Wheat straw lignin extraction with bio-based solvents using enabling technologies

UNIVERSITÀ DEGLI STUDI DI TORINO

Wheat straw lignin extraction with bio-based solvents using enabling technologies

Emanuela Calcio Gaudino[1], Silvia Tabasso[2], Giorgio Grillo[1], Giancarlo Cravotto[1,*], Thomas Dreyer[3], Gerhard Schories[4], Sven Altenberg[5], Lilija Jashina [6], Galina Telysheva[6]

[1] *Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Via P. Giuria 9, 10235 Turin, Italy*
[2] *Dipartimento di Chimica, University of Turin, Via P. Giuria 7, 10125 Turin, Italy*
[3] *Weber Ultrasonics AG, 76307 Karlsbad, Germany.*
[4] *TTZ Bremerhaven Am Lunedeich 12, 27572 Bremerhaven, Germany.*
[5] *Environmental Systems, Uhlandstrasse 22, 27576 Bremerhaven, Germany*
[6] *Latvian State Institute of Wood Chemistry, 1006 Riga, Latvia.*

* Corresponding author.
E-mail address: giancarlo.cravotto@unito.it (G. Cravotto)

ABSTRACT
Two bio-based solvents, natural deep eutectic solvents (NaDES), and γ-valerolactone (GVL), have been used under microwave (MW), and ultrasound (US) irradiation to design an efficient and sustainable process for wheat straw delignification and have been compared with the traditional alkali procedure. Best delignification (45%) was achieved with a three-component NaDES (lactic acid:glycerol:choline chloride) under MW (120 °C in 30 min), with a solid/liquid ratio of 1:50. A GVL/water mixture (8:2) also gave an efficient delignification (27%) under US (40 kHz, 200 W) at 50 °C for 60 min. Py-GC/MS/FID analyses provide valuable information on the extracts' chemical profile. DPPH and Folin-Ciocalteu tests highlighted the efficiency of MW- and US-assisted extraction as well as the extracts quality. The highest antioxidant activity was found for the NaDES extracts obtained under US irradiation.

KEY WORDS
Lignin, NaDES, GVL, Ultrasound, Microwaves, Antioxidant activity

1. Introduction

One of the hottest topics in current scientific literature is the design of sustainable procedures for the valorisation of lignocellulosic biomass from agricultural, industrial and forest residues [1][2], via extractions using bio-based solvents [3-6], and microwave (MW) and ultrasound irradiation (US) [7-10]. The major challenge in efficient biomass exploitation is still the reduction of lignin and hemicellulose within their complex lignocellulosic structure in order to facilitate subsequent cellulose conversion to value-added chemicals via either enzymatic hydrolysis or catalytic procedures [11]. Lignin is widely treated as a waste product in bioethanol production, it is mainly recycled as fuel for energy balance [12], but further valorisation is worthy of investigation [13,14], as it is the second most abundant natural polymer after cellulose [15]. Lignin is a three-dimensional, highly cross-linked macromolecule made up of three main phenyl propane units (monolignols), namely, coniferyl alcohol (G), sinapyl alcohol (S), and minor amounts of *p*-coumaryl alcohol (H), which are interwoven by a series of characteristic linkages, such as C–O–C and C–C bonds [16,17].
In particular, the destruction of the majority of lignin ether bonds, during the biomass delignification process, generally results in increased phenolic hydroxyl group content in the lignin structure. These compounds are

essential for the product's radical scavenging activity [18]; they can trap radicals and become phenoxyl radicals themselves. Furthermore, the methoxyl groups located in the *ortho* position play an important role in phenoxyl radical stabilization [19], which is also a step towards the capture of radicals and the subsequent antioxidant effect of lignin [20-23]. Lignin has two main advantages as an antioxidant; it is of natural origin and is a polymeric structure. Antioxidant polymers have been a topic of great interest for researchers in many industrial fields [24], meaning that lignin and lignin related polymers have started to see use as additives in biodegradable packaging materials [25], as food additives [26], and in cosmetics products [27]. However, the high chemical heterogeneity of lignin also makes it necessary to consider how antioxidant activity can be influenced by biomass processing conditions [28, 29]. Delignification processes may affect the functional groups and molecular weight of recovered lignins, conditioning their antioxidant properties. For this reason, finding an effective and sustainable means of recovering lignin from lignocellulosic biomass may be the first step towards its valorisation as an antioxidant. It has recently been demonstrated that the extraction of lignin from wheat straw, using organic solvents of different polarity and alkaline solutions, results in the isolation of products with good antioxidant activity [28], while a plethora of biological, chemical and physical methods have been reported for lignin extraction from a variety of biomass sources [30]. However, these methods are all arduous, time-consuming and entail high solvent and chemical consumption. Besides the complete redesign of all existing processes, the use of alternative solvents, together with so-called enabling technologies, may also be a means to increasing the efficiency of lignin extraction protocols [31]. Focusing on green extraction and mass transport enhancing methods, such as MW- and US-assisted extractions, may provide the necessary potential to accomplish faster and more gentle processes and that consume less solvent than conventional methods [32]. In fact, the last few decades have seen enabling technologies being widely exploited as efficient and sustainable alternatives to traditional lignocellulose pretreatment methods [33, 34]. In particular, the sonomechanical effect of US has been shown to enhance solvent penetration into cellular materials, thus improving the mass transfer of the extractive processes behind biomass pretreatment [35]. On the other hand, the MW-assisted technique has been postulated as an alternative heating technology and one that is able to reduce reaction times and improve extract efficiency and quality, compared with conventional extraction protocols [36] [37]. MW interact with polar molecules leading to the rapid and volumetric heating of irradiated mixtures and mass transfer optimization. With this efficient and selective heating, MW can also be considered a promising approach to the thermal treatment of bio-waste, especially lignocellulose [38]. However, of these numerous studies, only a few focus on MW-assisted lignin isolation and the majority of those work under acidic conditions [39,40]. Nevertheless, they do highlight the advantages that MW-assisted lignin isolation can offer, especially in terms of lignin purity and processing time. The present work reports the MW-assisted delignification of wheat straw (one of the most abundant agricultural residues), and demonstrates the synergism that can exist between so-called enabling technologies and bio-based solvents.

The concept of green solvents is strongly related to the principles of green chemistry, and natural deep eutectic solvents (NaDES), have received much more recent attention than the others available [41]. A deep eutectic solvent (DES), is a fluid that is generally composed of two or three inexpensive and safe components that are capable of self-association, often through hydrogen-bond interactions, to form a eutectic mixture with a melting point that is lower than that of each individual component. DES, similarly to the more traditionally-used ionic liquids (ILs), are generally liquid at temperatures below 100 °C. Their synthesis is 100% atom-economic and their purity is high. However, unlike ILs, they are nontoxic and biodegradable. A wide range of NaDES have been applied across a number of chemistry fields. Particularly noteworthy is the choline chloride series that are coupled with monosaccharides (mainly glucose, fructose), glycerol and organic acids, which act as hydrogen bond donors [42,43]. Moreover, NaDES have recently been utilised as extraction solvents for phenolic compounds [44].

