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Sustainable valorization of cherry (*Prunus avium* L.) pomace waste *via* the combined use of (NA)DESs and bio-ILs†

Angelica Mero,[a] Andrea Mezzetta,[a,b] Marinella De Leo,*[a,b,c] Alessandra Braca[a,b,c] and Lorenzo Guazzelli *[a,b]

Sweet cherry pomace waste (CPW) obtained after juice production still contains high amounts of dietary fiber and added value compounds such as polyphenols. For this reason, it can be employed as renewable feedstock to produce novel commodities, bioactive compounds and bio-functional materials. In this study, a sustainable biorefinery approach has been developed combining the use of natural deep eutectic solvents (NADESs) for the recovery of polyphenolic compounds and bio-based ionic liquids (bio-ILs) for the treatment and conversion of the remaining lignocellulosic residue into an ionogel. The extraction efficiency and selectivity of different classes of (NA)DESs were evaluated varying both the hydrogen bond acceptor, HBA (choline chloride (ChCl), betaine (Bet) and L-proline (Pro)) and the hydrogen bond donor, HBD (carboxylic acids and polyols). The extracted phenolic compounds were qualitatively and quantitatively analyzed by UHPLC-HR-ESI-MS leading to the identification of more than 25 compounds, classifiable into four main subclasses (phenolic acids, flavonoid glycosides, flavonoid aglycones, and anthocyanins). The majority of ChCl-based DESs improved the total phenol extraction compared to classical solvents (up to twice). ChCl : ethylene glycol 1 : 2 was identified as the best performing system in terms of total phenolic content (total phenol 759 ± 85 μg g^{-1} of dried CPW) and was employed as the extraction medium for further optimization of the operative conditions and recycling study. The solvent was quantitatively recovered for three cycles with retention of the extraction efficiency towards all the identified subclasses of phenols. Finally, cholinium arginate (ChArg) allowed for obtaining cellulose enriched material (35 wt% of dried biomass) that was subsequently dissolved at 2 wt% in cholinium levulinate (ChLev) to prepare a weak physical ionogel, a functional biomaterial potentially useful in medical and pharmaceutical sectors.

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Introduction

Sweet cherries (*Prunus avium* L.) with a global production of around 4.0 million tons per year[1] are one of the most appreciated and eaten fruits by consumers due to their organoleptic properties and nutritious nature.[2] Indeed, cherries contain a wealth of essential nutrients and biologically active molecules such as sugars, organic acids, carotenoids, melatonin, vitamin C, minerals, fibers and, most of all, high amount of phenolic compounds including anthocyanins, flavonoids, catechin and epicatechin, procyanidins and phenolic acids such as hydroxycinnamic acids.[3–6] Due to the short life (7–10 days) of sweet cherries, fresh fruits are widely processed into marmalades, juices or fruit cocktails and other food products[7] with the concomitant production of large volumes of waste which represent a potential source of high added value bioactive compounds. The most abundant components of these agri-food derived waste are all the typical dietary fibers such as cellulose, hemicellulose, pectin, and lignin.[8] Thus, following the concept of the circular economy, these wastes could be also used as valuable renewable feedstock for the production of novel commodities that are alternatives to fossil-based products. Nowadays, the extraction of phenolic compounds with high antioxidant capacity from sour cherry pomace has been much investigated[9–11] while there are very few studies about bioactive compounds in processed sweet cherries[12] or pomace wastes. Furthermore, the employment of green solvents instead of classical toxic organic solvents outlines one of the main challenges in the field of extraction processes or biomass treatments. Among the different sustainable extraction solvents already proposed in the literature, (natural) deep eutectic

[a]*Department of Pharmacy, Via Bonanno 33, 56126 Pisa, Italy. E-mail: lorenzo.guazzelli@unipi.it, marinella.deleo@unipi.it*
[b]*Centre for Instrumentation Sharing, University of Pisa (CISUP), Lungarno Pacinotti 43, 56127 Pisa, Italy*
[c]*Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy*

