure formed by the microwave improves the porosity of the matrix, thereby letting better contact of the solvent with the lignocellulosic material [113]. Related to these positive effects, microwave may be used either in the pretreatment of the biomass or in the extraction process directly. Ultrasound-assisted method is also advantageous when compared to classical methods. Similar to microwave-assisted procedure, it lets a better solvent penetration into the matrix. Ultrasound-assisted extraction creates microsteam in the cells that enhances the mass transfer. Ultrasonication creates longitudinal waves that form alternating compressions in the solvent, which leads to cavitation and gas bubbles. In this period, the bubbles expand, and at a point, the gas in the bubble condenses and a considerable amount of energy is released [114]. With the crashing of the condensed molecules, shock waves occur that lead to very high local temperature and pressure values. Regarding the effectiveness of the two nonconventional methods, we believe that researchers will pay attention to use them in the challenging delignification or extraction processes. Both microwave- and ultrasound-assisted extractions reduce working times and also increase the yield.

6. Different aspects of the utilization of deep eutectic solvents

After the use of DES in both fractionation of lignin and recovery of valuable compounds from lignin, DES can be recycled. The solvents that work as an antisolvent such as water, ethanol, or acetone result in the solidified form of DES and

can be easily removed from the media. After the evaporation or freeze drying of the antisolvent, DES can be obtained in a pure form as mentioned before by several authors [51, 102, 115]. The removal of water in DES can be performed by adding acetone, thereby precipitating DES. The precipitated DES can be reused again by heating.

Beyond the advantages of the use of DES in the treatment of lignocellulosic waste, some drawbacks of DESs were also reported and reviewed in the literature [116]. At high temperatures, DES was reported to decompose to the hydrogen bond donor and acceptor [117]. Moreover, low boiling point component was reported to evaporate. The decomposition temperatures of some DESs were also reported in the literature [117]. Another disadvantage of DESs was reported as the hygroscopicity; however, since certain amount of water is intendedly added during some treatments, this would not be a real disadvantage for many treatments to our opinion.

Another issue is the toxicity of DESs. As green solvents, they are assumed to be nontoxic, especially when natural components are used to form the eutectic mixtures. The most commonly used HBA in DESs is ChCl. Since cholinium is a component of vitamin B complex and the ever first DES was defined using ChCl, ChCl-based DESs are regarded as safe. However, according to the latest research articles, nontoxicity may not be generalized for all types of DESs, including the ones containing the common quaternary ammonium salt. Herein, some of the reports on the toxicity of different types of DESs are summarized.

Hayyan et al. [118] were among the first researchers working on the toxicity of DESs. Four different types of DESs [HBA: ChCl HBDs: glycerine, ethylene glycol, triethylene glycol, and urea (molar ratio: 1:3)] were studied for their toxicity toward *Bacillus subtilis* and *Staphylococcus aureus* as Gram-positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. The results showed that DESs were benign for these bacteria. However, the tests for the cytotoxicity of DESs that were performed using *Artemia salina* leach showed higher cytotoxic profile of DESs than their individual components. In their progressive search, Hayyan et al. [119] tested phosphonium-based DESs with different HBDs as glycerine, ethylene glycol, and triethylene glycol (molar ratio: 1:3) for their toxicity and cytotoxicity. They reported that the DESs showed antibacterial activity, and similar to the previous results, DESs and individual components showed different cytotoxicities toward brine shrimp. The authors also searched the toxicity of ammonium-based DESs using glycerine, ethylene glycol, triethylene glycol, and urea as HBDs [120]. The results obtained using *in vitro* cell lines and *in vivo* animal models showed that DESs were more toxic than their individual components; moreover, they enhanced reactive oxygen species production and induced apoptosis in treated cancer cells. However, they were found to be less cytotoxic than ionic liquids. Juneidi et al. [121] prepared different types of DESs [HBA: ChCl, HBDs: ethylene glycol, glycerol, urea (molar ratio, 1:2), and HBA: *N,N* diethyl ethanol ammonium chloride (EAC)-malonic acid (1:1), EAC-zinc nitrate hexahydrate (1:1), EAC-Gly (1:2), EAC-ZnCl2 (1:2)]. They tested the toxicity of DESs toward *Aspergillus niger* in addition to *Cyprinus carpio* fish. According to the results, Type I and II DESs showed higher toxicity than Type III DESs. The authors commented that this was due to the metal salts used in preparing DESs. On the other hand, the toxicity of DESs was found to be related to the concentration of DESs and the nature of the individual components. Similar findings on the toxicity of ChCl-ZnCl2 and acidic type of DESs were also reported by Juneidi et al. [122] in their progressive research. The largest inhibition zone for the tested fungi (*Phanerochaete chrysosporium, Aspergillus niger, Lentinus tigrinus*, and *Candida cylindracea*) was obtained by ChCl-ZnCl2 (1:2), followed by the acidic DESs, ChCl-*p*-toluene sulfonic acid (1:3), and ChCl-malonic acid (1:1). Hayyan et al. [123] claimed that ChCl-malonic acid