The non-volatility of NaDES is considered to be the most challenging issue in the design of separation processes, as solvents can no longer be separated by simple distillation. For this reason, other green solvents have been tested in lignin extraction experiments, including those that arise from the conversion of biomass itself. γ-Valerolactone (GVL), is a polar aprotic solvent that is conventionally produced from biomass-derived

levulinic acid [45], and its esters via (catalytic) hydrogenation [46]. GVL shows a number of interesting features [47]; its polarity is similar to those of the most common polar solvents (GVL's dielectric constant is 36.47, whereas those of CH_3CN, DMF, NMP and DMA are 37.5, 36.7, 32.0 and 37.8, respectively). Furthermore, it has low melting and high boiling points (-31 °C and 207 °C respectively), and a flash point (96 °C), that is generally higher than that of conventional dipolar aprotic solvents. It is also quite stable under neutral conditions. GVL has recently been used as a solvent in the extraction of lignin from biomass in a cascade MW-assisted integrated process [46].
In this work, the delignification power of NaDES and GVL on wheat straw has been maximised using US and MW irradiation. Moreover, the antioxidant property of extracts was employed to asses the best extraction procedure in term of product quality [48]. The extracts showed good antioxidant properties, thus paving the way for the application of lignin as an added value product, as an alternative to its combustion, according to the biorefinery approach. We herein demonstrate that non-conventional extraction techniques using bio-based solvents, GVL and NaDES, may be considered a valid alternative to classical methods which use organic solvents coupled with alkali or acid treatments.

2. Materials and methods

2.1. Plant material

Milled wheat straw homogenous powder (0.2 mm) was provided by Environmental System GmbH, (Germany) and was protected from light and humidity until use.

2.2. Chemicals

All chemicals were purchased from Sigma-Aldrich (Milan, Italy) and used without further purification. NaDES were obtained *via* heating: choline chloride (1 mol) was mixed with hydrogen bond donors; lactic acid (10 mol) to produce NaDES 1, and a combination of lactic acid (1 mol) and glycerol (1 mol) to produce NaDES 2. The mixtures were stirred for 2h at 100 °C until homogeneous liquids were obtained. NaDES 1 and NaDES 2 were finally collected for biomass extraction without further purification.

2.3. Biomass delignification procedures under non-conventional techniques

2.3.1. Ultrasound-assisted extraction (UAE)

UAE was performed in a high-power US bath (Weber Ultrasonics AG, Germany), composed of an inox cell (5 L), with three probes screwed into the bottom of the bath, which was operated at a frequency of 40 kHz and at maximum input power of 200 W. The suitable weight of wheat straw powder (0.2 mm particle size) was placed in a 250 mL round bottomed flask and immersed in the ultrasonic bath equipped with inlet and outlet for cooling water circulation. The temperature in the US bath was stabilized at either 35 or 50 °C during sonication (60-120 min). GVL and NaDES were tested at a solid/liquid ratio of 1:50 (200 mL maximum liquid volume), while a variety of GVL/H_2O ratios were investigated, as was a combination of bio-based solvents and NaOH (10% in the dry biomass). After UAE, the mixture was filtered off and washed with pure water.
Wheat straw (WS) solid fraction work up (after GVL extraction): the solid, recovered after filtration, was treated with US (40 kHz, 200 W for 30 min at 50 °C), in presence of an aqueous NaOH solution (1 % of NaOH in the dry biomass), in order to test the effectiveness of NaOH in degrafting GVL from the straw surface. This procedure was adopted for both the GVL-UAE5 and GVL-UAE6 samples. The suspension was then filtered and the treated wheat straw was freeze-dried (-70 °C, 0.2 mbar).
WS solid fraction work up (after NaDES extraction): The solid, recovered after filtration, was thoroughly washed with pure water until complete eutectic solvent removal was achieved, and the treated biomass was then freeze-dried under vacuum (-70 °C, 0.2 mbar).
WS liquid fraction work up (after GVL extraction): The recovered liquid fraction was used without any further purification.

WS liquid fraction work up (after NaDES extraction): An aqueous NaOH solution (1M), was added to the recovered liquid fraction, until neutrality was achieved, and then used without any further purification.

2.3.2. Microwave-assisted extraction (MAE)

MAE was performed by a multimode MW reactor in a closed vessel system, SynthWAVE (Milestone Srl). Reactor frequency was 2.45 GHz. The different bio-based solvents (GVL and NaDES), were tested for wheat straw pre-treatment at a solid/liquid ratio of 1:50 (20 mL maximum liquid volume). A variety of GVL/H_2O ratios were tested for wheat straw MAE at 90-120 °C under 1500 W, as was a combination of bio-based solvents and NaOH (10 % in the dry biomass).
WS solid fraction work up (after GVL extraction): the solid, recovered after filtration, was treated under conventional heating (for 30 min at 50 °C), in presence of an aqueous NaOH solution (1% of NaOH in the dry biomass), in order to test the effectiveness of NaOH in degrafting GVL from the straw surface. This procedure was adopted for both GVL-MAE2 and GVL-MAE4 samples. The suspension was then filtered and the treated wheat straw was freeze-dried (-70 °C, 0.2 mbar).
WS solid fraction work up (after NaDES extraction): The solid, recovered after filtration, was thoroughly washed with pure water until the eutectic solvent was completely removed and the treated biomass was freeze-dried (-70 °C, 0.2 mbar).
WS liquid fraction work up (after GVL extraction): The recovered liquid fraction was used without any further purification.
WS liquid fraction work up (after NaDES extraction): An aqueous NaOH solution (1M), was added to the recovered liquid fraction, until neutrality was achieved, which was then used without any further purification.

2.4. Liquid fraction antioxidant activity

2.4.1 DPPH tests

Antioxidant activity was determined using the radical scavenging activity method with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [49]. A lignin sample, recovered after water precipitation from each liquid extracts (NADEs and GVL both), was dissolved in 0.1 mL dioxane–water (9/1, v/v), and the solution was added to a 3.9 mL DPPH solution (25 mg/L in ethanol), used as the free radical source. The blank sample consisted of 0.1 mL methanol and a 3.9 mL DPPH solution. After a 30 min incubation period at room temperature in the dark, the decrease in solution absorbance, caused by proton donating activity, was measured immediately at 515 nm using a UV spectrometer (Cary 60 UV-Vis, Agilent Technologies). The tests were carried out in duplicate. Radical scavenging activity was calculated as follows: $I\ (\%) = (A0 - A1)/A0$; where A0 is the absorbance of the blank and A1 is the absorbance in the presence of the test compound at the various concentrations. IC50 values (concentrations that provide 50% inhibition), were calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding scavenging effect.