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solvents ((NA)DESs) play a prominent role. They are obtained by simple mixing of two or more components that are able to establish hydrogen bonds and thus form a homogeneous mixture for which the eutectic temperature is lower than that of an ideal eutectic.[13] (NA)DESs possess a lot of interesting features such as very simple preparation without the need of purification steps and the possibility of designing their properties by means of pairing the proper hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD).[14–16] The employment of natural or natural-derived compounds such as choline chloride (ChCl), amino acids, sugars, carboxylic acids, and other primary metabolites allows for the preparation of completely natural, biodegradable and generally low toxic solvents.[17,18] Another intriguing property of (NA)DESs is related to their great solvation power towards several classes of compounds giving them the applicability as extraction media in several fields. In particular, DESs, especially ChCl-based DESs, have shown great potential towards the extraction of bioactive compounds from disparate food products or wastes.[19–24] However, regarding cherry pomace, only one example of polyphenol extraction from sour cherry pomace using NADESs has been proposed to date[25] while no works on sweet cherry pomace and DESs have been reported so far. Before the discovery of DESs, ionic liquids (ILs) had been introduced as the first class of designer solvents thanks to the possibility of pairing a wide variety of cations and anions. ILs exhibit several appealing and tunable properties (negligible vapour pressure, high chemical and thermal stability, wide electrochemical range, and non-flammability) which make them suitable for a large range of applications.[26] One of the most appreciated properties of ILs is their capability for dissolving a wide range of materials, most importantly polymeric compounds such as cellulose, hemicellulose and lignin that are difficult to solubilize with classical organic solvents. In particular, these solvents are capable of fractionating biomass, selectively dissolving and so extracting one of the components.[27,28] This allows for better valorization of each component, especially regarding the cellulosic part, that can be derivatized to yield various useful novel cellulose-based products, *i.e.* gel-like materials (ionogels, hydrogels, aerogels, *etc.*).[29] Among them, ionogels are gel materials whose liquid phase is an IL while the matrix can be organic, inorganic, or hybrid organic–inorganic possessing a broad range of uses such as in electrochemical devices, energy storage, sensors and biosensors,[30,31] biomedical materials, catalysts *etc.*[32,33] In particular, cellulose is an excellent working building block for the preparation of ionogels due to the presence of three hydroxyl groups in each glucose unit. It can be used without any alterations or as modified by introducing functional groups for the formation of both physical or chemical ionogels.[34] ILs containing natural or bio-based ions, so-called bio-ILs, have been successfully tested in fractionation processes of lignocellulosic biomass[27] or in the preparation of new bio-materials starting from commercial microcrystalline cellulose (MCC).[34–36] To the best of our knowledge, while some examples about the preparation of aerogels or hydrogels *via* ILs starting from waste materials like waste newspapers[37] or hybrid poplar and biomass sorghum bagasse samples[38] or waste bamboo-based disposable paper cups[39] have been reported, only one work showed the preparation of an ionogel using cellulose-rich solids using a biorefinery approach. In this scenario, the development of a two-step biorefinery process for the valorisation of cherry pomace waste was the main objective of the present study (Fig. 1). (i) In the first part of the study, the effect of HBA (choline chloride, betaine, and proline) and HBD (ethylene glycol, glycerol, oxalic acid, malic acid, and citric acid) DES components on the extraction performance of high-value bioactive compounds and on the composition of the extracts was investigated. In this step, operating conditions were optimized for the best performing system in terms of the total amounts of polyphenols extracted. (ii) In the second part, the insoluble residue from the extraction process was fractionated using cholinium arginate (ChArg), and the cellulose-rich part obtained was further used for the preparation of an ionogel using cholinium levulinate (ChLev). The proposed biorefining process aims to valorise a waste product of the food industry exploiting solvents derived from natural sources and endowed with low environmental impact.