(1:1) was more toxic than the other acidic DESs they tested [ChCl-fructose-water (5:2:5), ChCl-glucose-water (5:2:5), ChCl-sucrose-water (4:1:4), and ChCl-glycerol-water (1:2:1)]. Glycerol, fructose, glucose, and sucrose containing NADESs showed significantly low toxicity as expected due to their nature, whereas the organic acid containing DES was quite toxic. Additionally, they reported that a trend could be observed between the cytotoxicity profile and the viscosity, the water content, and the nature of the components of NADESs. Ahmadi et al. [124] presented a research on the assessment of the cytotoxicity of 28 NADESs (HBA: ChCl, HBDs: ethylene glycol, glycerol, 1,2-propanediol, sorbitol, xylitol, xylose, fructose, glucose, mannose, glucosamine, sucrose, maltose, rhamnose, raffinose) by using quantitative structure activity relationship analysis. According to their findings, an increase in the glycerol molar ratio decreased the toxic effect of NADES, whereas an increase in the 1,2-propanediol molar ratio increased the toxic effect. On the other hand, the common ionic liquid [C8mim] [Cl] was found to be more toxic than all NADESs tested. This result was consistent with other studies compared the toxicity of different ILs with DESs [125, 126]. Macario et al. [127] prepared 23 DESs using ethylene glycol and 1-propanol as HBDs; [N1111]Cl, [N2222]Cl, and [N3333]Cl as HBAs, at four different molar ratios 1:2, 1:1, 2:1, and 4:1. They reported that these DESs were not harmful to *Aliivibrio fischeri*, whereas they showed the toxicity in the following order: [N1111]Cl-based DES < [N2222]Cl-based DES < [N3333]Cl-based DES. Mbous et al. [128] compared the cytotoxicity of NADESs prepared using choline chloride, fructose, or glucose with a DES (*N,N*-diethyl ethanolammonium chloride:triethylene glycol). NADESs were found to be less toxic than DES. Radosevic et al. [129] prepared 10 DESs as ChCl-oxalic acid (1:1), ChCl-urea (1:2), ChCl-xylitol (5:2), ChCl-sorbitol (2:3), betaine-glucose (5:2), betaine-malic acid-proline (1:1:1), betaine-malic acid-glucose (1:1:1), citric acid-proline (1:1), citric acid-glucose-glycerol (1:1:1), citric acid-fructose-glycerol (1:1:1). Citric acid-based NADESs did not show any cytotoxic effects on the tested cells, whereas ChCl-urea was toxic to only one kind of the cells (MCF-7 cells).

Regarding the above-mentioned reports, it can be concluded that the toxicity of a DES varies according to the type and molar ration of the components. The presence of an organic acid increases the overall toxicity of a DES. The eutectic mixture is generally more toxic than its individual components. This may be related to hydrogen bonds between the components. On the other hand, NADESs were shown to exhibit lower toxicity than DESs; moreover, natural based or not, all DESs exhibit lower toxicity than ILs. Therefore, "green solvent" characteristic should be proved, or the proven green solvents should be used especially in large-scale applications. On the other hand, new strategies should be developed to predict the toxicity level of a candidate green solvent in the near future.

7. Conclusions

Recent trends in the reduction of pollution effects of lignocellulosic waste are to convert them into useful products or to recover the natural components within them. Treatment of wastes provides the utilization of materials or substrates in the production of value-added products. However, most of the pretreatment methods used for lignocellulosics are energy consuming operations since they need to use high temperature and pressure for the removal of lignin. Deep eutectic solvents are new generation solvents that find several applications in chemistry and chemical engineering. Extraction methodology for the recovery of bioactive phenolic compounds from plants can also be used for agro-industrial wastes. Treatment with DESs requires less energy. Additionally, DESs facilitate accessibility to cellulose by

dissolving lignin at low temperatures and pressures. On the other hand, the task-specific DES for the target lignocellulosic waste differs according to the type of the waste. Therefore, one should search the literature and start with the promising DES and extend the research to similar kind of DESs to achieve high recovery yields. Apart from that, a new and target-specific DES may be synthesized since enormous number and type of components are candidates for new DESs.

DESs have unique properties such as polarity, conductivity, and viscosity depending on their composition. Therefore, novel DESs to be prepared and treatment conditions to be improved will help to solve environmental problems originated from agro-industrial wastes and also to develop new platforms for the production of valuable products such as chemicals, biofuels, and bioactive phenolic compounds.

Even promising results are published on the use of DESs for the lignocellulosic wastes, the mechanism of the treatment and the changes on the structure of DES still need to be clarified. Additionally, a detailed structural analysis on the extracted and purified biomass components relevant for the purpose should be revisited.