2.4.2 Total phenolic contents (TPC)

The TPCs of the liquid fractions were determined according to the method described by Singleton and Orthofer [50]. Briefly, each liquid sample was diluted with distilled water by 50 times and then 1 mL of this diluted liquid sample was added to 5 mL of 0.2 M Folin–Ciocalteu phenol reagent. After 5 min of gentle shaking, 4 mL of 7.5 % (w/w) sodium carbonate was added and mixed thoroughly. After 30 min storage in dark, optical density of the resulting blue complex was measured at 765 nm against the blank (without the analyzed sample) on a PerkinElmer Lambda 650 US/VIS spectrophotometer, using gallic acid as the standard. The total phenolic contents were expressed as milligrams (mg) of gallic acid equivalents (GAE) per 100 mL analyzed liquid fraction. .

2.5 Py-GC/MS/FID analysis

Py-GC/MS/FID analyses were performed using a Frontier Lab (Japan), Micro Double-shot Pyrolyser Py-3030D (pyrolysis temperature 500 °C, heating rate 600 °C s^{-1}), that was directly coupled with a Shimadzu 2D FID/MS gas chromatography system MSGC/GCMS–2010 (Japan), with a capillary column RTX-1701 (Restek, USA, 60 m x 0.25 mm x 0.25 μm film, injector temperature 250 °C, ion source 250 °C with an EI of 70 eV, MS scan range m/z 15 to 350, the carrier gas was helium at a flow rate of 1 mL min^{-1} and a split ratio of 1:30). Sample masses ranged from 1.00 to 2.00 mg. The oven program was as follows; 1 min at 60 °C, and the temperature was then increased at 6 °C min^{-1} to 270 °C, before it was finally held at 270 °C for 10 min. The identification of the individual compounds was performed on the basis of GC/MS chromatograms using Library MS NIST 14, whereas the relative peak areas of the individual compounds was calculated using the Shimadzu software on the basis of GC/FID data. The summed molar areas of the relevant peaks were normalized to 100%. Relative peak areas calculated in % rel, for the pyrolysis products of different origin, were used to assess the composition of the biomass samples.

2.6 ***Solid sample surface characterization***

The nitrogen adsorption-desorption isotherms method was used to characterise the micro surfaces of raw wheat straw and biomass samples. The isotherms were measured on a QUANTACHROME Gas Sorption System Nova 4000 (Quantachrome Instruments, USA), at a temperature of -196 °C, according to the recommendations of the International Union of Pure and Applied Chemistry [51].

3. Results and discussion

Bio-derived solvents and natural deep eutectic solvents (NaDES) are currently attracting increasing amounts of interest for use in biomass delignification since they exhibit low toxicity and are fully bio-degradable [52]. It has recently been proven that pretreatment with either choline chloride/glycerol or choline chloride/lactic acid boosts enzymatic digestibility and enhances the delignification of date palm residues and corncobs [53, 54]. Meanwhile, the use of GVL/H_2O mixtures for the fractionation of lignocellulosic biomass in a MW reactor has recently been reported [55]. Some recent papers have reported the use of US in biomass pretreatment [56], as well as that of NaDES for the US-assisted extraction of catechins from green tea [57]. However, this technology has yet to be studied in combination with biomass-derived solvents for biomass delignification to the best of our knowledge.

In this work, a variety of processes have been tested for their ability to perform wheat straw (containing 20.8% lignin) delignification in the presence of bio-based solvents and the results have been compared with those of more traditional methods.

Two different NaDES, a lactic acid /choline chloride mixture (10:1) (NaDES 1) and a three-component (lactic acid / choline chloride / glycerol 1:1:1) mixture (NaDES 2), have been used to study the synergism between organic acids and alcohols, and their effect on extraction efficacy. The two DES exhibit similar physicochemical properties to ILs (such as [BMIM][Cl])[58], while also being much cheaper and potentially environmentally friendly. The extraction capacity of NaDES is also correlated with their physical properties, including polarity and viscosity. DES display high ionic conductivity, as a result of their high ion concentrations, which is a positive feature in terms of their MW responsiveness. The major disadvantage of NaDES, when compared with conventional solvents, is their inherently high viscosity. Viscosity is known to hinder the efficiency of NaDES as extraction solvents since it results in slow mass transfer. This disadvantage has been negated here by the efficient heat and mass transfer provided by MW and US irradiation.

Despite extensive research into NaDES, there is still a lack of information as to the practical issues, including their efficiency, optimal water content and the recovery of extracted compounds, related to their use as extraction solvents. The inherent low vapour pressure that NaDES display hampers evaporation, meaning that extracted compounds can be only recovered via selective adsorption onto a suitable stationary phase. However, the whole NaDES extract can be used in non-food applications [54].

Two different GVL/H_2O mixtures (1:1 and 8:2) were adopted for lignin extraction from wheat straw under MW and US. These ratios were chosen in order to test the specific role that GVL plays with respect to water. The delignification and antioxidant activity of liquor recovered from these alternative lignin extraction protocols were compared with those of the extracts of the water/alkali treatment.

Wheat straw was US irradiated for 60 min at 40 kHz and 200 W power (temperature 35 °C or 50 °C), at a solid-liquid ratio of (1:50), using water as reference solvent (Table 1, entries 2 and 3). The same treatment was reproduced, but 10% of NaOH was added to the dry biomass (Table 1, entries 4 and 5), while bio-derived solvents were also used for comparison (Table 1).

Although it has been reported in the literature that maximum cavitation is achieved at 50 °C [59], lower temperatures were tested here to increase the sustainability of the process. Moreover, it is important to note that sonication time has the largest effect on biomass delignification. However, prolonging sonication beyond a certain limit has no additional effect in terms of delignification and post-sugar release [60]. Besides duration, another parameter to directly determine the power of sonication is sonication frequency and it is also important here as it affects lignocellulosic feedstock delignification. The majority of research in the field has used frequencies in the 10-100 kHz range, which is sufficient for cell rupture and polymer degradation [61]. NaDES were exposed to US irradiation for longer time periods in order to overcome the problems related to their viscosity.