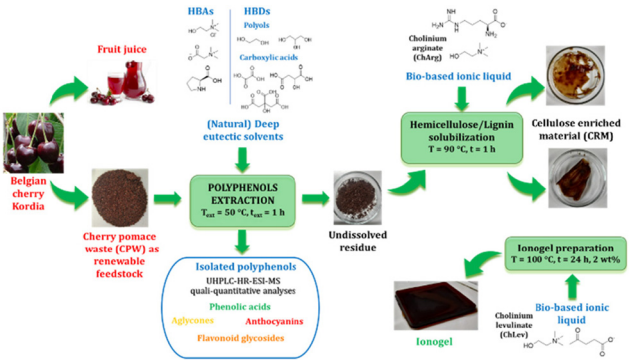

Fig. 1 Scheme of the developed biorefinery approach aimed at the complete valorization of cherry pomace waste.

Experimental

Materials and methods

UHPLC grade acetonitrile, formic acid, and water were purchased from Romil-Deltek (Pozzuoli, Italy). Betaine anhydrous (>97%) and choline hydroxide in water solution (47–50%) were purchased from TCI Europe (Zwijndrecht, Belgium). Choline chloride (98%), L-proline (99%), oxalic acid dihydrate (98%), citric acid (99%), DL-malic acid (98%), ethylene glycol (99%), levulinic acid (98%) were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). L-Arginine (≥98%), methanol, *n*-BuOH, glycerol (99%) and Amberlite XAD-7 resin were purchased from Merck KGaA (Darmstadt, Germany). Chlorogenic acid (≥95%) and rutin (>98%) used as reference standards were purchased from Extrasynthese (Genay, France), while naringenin (≥95%) and cyanidin 3-*O*-glucoside (≥98%) were from Merck KGaA (Darmstadt, Germany).

The cherry pomace waste (CPW) was provided by "ILVO" (Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium) which destoned the cherries with a

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cherry destoner of Enotecnica Pillan and pressed them with the VaculIQ for the production of cherry juice. Before using, the pomace was dried at 40 °C in an oven for 48 h and was ground using an MF 10 basic microfine grinder drive (IKA-Werke, Staufen, Germany) with a 0.25 mm sieve hole.

Preparation of classical extracts

The extraction with a classical solvent was performed by dynamic maceration (150 rpm) of 1 g of dried CPW in 10 g of MeOH for 1 h. For LC-MS analyses, the methanolic extract was first partitioned between *n*-BuOH and H_2O to remove unwanted metabolites (*e.g.*, sugars). After centrifugation (2710*g* for 5 min), the *n*-butanolic phase was dried under vacuum to obtain a residue, and subsequently used for LC-MS analyses.

Anthocyanins were extracted by dynamic maceration (150 rpm) of 1 g dried CPW in 6 mL of 2% HCl methanol solution for 1 h. The extract solutions were then centrifuged (2710*g* for 5 min) and the obtained supernatants injected into the LC-MS system.

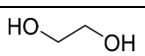
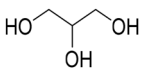
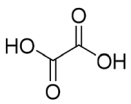
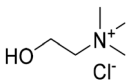
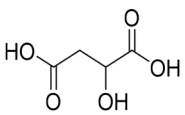
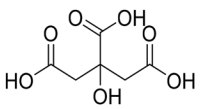
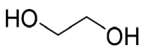
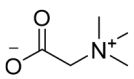
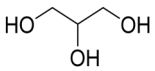
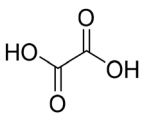
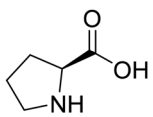
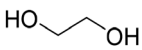
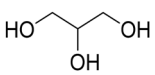
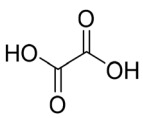
Preparation of (NA)DESs

Before preparation, choline chloride (ChCl), ethylene glycol (EG), and glycerol (Gly) were dried under vacuum for 6 h at 80 °C. The (NA)DESs were prepared by mixing the different HBAs (ChCl, Bet or L-Pro) and the corresponding HBD (carboxylic acid or polyol) at certain molar ratios, as shown in Table 1. The mixtures were stirred at the appropriate temperature (80 °C for DL-malic and citric acid-based DESs, 40 °C for oxalic acid-based DES and room temperature for all the polyol-based DESs) until a homogeneous transparent liquid was formed. After the (NA)DES formation, no purification step was needed, and the solvents were kept at room temperature in sealed vessels until their use. The DESs with a carboxylic acid as the HBD were mixed with water at 25 wt% water in order to reduce their viscosity. ^{1}H-NMR spectra (D_2O) were obtained to assess the composition and the purity of the prepared DESs (Fig. S2–S12†).