Conflict of interest

The authors declare no conflict of interest.

Author details

Ayşe Ezgi Ünlü and Serpil Takaç*
Faculty of Engineering, Department of Chemical Engineering, Ankara University, Tandoğan, Ankara, Turkey

*Address all correspondence to: takac@eng.ankara.edu.tr

IntechOpen

References

[1] Hoornweg D, Bhada-Tat P. What a waste: A global review of solid waste management. Urban Development Series Knowledge Papers. 2012;**15**:1-98

[2] Zheng W, Phoungthong K, Lü F, Shao LM, He PJ. Evaluation of a classification method for biodegradable solid wastes using anaerobic degradation parameters. Waste Management. 2013;**33**:2632-2640

[3] EPA. Municipal Solid Waste (MSW) in the United States: Facts and Figures. Washington, D.C.; 2010

[4] Tan KT, Lee KT, Mohamed AR. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Policy. 2008;**36**:3360-3365

[5] Isikgor FH, Becer CR. Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers. Polymer Chemistry. 2015;**6**:4497-4559

[6] Brandt A, Gräsvik J, Hallett JP, Welton T. Deconstruction of lignocellulosic biomass with ionic liquids. Green Chemistry. 2013;**15**(3):537-848

[7] Sun N, Rodríguez H, Rahman M, Rogers RD. Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? Chemical Communications. 2011;**47**:1405-1421

[8] Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial and Engineering Chemistry Research. 2009;**48**:3713-3729

[9] Zakzeski J, Bruijnincx PCA, Jongerius AL, Weckhuysen BM. The catalytic valorization of lignin for the production of renewable chemicals. Chemical Reviews. 2010;**110**(6):3552-3599

[10] Adorjan I, Potthast A, Rosenau T, Sixta H, Kosma P. Discoloration of cellulose solutions in N-methylmorpholine-N-oxide (lyocell). Part 1: Studies on model compounds and pulps. Cellulose. 2005;**12**(1):51-57

[11] Ralph J, Lapierre C, Boerjan W. Lignin structure and its engineering. Current Opinion in Biotechnology. 2019;**56**:240-249

[12] Van Osch DJGP, Kollau LJBM, Van Den Bruinhorst A, Asikainen S, Rocha MAA, Kroon MC. Ionic liquids and deep eutectic solvents for lignocellulosic biomass fractionation. Physical Chemistry Chemical Physics. 2017;**19**:2636-2665

[13] Niaounakis M, Halvadakis CP. Olive processing waste management. In: Literature Review and Patent Survey. 2nd ed. Pergamon: Elsevier; 2006

[14] Shalini R, Gupta DK. Utilization of pomace from apple processing industries: A review. Journal of Food Science and Technology. 2010;47(4):365-371

[15] Tuck CO, Pérez E, Horváth IT, Sheldon RA, Poliakoff M. Valorization of biomass: Deriving more value from waste. Science. 2012;**337**:695-699

[16] Li X, Row KH. Development of deep eutectic solvents applied in extraction and separation. Journal of Separation Science. 2016;**39**(18):3505-3520

[17] Li Y, Fabiano-Tixier AS, Tomao V, Cravotto G, Chemat F. Green ultrasound-assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an alternative solvent. Ultrasonics Sonochemistry. 2013;**20**(1):12-18

[18] Abbott AP, Capper G, Davies DL, Rasheed RK, Tambyrajah V. Novel solvent properties of choline chloride/urea mixtures. Chemical Communications. 2003;**1**:70-71

[19] Zhang Q, De Oliveira VK, Royer S, Jerome F. Deep eutectic solvents: Syntheses, properties and applications. Chemical Society Reviews. 2012;**41**:7109-7146

[20] García G, Aparicio S, Ullah R, Atilhan M. Deep eutectic solvents: Physicochemical properties and gas separation applications. Energy & Fuels. 2015;**29**(4):2616-2644

[21] Dai Y, Witkamp GJ, Verpoorte R, Choi YH. Natural deep eutectic solvents as a new extraction media for phenolic metabolites in carthamus tinctorius L. Analytical Chemistry. 2013;**85**(13):6272-6278

[22] Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH. Natural deep eutectic solvents as new potential media for green technology. Analytica Chimica Acta. 2013;**766**:61

[23] Gutiérrez MC, Ferrer ML, Mateo CR, Del Monte F. Freeze-drying of aqueous solutions of deep eutectic solvents: A suitable approach to deep eutectic suspensions of self-assembled structures. Langmuir. 2009;**25**(10):5509-5515

[24] Florindo C, Oliveira FS, Rebelo LPN, Fernandes AM, Marrucho IM. Insights into the synthesis and properties of deep eutectic solvents based on cholinium chloride and carboxylic acids. ACS Sustainable Chemistry & Engineering. 2014;**2**(10):2416-2425