As regards MAE, the same GVL/H_2O mixtures were tested under 30 min irradiation at 90 °C. NaDES were tested under MW irradiation for 30 minutes at 120 °C. Higher temperatures were required to reduce NaDES viscosity. In summary, longer reaction times at lower temperatures were used with US, while the MW-assisted treatments saw shorter reaction times and higher temperatures, owing to the specific features of the two technologies. In order to better understand the influence that the processes had on the chemical composition of the biomass, analytical pyrolysis (Py-GC/MS/FID), was applied to wheat straw before and after treatment. The analytical data (Table 1), show that the lignin content in the residual solids decreased after extraction. In particular, it can be observed that the addition of 10% NaOH had a surprising negative effect on the GVL/water mixture-mediated US-assisted delignification (Table 1, entries 8, 9, 14 and 15). Meanwhile, temperature seems not to influence delignification when GVL content is higher (Table 1, entries 6 and 7). However, it does have an effect when the amounts of GVL and water are the same (Table 1, entries 12 and 13). Almost identical results were achieved by the two mixtures under MAE, indicating that the temperature may be the most important parameter to influence delignification in MW-assisted processes (Table 1, entries 16 and 18).

NaDES are generally more promising extraction solvents than the GVL/H_2O mixtures. Interestingly, NaDES 1 is more efficient under US irradiation (Table 1, entry 20), while NaDES 2 leads to higher delignification under MAE (Table 1, entry 23). This may be due to the synergism between two polar compounds enhancing the dielectric constant of the system and thus improving their interaction with MW.

Analytical pyrolysis data for US- and MW-pretreated wheat straw in bio-based solvents demonstrated that the most prominent decrease in lignin content was found in the MW method, while guaiacyl- and syringyl-type phenols (G units and S units, respectively), were found to be the dominant lignin-related analytic pyrolysis products in both untreated and pretreated biomass. The obtained data show the variations in relative S- and G-type phenol contents (S/G ratio) in the volatile products of analytical biomass pyrolysis (Table 1), indicating that the aromatic lignin structure was modified by the extraction process.

Table 1. Py-GC/MS/FID results: Summarized relative abundance (%) of lignin derived pyrolysis products and the ratio of relative S- and G-type phenol contents (S/G ratio), as detected for untreated and pretreated wheat straw biomass.

Entry	Sample	Extraction method	lignin deriv. in volatiles (% rel.)	S/G ratio
1	**WS**	Non-treated	20.8±0.1	1/1.7

2	**H_2O-UAE1**	35 °C, 60 min, H_2O, US (40kHz-200W)	18.2±0.1	1/1.8?
3	**H_2O-UAE2**	50 °C, 60 min, H_2O, US (40kHz-200W)	19.0±0.2	1/1.8
4	**NaOH-UAE1**	35°C, 60 min, H_2O +10 % NaOH, US (40 kHz-200 W)	17.0±0.1	1/1.7
5	**NaOH-UAE2**	50°C, 60 min, H_2O +10 % NaOH, US (40 kHz-200 W)	16.0±0.1	1/1.6
6	**GVL-UAE1**	35°C, 60 min, 8:2 GVL:H_2O, US (40 kHz-200 W)	16.1±0.2	1/1.8
7	**GVL-UAE2**	50°C, 60 min, 8:2 GVL:H_2O, US (40 kHz-200 W)	16.2±0.1	1/1.8
8	**GVL-UAE3**	35°C, 60 min, 8:2 GVL:H_2O +10 % NaOH, US (40 kHz-200 W)	20.1±0.1	1/1.5
9	**GVL-UAE4**	50 °C, 60 min, 8:2 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	18.6±0.1	1/1.6
10	**GVL-UAE5**	35°C, 60 min, 8:2 GVL:H_2O, US (40 kHz-200 W)*	-	-
11	**GVL-UAE6**	50 °C, 60 min, 8:2 GVL:H_2O, US (40 kHz-200 W)*	-	-
12	**GVL-UAE7**	35°C, 60 min, 1:1 GVL:H_2O, US (40 kHz-200 W)	17.9±0.2	1/1.6
13	**WS GR 16 GVL-UAE8**	50 °C, 60 min, 1:1 GVL:H_2O, US (40 kHz--200 W)	15.3±0.1	1/1.9
14	**GVL-UAE9**	35°C, 60 min, 1:1 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	19.2±0.2	1/1.5
15	**GVL-UAE10**	50 °C, 60 min, 1:1 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	18.0±0.2	1/1.6
16	**GVL-MAE1**	90 °C, 30 min, 1:1 GVL:H_2O, MW (1500 W)	15.1±0.1	1/1.8
17	**GVL-MAE2**	90 °C, 30 min, 1:1 GVL:H_2O, MW (1500 W)**	-	-
18	**GVL-MAE3**	90 °C, 30 min, in 8:2 GVL:H_2O, MW (1500 W)	15.2±0.1	1/1.8
19	**GVL-MAE4**	90 °C, 30 min, in in 8:2 GVL:H_2O, MW (1500 W)**	-	-
20	**NaDES-UAE1**	50 °C, 120 min, in NaDES1 (lactic acid:choline chloride (10:1)), US (40 kHz- 200 W)	14.6±0.1	1/1.7
21	**NaDES-UAE2**	50 °C, 120 min, in NaDES2 (lactic acid:glycerol: choline chloride (1:1:1), US (40 kHz-200 W)	15.5±0.1	1/1.9
22	**NaDES-MAE1**	120 °C, 30 min, in NaDES1 (lactic acid:choline chloride (10:1), MW (1500 W)	14.5±0.2	1/1.7
23	**NaDES-MAE2**	120 °C, 30 min, in NaDES2 (lactic acid:glycerol: choline chloride (1:1:1), MW (1500 W)	11.3±0.1	1/1.2

*The solid, recovered after filtration, was post-treated with US (40 kHz, 200 W for 30 min at 50 °C), in the presence of an aqueous NaOH solution (1% of NaOH in dry biomass); ** The solid, recovered after filtration, was treated under conductive heating at 50 °C (for 30 min), in presence of an aqueous NaOH solution (1% of NaOH in dry biomass).

The clear differences in the influence exerted on the extractability of lignin from wheat straw by the tested GVL-H_2O mixtures, NaDES and water treatments were also reflected in the colour of the obtained extracts. The GVL and NaDES extracts exhibited the most intense colour (Fig. 1), which relates to their better delignification activity.