Synthesis of bio-ILs

Cholinium arginate (ChArg). ChArg (Fig. 2) was synthesised as described previously by To *et al.*[40,41] Briefly, prior to use,

Table 1 (NA)DES molar ratio and preparation conditions

Name	HBA	HBD	Molar ratio	*T* (°C)
ChCl : EG (choline chloride : ethylene glycol)			1 : 2	25
ChCl : Gly (choline chloride : glycerol)			1 : 2	25
ChCl : OA + 25% H_2O (choline chloride : oxalic acid)			1 : 1	40
ChCl : MA + 25% H_2O (choline chloride : malic acid)			1 : 1	80
ChCl : CA + 25% H_2O (choline chloride : citric acid)			1 : 1	80
Bet : EG (betaine : ethylene glycol)			1 : 3	25
Bet : Gly (betaine : glycerol)			1 : 2	25
Bet : OA + 25% H_2O (betaine : oxalic acid)			1 : 1	80
L-Pro : EG (L-proline : ethylene glycol)			1 : 3	25
L-Pro : Gly (L-proline : glycerol)			1 : 2	25
L-Pro : OA + 25% H_2O (L-proline : oxalic acid)			1 : 1	80

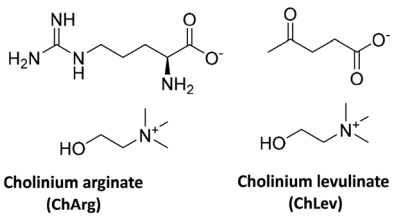


Fig. 2 Chemical structure of the employed bio-ILs.

commercial choline hydroxide (ChOH) aqueous solution (*ca.* 47–50%) was titrated with 1 M HCl solution giving the exact percent of ionic liquid in water (49.15%). Equal amounts (mol%) of ChOH and aqueous solution of the amino acid were mixed and then the mixture was heated to 70 °C and stirred for 3 h. At the end of the reaction, water was removed under reduced pressure. The correct stoichiometry and purity of the synthesized IL was verified by means of the ^{1}H-NMR and ^{13}C-NMR spectra registered in D_2O and the data are in accordance with those reported in the literature.[42]

Cholinium levulinate (ChLev). The synthesis of ChLev (Fig. 2) was performed starting again from commercial ChOH aqueous solution (*ca.* 47–50%) that had prior to use been titrated with 1 M HCl solution giving the exact percent of ionic liquid in water (49.15%). An equal amount (mol%) of levulinic acid was added to the stirred aqueous solution of ChOH at room temperature and then the mixture was stirred for 1 h. At the end of the reaction, water was removed under reduced pressure. The correct stoichiometry and purity of the synthesized IL was verified by means of the ^{1}H-NMR spectrum registered in D_2O and the data are reported in the ESI (Fig. S13†). The synthesized IL was also analyzed by FTIR (Fig. S14†) and TGA (Fig. S15†).

Preparation of (NA)DES extracts

The extraction with (NA)DES media was performed in a round-bottomed glass flask by the stirring-heating method as reported in our previous works with slight modifications.[22,43] Briefly, for the initial screening of the best performing system, fixed conditions were applied: 1 g of dried CPW was treated with 10 g of (NA)DES for 1 h at 50 °C. Afterwards, the mixture was cooled down to room temperature, diluted with MeOH/H_2O (70/30) to reduce the viscosity and facilitate the subsequent filtration step for the separation of the undissolved residue that was collected and dried in an oven at 60 °C. The supernatant was concentrated under reduced pressure to recover the MeOH and then, a polymeric resin, Amberlite XAD-7 (50 g), was used to separate phenolic compounds from the recovered water phase containing the (NA)DES. Prior to use, the resin was washed and activated by stirring it with acidified water (0.01 M HCl) for 30 min. The activated resin was put into a column and the extraction mixture was added. The (NA)DES was collected by washing the absorbed polyphenol–polymeric resin with water. The aqueous fraction thus obtained was evaporated under reduced pressure for recovering the (NA)DES. Subsequently, after drying the resin under airflow, the extracted polyphenols were desorbed from the resin with MeOH (100 mL). Following that, the methanolic extract was evaporated under reduced pressure for recovering the polyphenolic extracts as solid residues, and subsequently used for UHPLC-MS analyses (the method is described in detail in the ESI†).