[25] Smith EL, Abbott AP, Ryder KS. Deep eutectic solvents (DESs) and their applications. Chemical Reviews. 2014;**114**(21):11060-11082

[26] Durand E, Lecomte J, Villeneuve P. Deep eutectic solvents: Synthesis, application, and focus on lipase-catalyzed reactions. European Journal of Lipid Science and Technology. 2013;**115**(4):379-385

[27] Zubeir LF, Lacroix MHM, Kroon MC. Low transition temperature mixtures as innovative and sustainable CO_2 capture solvents. The Journal of Physical Chemistry. B. 2014;**118**(49):14429-14441

[28] Dean PM, Pringle JM, MacFarlane DR. Structural analysis of low melting organic salts: Perspectives on ionic liquids. Physical Chemistry Chemical Physics. 2010;**12**:9144-9153

[29] Fredlake CP, Crosthwaite JM, Hert DG, Aki SNVK, Brennecke JF. Thermophysical properties of imidazolium-based ionic liquids. Journal of Chemical & Engineering Data. 2004;**49**(4):954-964

[30] Almeida HFD, Passos H, Lopes-Da-Silva JA, Fernandes AM, Freire MG, Coutinho JAP. Thermophysical properties of five acetate-based ionic liquids. Journal of Chemical & Engineering Data. 2012;**57**(11):3005-3013

[31] Van Osch DJGP, Zubeir LF, Van Den Bruinhorst A, Rocha MAA, Kroon MC. Hydrophobic deep eutectic solvents as water-immiscible extractants. Green Chemistry. 2015;**17**:4518-4521

[32] Van Osch DJGP, Parmentier D, Dietz CHJT, Van Den Bruinhorst A, Tuinier R, Kroon MC. Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents. Chemical Communications. 2016;**52**:11987-11990

[33] Ribeiro BD, Florindo C, Iff LC, Coelho MAZ, Marrucho IM. Menthol-based eutectic mixtures: Hydrophobic low viscosity solvents. ACS Sustainable Chemistry & Engineering. 2015;**3**(10):2469-2477

[34] Zainal-Abidin MH, Hayyan M, Hayyan A, Jayakumar NS. New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. Analytica Chimica Acta. 2017;**979**:1-23. DOI: 10.1016/j. aca.2017.05.012

[35] Shishov A, Bulatov A, Locatelli M, Carradori S, Andruch V. Application of deep eutectic solvents in analytical chemistry. A review. Microchemical Journal. 2017;**135**:33-38

[36] Hadj-Kali M. Separation of ethylbenzene and n-octane using deep eutectic solvents. Green Processing and Synthesis. 2015;**4**(2):117-123

[37] Lee JS. Deep eutectic solvents as versatile media for the synthesis of noble metal nanomaterials. Nanotechnology Reviews. 2017;**6**(3):271-278

[38] Isaifan RJ, Amhamed A. Review on carbon dioxide absorption by choline chloride/urea deep eutectic solvents. Advances in Chemotherapy. 2018;**2018**:1-6

[39] Wang L, Zhou M, Chen Q , He M-Y. Brønsted acidic deep eutectic solvent catalysed the one-pot synthesis of 2H-indazolo[2,1-b]phthalazine-triones. Journal of Chemical Research. 2013;**37**(10):598-600

[40] Tran PH, Nguyen HT, Hansen PE, Le TN. An efficient and green method for regio-and chemo-selective Friedel–Crafts acylations using a deep eutectic solvent ([CholineCl][ZnCl$_2$]$_3$). RSC Advances. 2016;**6**(43):37031-37038

[41] De Santi V, Cardellini F, Brinchi L, Germani R. Novel Brønsted acidic deep eutectic solvent as reaction media for esterification of carboxylic acid with alcohols. Tetrahedron Letters. 2012;**53**(38):5151-5155

[42] Ünlü AE, Prasad B, Anavekar K, Bubenheim P, Liese A. Investigation of a green process for the polymerization of catechin. Preparative Biochemistry & Biotechnology. 2017;**47**(9):918-924

[43] Arıkaya A, Ünlü AE, Takaç S. Use of deep eutectic solvents in the enzyme catalysed production of ethyl lactate. Process Biochemistry. 2019;**84**:53-59

[44] Musale RM, Shukla SR. Deep eutectic solvent as effective catalyst for aminolysis of polyethylene terephthalate (PET) waste. International Journal of Plastics Technology. 2016;**20**(1):106-120

[45] Patil UB, Singh AS, Nagarkar JM. Choline chloride based eutectic solvent: An efficient and reusable solvent system for the synthesis of primary amides from aldehydes and from nitriles. RSC Advances. 2014;**4**(3):1102-1106

[46] Mondal D, Sharma M, Wang C-H, Lin Y-C, Huang H-C, Saha A, et al. Deep eutectic solvent promoted one step sustainable conversion of fresh seaweed biomass to functionalized graphene as a potential electrocatalyst. Green Chemistry. 2016;**18**(9):2819-2826