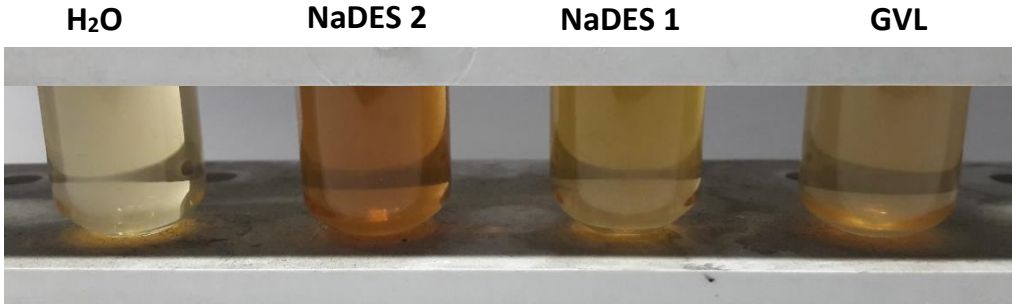

Fig. 1. Delignification solvents

Moreover, the content of levoglucosan (LG), in the analytical pyrolysis volatiles data, was lowered upon MW treatment in the presence of GVL. This becomes evident when compared with the data from the H_2O solvent treatment (Fig. 2). This reveals that the proportion of ordered cellulose in the solid pre-treated products decreased, making cellulose more prone to enzyme attack, which could be very interesting for the further valorisation of wheat straw by enzymatic digestion. On the other hand, LG content increased with NaDES 1 and 2, except for the NaDES-MAE 2 sample, thus making NADES less useful for further enzymatic treatment (Figure 3).

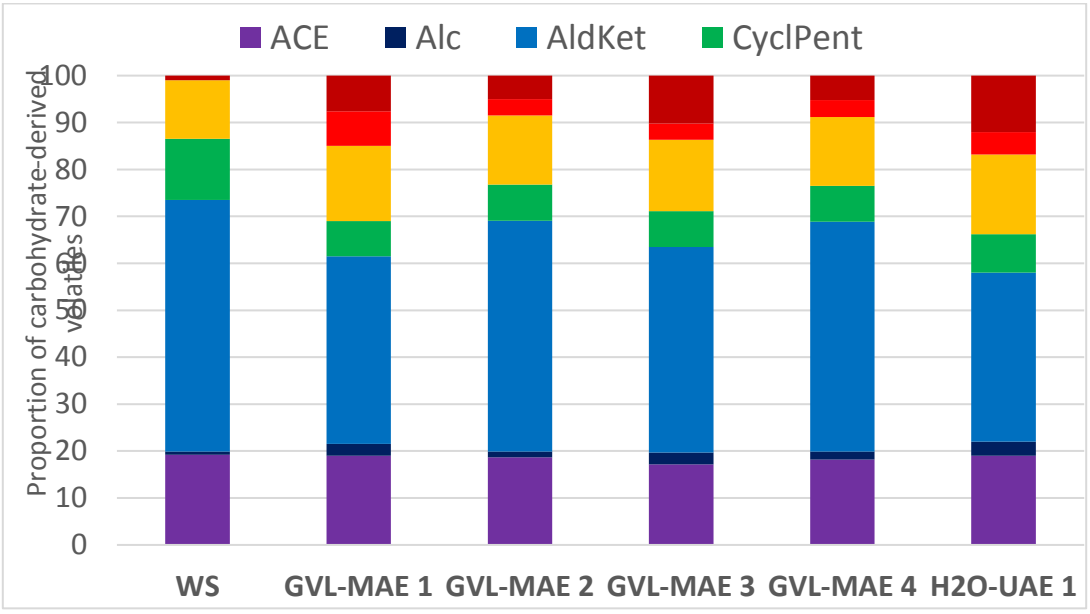

Fig. 2. Aliphatic alcohol (Alc), aliphatic aldehydes and ketones (AldKet), cyclopentane derivatives (CyclPent), aliphatic acids and esters (ACE), furan derivatives (FuD), pyran derivatives (PyD), and levoglucosan (LG) (after normalization to 100%). Proportions of carbohydrate-derived products of the analytical pyrolysis of wheat straw samples treated in the presence of GVL.

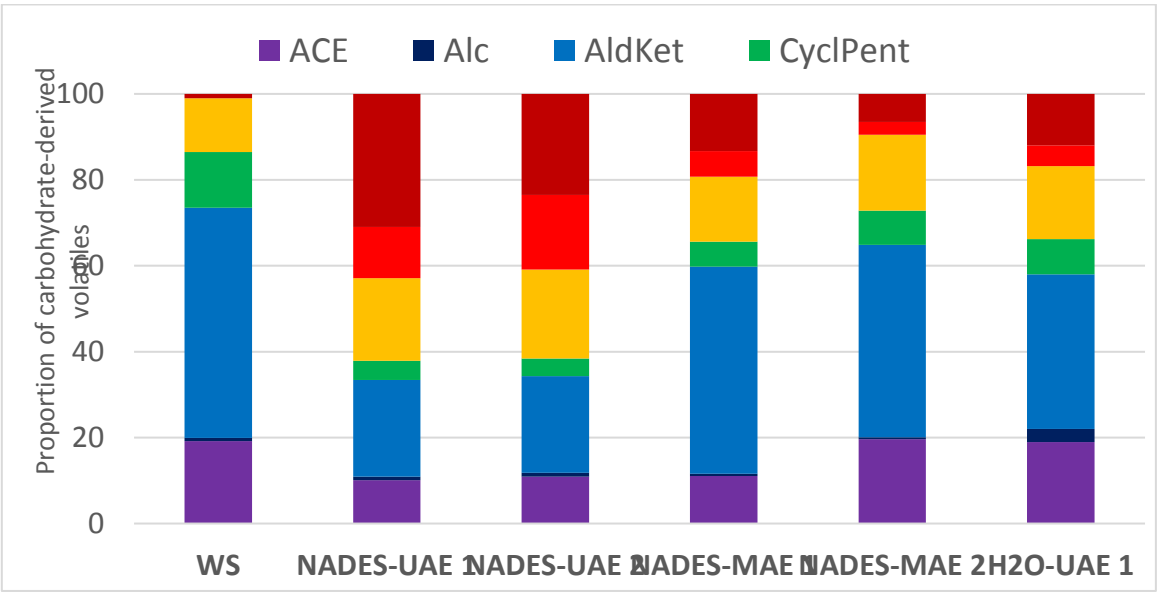

Fig. 3. Aliphatic alcohol (Alc), aliphatic aldehydes and ketones (AldKet), cyclopentane derivatives (CyclPent), aliphatic acids and esters (ACE), furan derivatives (FuD), pyran derivatives (PyD), and levoglucosan (LG) (after normalization to 100%). Proportions of carbohydrate-derived products of the analytical pyrolysis of wheat straw samples treated in the presence of NaDES.

The radical scavenging capacity (by DPPH test, Table 2), and total phenolic content (by Folin-Ciocalteu colorimetric method, Table 3) of the crude US and MW extracts were also evaluated. The samples that provided the best delignification data, according to the results reported in Table 1, were analyzed.

Table 2. Antioxidant activity of US and MW extracts.