[47] Singh B, Lobo H, Shankarling G. Selective N-alkylation of aromatic primary amines catalyzed by bio-catalyst or deep eutectic solvent. Catalysis Letters. 2011;**141**(1):178-182

[48] Keshavarzipour F, Tavakol H. Deep eutectic solvent as a recyclable catalyst for three-component synthesis of β-amino carbonyls. Catalysis Letters. 2015;**145**(4):1062-1066

[49] Tang B, Row KH. Recent developments in deep eutectic solvents in chemical sciences. Monatshefte für Chemie. 2013;**144**(10):1427-1454

[50] Satlewal A, Agrawal R, Bhagia S, Sangoro J, Ragauskas AJ. Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel

opportunities. Biotechnology Advances. 2018;**36**(8):2032-2050

[51] Luo J, Cai M, Gu T. Pretreatment of lignocellulosic biomass using green ionic liquids. In: Gu T, editor. Green Biomass Pretreatment for Biofuels Production. Dordrecht: Springer, Dordrecht; 2013

[52] Casal F, Bruinhorts A, Rocha MA, Kroon MC. Suitability of deep eutectic solvents (organic salt and natural carboxylic acid mixtures) for lignocellulosic biomass processing. In: 26th European Symposium on Applied Thermodynamics. Potsdam; 2012

[53] Francisco M, van den Bruinhorst A, Kroon MC. New natural and renewable low transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic biomass processing. Green Chemistry. 2012;**14**(8):2153-2157

[54] Procentese A, Johnson E, Orr V, Garruto Campanile A, Wood JA, Marzocchella A, et al. Deep eutectic solvent pretreatment and subsequent saccharification of corncob. Bioresource Technology. 2015;**192**:31-36

[55] Zhang CW, Xia SQ, Ma PS. Facile pretreatment of lignocellulosic biomass using deep eutectic solvents. Bioresource Technology. 2016;**219**:1-5

[56] Kumar AK, Parikh BS, Pravakar M. Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. Environmental Science and Pollution Research. 2016;**23**(10):9265-9275

[57] Procentese A, Raganati F, Olivieri G, Russo ME, Rehmann L, Marzocchella A. Low-energy biomass pretreatment with deep eutectic solvents for bio-butanol production. Bioresource Technology. 2017;**243**:464-473

[58] Hou XD, Feng GJ, Ye M, Huang CM, Zhang Y. Significantly enhanced enzymatic hydrolysis of rice straw via a high-performance two-stage deep eutectic solvents synergistic pretreatment. Bioresource Technology. 2017;**238**:139-146

[59] Procentese A, Raganati F, Olivieri G, Russo ME, Rehmann L, Marzocchella A. Deep eutectic solvents pretreatment of agro-industrial food waste. Biotechnology for Biofuels. 2018;**11**(37):1-12

[60] Liu Y, Zheng J, Xiao J, He X, Zhang K, Yuan S, et al. Enhanced enzymatic hydrolysis and lignin extraction of wheat straw by triethylbenzyl ammonium chloride/lactic acid-based deep eutectic solvent pretreatment. ACS Omega. 2019;**4**:19829-19839

[61] New EK, Wu TY, Tien Loong Lee CB, Poon ZY, Loow YL, Wei Foo LY, et al. Potential use of pure and diluted choline chloride-based deep eutectic solvent in delignification of oil palm fronds. Process Safety and Environment Protection. 2019;**123**:190-198

[62] Ong VZ, Wu TY, Lee CBTL, Cheong NWR, Shak KPY. Sequential ultrasonication and deep eutectic solvent pretreatment to remove lignin and recover xylose from oil palm fronds. Ultrasonics Sonochemistry. 2019;**58**:1-10

[63] Tan YT, Ngoh GC, Chua ASM. Effect of functional groups in acid constituent of deep eutectic solvent for extraction of reactive lignin. Bioresource Technology. 2019;**281**:359-366

[64] Fang C, Thomsen MH, Frankær CG, Brudecki GP, Schmidt JE, Alnashef IM. Reviving pretreatment effectiveness of deep eutectic solvents on lignocellulosic date palm residues by prior recalcitrance reduction. Industrial

and Engineering Chemistry Research. 2017;**56**:3167-3174

[65] Tian D, Guo Y, Hu J, Yang G, Zhang J, Luo L, et al. Acidic deep eutectic solvents pretreatment for selective lignocellulosic biomass fractionation with enhanced cellulose reactivity. International Journal of Biological Macromolecules. 2020;**142**:288-297

[66] Chen Z, Bai X, Lusi A, Wan C. High-solid lignocellulose processing enabled by natural deep eutectic solvent for lignin extraction and industrially relevant production of renewable chemicals. ACS Sustainable Chemistry & Engineering. 2018;**6**:12205-12216

[67] Lim WL, Gunny AAN, Kasim FH, AlNashef IM, Arbain D. Alkaline deep eutectic solvent: A novel green solvent for lignocellulose pulping. Cellulose. 2019;**26**:4085-4098

[68] Wan YL, Mun YJ. Assessment of natural deep eutectic solvent pretreatment on sugar production from lignocellulosic biomass. In: MATEC Web of Conferences. 2018. pp. 1-10

[69] Yu Q, Qin L, Liu Y, Sun Y, Xu H, Wang Z, et al. In situ deep eutectic solvent pretreatment to improve lignin removal from garden wastes and enhance production of bio-methane and microbial lipids. Bioresource Technology. 2019;**271**:210-217

[70] Shen XJ, Wen JL, Mei QQ, Chen X, Sun D, Yuan TQ, et al. Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization. Green Chemistry. 2019;**21**:275-283

[71] Ho MC, Wu TY, Chee SWQ, Ngang CY, Chew IML, Teoh WH, et al. An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds. Cellulose. 2019;**26**:8557-8573

[72] Liu Q, Yuan T, Jin FQ, Yuan BY, Peng F, Li YC. Choline chloride-lactic acid deep eutectic solvent for delignification and nanocellulose production of moso bamboo. Cellulose. 2019;**26**:9447-9462

[73] Tan YT, Ngoh GC, Chua ASM. Evaluation of fractionation and delignification efficiencies of deep eutectic solvents on oil palm empty fruit bunch. Industrial Crops and Products. 2018;**123**:271-277

[74] Kandanelli R, Thulluri C, Mangala R, Rao PVC, Gandham S, Velankar HR. A novel ternary combination of deep eutectic solvent-alcohol (DES-OL) system for synergistic and efficient delignification of biomass. Bioresource Technology. 2018;**265**:573-576

[75] Jablonsky M, Majova V, Ondrigova K, Sima J. Preparation and characterization of physicochemical properties and application of novel ternary deep eutectic solvents. In: Cellulose. 2019. pp. 3031-3045

[76] Mamilla JLK, Novak U, Grilc M, Likozar B. Natural deep eutectic solvents (DES) for fractionation of waste lignocellulosic biomass and its cascade conversion to value-added bio-based chemicals. Biomass and Bioenergy. 2019;**120**:417-425

[77] Muley PD, Mobley JK, Tong X, Novak B, Stevens J, Moldovan D, et al. Rapid microwave-assisted biomass delignification and lignin depolymerization in deep eutectic solvents. Energy Conversion and Management. 2019;**196**:1080-1088

[78] Smink D, Juan A, Schuur B, Kersten SRA. Understanding the role of choline chloride in deep eutectic solvents used for biomass delignification. Industrial and Engineering Chemistry Research. 2019;**58**(66):16348-16357

[79] Quek JD, Lee KM, Lim S, Tey WY, Kang HS, Quen LK. Delignification of oil palm empty fruit bunch via ultrasound-assisted deep eutectic solvent pretreatment. In: International Conference on Sustainable Energy and Green Technology. IOP Publishing; 2019, 2020. pp. 1-6

[80] Loow YL, New EK, Yang GH, Ang LY, Foo LYW, Wu TY. Potential use of deep eutectic solvents to facilitate lignocellulosic biomass utilization and conversion. Cellulose. 2017;**24**:3591-3618

[81] Tang X, Zuo M, Li Z, Liu H, Xiong C, Zeng X, et al. Green processing of lignocellulosic biomass and its derivatives in deep eutectic solvents. ChemSusChem. 2017;**10**(13):2696-2706

[82] Zdanowicz M, Wilpiszewska K, Spychaj T. Deep eutectic solvents for polysaccharides processing. A review. Carbohydrate Polymers. 2018;**15**:361-380

[83] Jouyban A. Handbook of Solubility Data for Pharmaceuticals. Boca Raton: CRC Press; 2009

[84] Soares B, Tavares DJP, Amaral JL, Silvestre AJD, Freire CSR, Coutinho JAP. Enhanced solubility of lignin monomeric model compounds and technical lignins in aqueous solutions of deep eutectic solvents. ACS Sustainable Chemistry & Engineering. 2017;**5**:4056-4065

[85] Xia Q, Liu Y, Meng J, Cheng W, Chen W, Liu S, et al. Multiple hydrogen bond coordination in three-constituent deep eutectic solvents enhances lignin fractionation from biomass. Green Chemistry. 2018;**20**:2711-2721

[86] Alvarez-Vasco C, Ma R, Quintero M, Guo M, Geleynse S, Ramasamy KK, et al. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): A source of lignin for valorization. Green Chemistry. 2016;**18**:5133-5141