Entry	Sample	Extraction method		DPPH results IC50
1	**NaOH-UAE2**	10% NaOH	US	**0.2451** (0.2131÷0.2917)
2	**NaOH-MW2**	10% NaOH	MW	**0.2863** (0.2383÷0.3514)
3	**GVL-UAE8**	GVL:H_2O	US	**0.3129** (0.2604÷0.3742)
4	**GVL-MAE1**	GVL:H_2O	MW	**0.3617** (0.3117÷0.4235)
5	**NaDES-UAE1**	NaDES 1	US	**0.2713** (0.2493÷0.3063)
6	**NaDES-MAE1**	NaDES 1	MW	**0.3357** (0.3061÷0.3717)
7	**NaDES-UAE2**	NaDES 2	US	**0.2619** (0.1235÷0.4461)
8	**NaDES-MAE2**	NaDES 2	MW	**0.3652** (0.3244÷0.4163)

Lower IC50 values indicate higher radical scavenging activity, as the IC50 indicates the concentration of the tested antioxidant samples required for a 50% inhibition of the radical species [21]. Despite MAE showing the best results in terms of delignification, antioxidant activity was higher in the extracts obtained under US treatment. This is probably due to the higher lignin depolymerisation induced by cavitation. The MW-extracted lignin most likely still displayed a high polymerization degree, owing to the reduced reaction time. Once more, NaDES proved to be more efficient than GVL/H_2O mixtures, with the three-component NaDES 2 showing the best results. The higher extractability that phenolic compounds show in NaDES may be attributed to H-bonding interactions that occur between the NaDES molecules and phenolic compounds. The functional groups that are generally involved in H-bonds are hydroxyl, carboxylic and amine groups, all of which are abundant in NaDES, while hydroxyl groups are obviously available in phenolic compounds. Furthermore, it can be observed that the alkaline extracts showed the best antioxidant activity.
The data on total phenolic content in the liquid fractions obtained after the wheat straw US and MW extraction processes (Table 3) did not show high values.

Table 3. Total phenolic content of the liquid fractions obtained from wheat straw delignification in the presence of green solvents.

Entry	Sample	Wheat straw pre-treatment	Total phenolic content (mg of GAE per 100 mL)
1	**GVL-UAE1**	35°C, 60 min, in 8:2 GVL:H_2O, US (40 kHz-200 W)	10 ± 1
2	**GVL-UAE2**	50°C, 60 min, in 8:2 GVL:H_2O, US (40 kHz-200 W)	17 ± 2
3	**GVL-UAE3**	35°C, 60 min, in 8:2 GVL:H_2O +10 % NaOH, US (40 kHz-200 W)	14 ± 1
4	**GVL-UAE4**	50°C, 60 min, in 8:2 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	14 ± 1
5	**GVL-UAE7**	35°C, 60 min, in 1:1 GVL:H_2O, US (40 kHz-200 W)	29 ± 2
6	**GVL-UAE8**	50°C, 60 min, in 1:1 GVL:H_2O, US (40 kHz-200 W)	17 ± 1
7	**GVL-UAE9**	35°C, 60 min, in 1:1 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	21 ± 1

8	**GVL-UAE10**	50°C, 60 min, in 1:1 GVL:H_2O+10 % NaOH, US (40 kHz-200 W)	25 ± 1
9	**GVL-MAE1**	90°C, 30 min, in 1:1 GVL:H_2O, MW (1500 W)	20 ± 1
10	**GVL-MAE2**	90°C, 30 min, in 1:1 GVL:H_2O, MW (1500 W)*	25 ± 1
11	**GVL-MAE3**	90°C, 30 min, in 8:2 GVL:H_2O, MW (1500 W)	20 ± 2
12	**GVL-MAE4**	90°C, 30 min, in 8:2 GVL:H_2O, MW (1500 W)*	24 ± 2

* Samples for which post-treatment was performed as reported in the experimental section.

It should be noted that it was not possible to carry out this analysis on the samples obtained using NaDES 1 and NaDES2 because of the stratification of the end products of the Folin reaction, meaning that their phenolic content could not be detected by UV/VIS spectrophotometry.
Finally, the surface characteristics of the recovered US and MW pre-treated wheat straw samples were investigated. The results of the nitrogen gas adsorption-desorption experiments are summarized in Table 4.

Table 4. The surface characteristics of wheat straw biomass before and after US and MW extraction processes, calculated from the results of a nitrogen gas adsorption-desorption experiment.

Entry	**Sample**	**Surface characteristics**				
		Specific surface area (S_{BET}, m^2/g)	**Total pore volume** (mm^3/g)	**Micropore volume** (mm^3/g)	**Average pore width** (nm) **DR**	**BJH**
1	**WS**	**1.3 ± 0.2**	**5.1 ± 0.7**	**0.35 ± 0.05**	**3.7 ± 0.7**	**3.7 ± 0.4**
2	H_2O-UAE1	1.2 ± 0.2	11.1 ± 2.0	0.80 ± 0.03	3.5 ± 0.1	3.5 ± 0.1
3	H_2O-UAE2	2.3 ± 0.1	9.8 ± 0.2	0.78 ± 0.05	3.6 ± 0.1	3.3 ± 0.2
4	GVL-UAE1	1.3 ± 0.2	4.7 ± 0.1	0.43 ± 0.03	3.2 ± 0.1	3.4 ± 0.1
5	GVL-UAE2	1.1 ± 0.1	4.3 ± 0.1	0.31 ± 0.02	3.2 ± 0.1	3.3 ± 0.2
6	GVL-UAE3	1.6 ± 0.1	5.1 ± 0.1	0.61 ± 0.04	3.2 ± 0.1	3.5 ± 0.5
7	GVL-UAE4	2.0 ± 0.1	7.0 ± 0.4	0.74 ± 0.02	3.1 ± 0.1	3.3 ± 0.2
8	GVL-UAE5	2.7 ± 0.1	8.2 ± 0.1	0.84 ± 0.01	3.2 ± 0.1	4.1 ± 0.4
9	GVL-UAE6	2.7 ± 0.3	9.2 ± 2.0	0.88 ± 0.08	3.2 ± 0.1	3.7 ± 0.3
10	GVL-UAE7	3.1 ± 0.1	9.6 ± 0.6	1.22 ± 0.01	2.7 ± 0.4	3.9 ± 0.2
11	GVL-UAE8	2.0 ± 0.1	9.0 ± 0.6	0.82 ± 0.01	2.9 ± 0.1	3.1 ± 0.2
12	GVL-UAE9	3.3 ± 0.1	10.2 ± 1.4	1.14 ± 0.01	2.9 ± 0.1	3.9 ± 0.1
13	GVL-UAE10	2.0 ± 0.1	7.5 ± 0.1	0.86 ± 0.03	3.0 ± 0.1	3.9 ± 0.1
14	GVL-MAE1	1.6 ± 0.2	5.8 ± 0.4	0.45 ± 0.04	3.5 ± 0.2	3.1 ± 0.1
15	GVL-MAE2	1.2 ± 0.2	7.0 ± 0.4	0.38 ± 0.04	3.4 ± 0.2	3.9 ± 0.2
16	GVLMAE3	1.8 ± 0.2	6.3 ± 0.4	0.52 ± 0.04	3.7 ± 0.3	3.4± 0.1
17	GVL-MAE4	1.6 ± 0.2	6.2 ± 0.6	0.49 ± 0.03	3.2 ± 0.2	3.5 ± 0.3
18	NaDES-UAE1	2.0 ± 0.2	10.0 ± 1.6	0.54 ± 0.04	--	4.3 ± 0.4
19	NaDES-UAE2	1.1 ± 0.2	8.4 ± 0.8	0.32 ± 0.04	3.7 ± 0.1	3.1 ± 0.2
20	NaDES-MAE1	--	--	--	--	--
21	NaDES-MAE2	--	--	--	--	--

US lignin extraction, in the presence of bio-based solvents, generally increased wheat straw porosity both in the GVL (entries 6 - 9 and 10 - 13), and NaDES samples (entry 18). Moreover, the addition of NaOH (10% in dry biomass), positively influenced the surface parameters of recovered wheat straw (entries 5 and 7, Table 4). A further improvement in the surface characteristics of the GVL:H_2O treated wheat straw (more than obtained by adding NaOH to bio based solvents), was recorded when the solid was further sonicated in the presence of NaOH (1% on dry material), as can be seen by the results for entry 5 *vs* 7 and 9 (Table 4). This result was probably due to the degrafting of GVL from wheat straw surface, as mediated by NaOH. NaDES 1 was more effective than NaDES 2 in improving the surface characteristics of US pre-treated biomass (Table 4, entries 18 *vs* 19).

MW pre-treatment of wheat straw biomass in green solvents (Table 4, entries 14, 16, 15 and 17), did not significantly influence wheat straw porosity.

4. Conclusions

In comparison with conventional extraction, the proposed MAE and UAE with green solvents presents significant advantages in terms of sustainability. These findings suggest that US and MW are eco-friendly techniques suitable for wheat straw delignification.

A mixture of natural compounds, NaDES and bio-derived GVL, have been tested as solvents for the extraction of lignin derived compounds as an alternative to alkali treatment.

The highest delignification (45%), for unconventionally pre-treated wheat straw in the presence of bio-derived solvents was reached under MW irradiation at 120 °C in only 30 min, in the presence of three-component NaDES. A GVL/water mixture also gave efficient delignification (27%), under US mild pre-treatment (50 °C for 60 min). Antioxidant activity, measured using the DPPH test, was higher in the extracts obtained under US, and in particular, using NaDES. This holds promise for further applications of NaDES in the valorisation of lignin as an antioxidant additive in pharmaceuticals, cosmetics and the food industries. Furthermore, GVL/water mixtures proved efficient in decreasing the portion of ordered cellulose in wheat straw. According to these findings, NADES could be used for liquid fraction valorisation, while GVL could be the solvent of choice for further solid applications. These non-conventional treatments can lead to the valorisation of both the lignin and the cellulose in wheat straw and, as such, fulfil the requirements the biorefinery concept.

Acknowledgments

The H2020 Project US4GREENCHEM (Grant Agreement n° 669055) “Combined Ultrasonic and Enzyme Treatment of Lignocellulosic Feedstock as Substrate for Sugar Based Biotechnological Applications” is warmly acknowledged for the funds provided by the BBI-JU.

Reference

[1] J. C. Serrano-Ruiz, R. Luque, A. Sepúlveda-Escribano, Chem. Soc. Rev. 40 (2011) 5266.
[2] E. Roselló-Soto, M. M. Poojary, F. J. Barbaa, J. M. Lorenzo, J. Mañes, J. C. Moltó, Innov. Food Sci. Emerg. 45 (2018) 306.
[3] (a) J.G. Lynam, N. Kumar, M.J. Wong, Biores. Technol. 238 (2017) 684.
[4] (b) R.M. Trevoraha, T. Huynhb, T. Vancovc, M.Z. Othmana, Biores. Technol. 250 (2018) 673.
[5] (c) M. Pan, G. Zhao, C. Ding, B. Wu, Z. Lian, H. Lian, Carbohydr. Polym. 176 (2017) 307.
[6] (d) W. Xing, G. Xu, J. Dong, R. Han, Y. Ni Chem. Eng. J. 333 (2018) 712.
[7] (a) D. Duana, R. Ruana, Y. Wanga, Y. Liua, L. Daia, Y. Zhao, Y. Zhou, Q. Wu, Biores. Technol. 251 (2018) 57.
[8] (b) C.L. Yiin, A.T. Quitain, S. Yusup, Y. Uemura, M. Sasaki, T. Kida, Biores. Technol. 244 (2017) 941.
[9] (c) Z. Chen, C. Wan, Biores. Technol. 250 (2018) 532.