[87] Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006;**99**(1):191-203

[88] Vodnar DC, Călinoiu LF, Dulf FV, Ştefănescu BE, Crişan G, Socaciu C. Identification of the bioactive compounds and antioxidant, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste. Food Chemistry. 2017;**231**:131-140

[89] El-hawary SS, Rabeh MA. *Mangifera indica* peels: A common waste product with impressive immunostimulant, anticancer and antimicrobial potency. Journal of Natural Science Research. 2014;**4**(3):102-115

[90] Tanase C, Cosarcă S, Muntean DL. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules. 2019;**24**(6):1182

[91] Ruesgas-Ramón M, Figueroa-Espinoza MC, Durand E. Application of deep eutectic solvents (DES) for phenolic compounds extraction: Overview, challenges, and opportunities. Journal of Agricultural and Food Chemistry. 2017;**65**(18):3591-3601

[92] Mišan A, Nađpal J, Stupar A, Pojić M, Mandić A, Verpoorte R, et al. The perspectives of natural deep

eutectic solvents in agri-food sector. Critical Reviews in Food Science and Nutrition. 2019;**59**:1-29. DOI: 10.1080/10408398.2019.1650717

[93] Torres-Valenzuela LS, Ballesteros-Gomez A, Rubio S. Green solvents for the extraction of high added-value compounds form agri-food waste. Food Engineering Reviews. 2020;**12**:83-100. DOI: 10.1007/s12393-019-09206-y

[94] Kalhor P, Ghandi K. Deep eutectic solvents for pretreatment, extraction, and catalysis of biomass and food waste. Molecules. 2019;**24**(22):4012

[95] Jeong KM, Zhao J, Jin Y, Heo SR, Han SY, Yoo DE, et al. Highly efficient extraction of anthocyanins from grape skin using deep eutectic solvents as green and tunable media. Archives of Pharmacal Research. 2015;**38**(12):2143-2152

[96] Radošević K, Ćurko N, Gaurina Srček V, Cvjetko Bubalo M, Tomašević M, Kovačević Ganić K, et al. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. LWT- Food Science and Technology. 2016;**73**:45-51

[97] Panić M, Radić Stojković M, Kraljić K, Škevin D, Radojčić Redovniković I, Gaurina Srček V, et al. Ready-to-use green polyphenolic extracts from food by-products. Food Chemistry. 2019;**283**(December 2018):628-636. DOI: 10.1016/j. foodchem.2019.01.061

[98] Chanioti S, Tzia C. Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. Innovative Food Science and Emerging Technologies. 2018;**48**(June):228-239

[99] Ozturk B, Parkinson C, Gonzalez-Miquel M. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents.

Separation and Purification Technology. 2018;**206**:1-13

[100] Pal CBT, Jadeja GC. Microwave-assisted extraction for recovery of polyphenolic antioxidants from ripe mango (*Mangifera indica* L.) peel using lactic acid/sodium acetate deep eutectic mixtures. Food Science and Technology International. 2019;**26**(1):78-92

[101] Fernández d l ÁM, Espino M, FJV G, Silva MF. Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products. Food Chemistry. 2018;**239**:671-678

[102] Mouratoglou E, Malliou V, Makris DP. Novel glycerol-based natural eutectic mixtures and their efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from agri-food waste biomass. Waste and Biomass Valorization. 2016;7(6):1377-1387

[103] Stefou I, Grigorakis S, Loupassaki S, Makris DP. Development of sodium propionate-based deep eutectic solvents for polyphenol extraction from onion solid wastes. Clean Technologies and Environmental Policy. 2019;**21**(8):1563-1574

[104] Pal CBT, Jadeja GC. Deep eutectic solvent-based extraction of polyphenolic antioxidants from onion (*Allium cepa* L.) peel. Journal of the Science of Food and Agriculture. 2019;**99**(4):1969-1979

[105] Ruesgas-Ramón M, Suárez-Quiroz ML, González-Ríos O, Baréa B, Cazals G, Figueroa-Espinoza MC, et al. Biomolecules extraction from coffee and cocoa by- and co-products using deep eutectic solvents. Journal of the Science of Food and Agriculture. 2020;**100**:81-91

[106] Lakka A, Grigorakis S, Karageorgou I, Batra G, Kaltsa O,

Bozinou E, et al. Saffron processing wastes as a bioresource of high-value added compounds: Development of a green extraction process for polyphenol recovery using a natural deep eutectic solvent. Antioxidants. 2019;**8**(12):586

[107] Athanasiadis V, Grigorakis S, Lalas S, Makris DP. Highly efficient extraction of antioxidant polyphenols from *Olea europaea* leaves using an eco-friendly glycerol/glycine deep eutectic solvent. Waste and Biomass Valorization. 2018;**9**(11):1985-1992

[108] Alañón ME, Ivanović M, Gómez-Caravaca AM, Arráez-Román D, Segura-Carretero A. Choline chloride derivative-based deep eutectic liquids as novel green alternative solvents for extraction of phenolic compounds from olive leaf. Arabian Journal of Chemistry. 2018. DOI: 10.1016/j.arabjc.2018.01.003