[10] N. N. Misra, A. Martynenko, F. Chemat, L. Paniwnyk, F. J. Barba, A. R. Jambrak, Crit. Rev. Food Sci. DOI: 10.1080/10408398.2017.1287660.
[11] B. B. Hewetson, X. Zhang, N. S. Mosier, Energy Fuels, 30 (2016) 9975.
[12] P. Sannigrahi, A. J. Ragauskas, J. Biobased Mat. Bioeng. 5 (2011) 514.
[13] W. Thielemans, E. Can, S. Morye, R. Wool J. Appl. Polym. Sci. 83 (2002) 323.
[14] M. Kleinert, T. Barth, Energy Fuels, 22 (2008) 1371.
[15] R. Kuhad, A. Singh, Lignocellulose biotechnology: future prospects. I.K. International Publishing House, Put. Ltd., India, 2007.
[16] J. M. Humphreys, C. Chapple, Curr. Opin. Plant Biol. 5 (2002) 224.
[17] A. Naseem, S. Tabasuma, K. M. Zia, M. Zuber, M. Ali, A. Noreen, Int. J. Biol. Macromol. 93 (2016) 296.
[18] D. Watkins, Md. Nuruddin, M. Hosur, A. Tcherbi-Narteh, S. Jeelani, J. Mater. Res. Technol. 4 (2015) 26.
[19] X. Pan, J.F. K. Ehara , N. Gilkes , J.N. Saddler, J. Agric. Food Chem. 54 (2006) 5806.
[20] X. Wanga, S. Raya, R.P. Cooneya, P.A. Kilmartina, G.I.N.Waterhouseb, A.J. Easteala, Synth. Met. 162 (2012) 1084.
[21] J. Ponomarenko, T. Dizhbite, M. Lauberts, A. Viksna, G. Dobele, O. Bikovens, G. Telysheva, BioResources, 9 (2014) 2051.
[22] H. Faustino, N. Gil, C. Baptista, A.P. Duarte, Molecules, 15 (2010) 9308.
[23] X. Wanga, S. Raya, R. P. Cooneya, P. A. Kilmartina, G. I. N. Waterhouseb, A. J. Easteala, Synth. Met. 162 (2012) 1084;
[24] G. Cirillo, F. Iemma, Antioxidant Polymers: Synthesis, Properties and Applications, Wiley and Scrivener, New York 2012.
[25] S. Domenek, A. Louaifi, A. Guinault S. Baumberger, J. Polym. Environ. 21 (2013) 692.
[26] M. Bunzel, J. Ralph. J. Agric. Food. Chem. 54 (2006) 8352.
[27] R.L. Shogren, A. Biswas, Carbohydr. Polym. 91 (2013) 581.
[28] P. Ma, Y. Gao, H. Zhai, BioResources, 8 (2013) 5581.
[29] T. Dizhbite, G. Telysheva, V. Jurkjane, U. Viesturs. Bioresource Technol. 95 (2004) 309.
[30] A. Bhat, Y. Dasan I. Khan,Extraction of Lignin from Biomass for Biodiesel Production. In: Hakeem K., Jawaid M., Y. Alothman O. (eds) Agricultural Biomass Based Potential Materials. Springer, Cham, Switzerland (2015);
[31] C. Dary, B. Baghdikian, S. Kim, F. Mabrouki, S. Hul, F. Jabbour, E. Ollivier , S.-S. Bun. C. R. Chimie, (2017) 1.
[32] M. Kouba, H. Mhemdi, F. J. Barba, S. Roohinejad, R. Greiner, E. Vorobiev, Food Res. Int. 85 (2016) 59.
[33] R. Singh, B. B. Krishna, J. Kumar, T. Bhaskar, Bioresource Technol. 199 (2016) 398.
[34] N. D. Vu, H. T. Tran, N.D. Bui, C. D. Vu, H. V. Nguyen, Int. J. Polym. Sci. (2017) doi:10.1155/2017/1063695.
[35] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, M. Abert-Vian, Ultrason. Sonochem. 34 (2017) 540.
[36] F. Chemat, G. Cravotto, (Eds.) Microwave-assisted Extraction for Bioactive Compounds, Springer US, New York, 2013.
[37] M. Bouras , M. Chadni, F. J. Barbaa, N. Grimi , O. Bals, E. Vorobiev, Ind. Crop. Prod. 77 (2015) 590.
[38] J. Fan, M. De Bruyn, V. L. Budarin, M. J. Gronnow, P. S. Shuttleworth, S.Breeden,D. J. Macquarrie, J. H. Clark, J. Am. Chem. Soc. 135 (2013) 11728.
[39] (a) L. Zoia, M. Orlandi, D. S. Argyropoulos, J. Agric. Food Chem. 56 (21) (2008), 10115;
[40] (c) L. Zhou, V. Budarin, J. Fan, R. Sloan, D. Macquarrie, ACS Sustainable Chem. Eng. 5 (2017) 3768.
[41] A. Farra, C. Cai, M. Sandoval, Y. Xu, J. Liu, M. J. Hernáiz, R. J. Linhardt, Chem. Rev. 115 (2015) 6811.
[42] (a) A. P. Abbott, D. Boothby, G. Capper, D. L. Davies, R. K. Rasheed, J. Am. Chem. Soc. 126 (2004) 9142.
[43] (c) Y. Dai, J. van Spronsen, G.-J. Witkamp, R. Verpoorte, Y. H. Choi, J. Nat. Prod. 76 (2013) 2162.
[44] M. Ruesgas-Ramón M. C. Figueroa-Espinoza, E Durand, J. Agric. Food Chem. 65 (2017) 3591.
[45] A. M. Raspolli Galletti, C. Antonetti, V. De Luise, M. Martinelli, Green Chem. 14 (2012) 688.
[46] S. Tabasso, G. Grillo, D. Carnaroglio, E. Calcio Gaudino, G. Cravotto, Molecules 21 (2016) 413.
[47] G. Strappaveccia, L. Luciani, E. Bartollini, A. Marrocchi, F. Pizzo, L. Vaccaro, Green Chem. 17 (2015) 1071.
[48] Touati, F. Jose Barba, H. Louaileche, A. Frigola, M. J. Esteve, J. Food Quality, 39 (2016) 209. .
[49] W. Brand-Williams, M. E. Cuvelier, C. Berset, Lebensm.-Wiss. u.-Technol. 28 (1995) 25.

[50] V. F. Singleton, R. Orthofer, Methods Enzymol. 299 (1999) 152.
[51] K.S.W. Sing, D.H. Everett, R.A.W. Haul, L. Moscou, R.A. Pierotti, J. Rouquerol, T. Siemieniewska, Pure Appl. Chem. 57 (1985) 603.
[52] Y. Dai, G.-J. Witkamp, R. Verpoorte, Y. H. Choi, Anal. Chem. 85 (2013) 6272.
[53] C. Fang, M. H. Thomsen, C. G. Frankær, G. P. Brudecki,J. E. Schmidt, I. M. AlNashef, Ind. Eng. Chem. Res. 56 (2017) 31674.
[54] C.-W. Zhang, S.-Q. Xia, P.-S Ma, Biores. Technol. 219 (2016) 1.
[55] W. Fang, H. Sixta, ChemSusChem 8 (2015) 73.
[56] A.K. Kumar, S. Sharma, Bioresource Bioprocess (2017) 4.
[57] K. M. Jeong, J. Ko, J. Zhao, Y. Jin, D. E. Yoo, S. Y. Han, J. Lee, J. Cleaner Prod. 151 (2017) 87.
[58] A. Farrán, C. Cai, M. Sandoval, Y. Xu, J. Liu, M. J. Hernáiz, R. J. Linhardt, Chem Rev 115 (2015) 6811.
[59] V. Yachmenev, B. Condon, T. Klasson, A. Lambert, J. Biobased Mater. Bioenergy 3 (2009) 25.
[60] M.S.U. Rehman, I. Kim, Y. Chisti, J.I. Han, EEST Part A, Energy Sci. Res. 30 (2013) 1391.
[61] P.R. Gogate, V.S. Sutkar, A.B. Pandit, Chem. Eng. J. 166 (2011) 1066.