[109] Dedousi M, Mamoudaki V, Grigorakis S, Makris D. Ultrasound-assisted extraction of polyphenolic antioxidants from olive (*Olea europaea*) leaves using a novel glycerol/sodium-potassium tartrate low-transition temperature mixture (LTTM). Environments. 2017;**4**(2):31

[110] Cui Q, Peng X, Yao XH, Wei ZF, Luo M, Wang W, et al. Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots. Separation and Purification Technology. 2015;**150**:63-72

[111] Wei Z, Qi X, Li T, Luo M, Wang W, Zu Y, et al. Application of natural deep eutectic solvents for extraction and determination of phenolics in *Cajanus cajan* leaves by ultra performance liquid chromatography. Separation and Purification Technology. 2015;**149**:237-244

[112] Smink D, Kersten SRA, Schuur B. Recovery of lignin from deep eutectic solvents by liquid-liquid extraction. Separation and Purification Technology. 2020;**235**:116127

[113] Routray W, Orsat V. Microwave-assisted extraction of flavonoids: A review. Food and Bioprocess Technology. 2012;**5**:409-424

[114] Picó Y. Ultrasound-assisted extraction for food and environmental samples. TrAC, Trends in Analytical Chemistry. 2013;**43**:84-99

[115] Jeong KM, Lee MS, Nam MW, Zhao J, Jin Y, Lee DK, et al. Tailoring and recycling of deep eutectic solvents as sustainable and efficient extraction media. Journal of Chromatography. A. 2015;**1424**:10-17

[116] Chen Y, Mu T. Application of deep eutectic solvents in biomass pretreatment and conversion. Green Energy & Environment. 2019;**4**:95-115

[117] Chen W, Xue Z, Wang J, Jiang J, Zhao X, Mu T. Investigation on the thermal stability of deep eutectic solvents. Huaxue Xuebao [Acta Chimica Sinica]. 2018;**34**(8):904-911

[118] Hayyan M, Hashim MA, Hayyan A, Al-Saadi MA, AlNashef IM, Mirghani MES, et al. Are deep eutectic solvents benign or toxic? Chemosphere. 2013;**90**:2193-2195

[119] Hayyan M, Hashim MA, Al-Saadi MA, Hayyan A, AlNashef IM, Mirghani MES. Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. Chemosphere. 2013;**93**:455-459

[120] Hayyan M, Looi CY, Hayyan A, Wong WF, Hashim MA. In vitro and in vivo toxicity profiling of ammonium-based deep eutectic solvents. PLoS One. 2015;**10**(2):1-18

[121] Juneidi I, Hayyan M, Hashim MA. Evaluation of toxicity and

biodegradability for cholinium-based deep eutectic solvents. RSC Advances. 2015;**5**:83636-83647

[122] Juneidi I, Hayyan M, Mohd AO. Toxicity profile of choline chloride-based deep eutectic solvents for fungi and Cyprinus carpio fish. Environmental Science and Pollution Research. 2016;**23**:7648-7659

[123] Hayyan M, Mbous YP, Looi CY, Wong WF, Hayyan A, Salleh Z, et al. Natural deep eutectic solvents: Cytotoxic profile. Springerplus. 2016;**5**:913

[124] Ahmadi R, Hemmateenejad B, Safavi A, Shojaeifard Z, Mohabbati M, Firuzi O. Assessment of cytotoxicity of choline chloride-based natural deep eutectic solvents against human HEK-293 cells: A QSAR analysis. Chemosphere. 2018;**209**:831-838

[125] García-Lorenzo A, Tojo E, Tojo J, Teijeira M, Rodríguez-Berrocal FJ, González MP, et al. Cytotoxicity of selected imidazolium-derived ionic liquids in the human Caco-2 cell line. Sub-structural toxicological interpretation through a QSAR study. Green Chemistry. 2008;**10**:508-516

[126] Ranke J, Müller A, Bottin-Weber U, Stock F, Stolte S, Arning J, et al. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. Ecotoxicology and Environmental Safety. 2007;**67**(3):430-438

[127] Macário IPE, Jesus F, Pereira JL, Ventura SPM, Gonçalves AMM, Coutinho JAP, et al. Unraveling the ecotoxicity of deep eutectic solvents using the mixture toxicity theory. Chemosphere. 2018;**212**:890-897

[128] Mbous YP, Hayyan M, Wong WF, Looi CY, Hashim MA. Unraveling the cytotoxicity and metabolic pathways of binary natural deep eutectic solvent systems. Scientific Reports. 2017;**7**(41257):1-14

[129] Radošević K, Čanak I, Panić M, Markov K, Bubalo MC, Frece J, et al. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. Environmental Science and Pollution Research. 2018;**25**:14188-14196