Recovery and reuse of the DES

The possibility of recycling the DES was evaluated for the best performing system studied for the extraction of polyphenols (ChCl : EG 1 : 2). In particular, at the end of the process, the aqueous fraction obtained after washing the polymeric resin for recovering the DES was evaporated under reduced pressure. Subsequently, the recovered DES was reused without any modification for a new extraction process with fresh biomass waste as described in the previous section. The solvent was recycled three times and it was analyzed by ^{1}H-NMR in D_2O before each cycle. The extraction efficiency of the recovered DES was evaluated by determining the phenol content of the methanolic extracts by UHPLC-MS analyses as described in the previous section.

Determination of the chemical composition

The chemical compositions of the starting CPW biomass and of the residue obtained after polyphenol extraction were determined according to the classical methods reported in the literature as described in detail in the ESI.† In particular, free water-soluble sugars, pectin and holocellulose contents were measured and compared.

Treatment with bio-ILs for the valorization of the undissolved residue obtained after polyphenol extraction

Treatment with ChArg. After polyphenol extraction, the undissolved residue was treated with ChArg following the procedure previously described by Hou *et al.*,[44] An *et al.*[42] and in our previous work[41,43] with some modifications. The treatment was performed under milder condition than those described by the authors. Briefly, IL was heated under stirring at 90 °C and then biomass samples were added with a biomass/IL ratio 1/10 (w/w) and stirred at the same temperature for 1 h. Subsequently, the suspension was cooled to room temperature and diluted with 20 mL of NaOH solution (0.1 M). The undissolved residue (cellulose enriched material – CRM) was recovered by filtration and washed with the same basic solution and then water until neutral pH. Afterwards, the solid residue was dried for 24 h in an oven at 45 °C and stored in a sealed vial prior to characterization and final dissolution in ChLev. The remaining filtrate was acidified to pH 2 with (HCl, 4 and 1 M) and stored at 4 °C for a night to precipitate a second fraction. The recovered material was separated by centrifugation and washed with the same acidic solution and then with water and finally dried in an oven at 45 °C for 24 h. The two fractions were then analyzed by FTIR and TGA. Furthermore, the IL was recovered; after the separation of the second fraction, the filtrate was basified to pH 11 (pH of the aqueous solution of the IL) with NaOH (4 and 1 M). Water was removed by evaporation under reduced pressure. Anhydrous MeOH was added to the residue, leaving NaCl as the insoluble solid, which was removed by filtration. MeOH was evaporated under reduced pressure and the recovered IL was analyzed by ^{1}H-NMR spectroscopy.

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Treatment with ChLev: dissolution and ionogel formation. The cellulose enriched material obtained from the previous treatment with ChArg was dissolved as described in our previous work[45] with slight modifications. Briefly, the CRM was triturated and then slowly added at 2 wt% load under stirring to ChLev that had been previously heated to 100 °C. Before using, ChLev was thoroughly dried under vacuum to reduce the presence of water. Then, the mixture was stirred at the selected temperature (100 °C) until complete dissolution of the CRM (24 h). After dissolution, the warm mixture was poured in a suitable mold and kept at room temperature until the formation of an ionogel. After 3 d, formation of the gel was observed. The ionic liquid not incorporated in the gel was removed and recovered and the obtained gel was washed with a minimum amount of water in order to remove the excess of IL that had exuded after water absorption. Finally, the obtained ionogel was saturated with water at 30 °C (it was put in an enclosed environment with water in an oven) before analysis. The ionogel was characterized by FTIR spectroscopy, TGA and rheological analyses. The excess of IL was collected, evaporated under reduced pressure and analyzed by ^{1}H-NMR.

Results and discussion

Polyphenol extraction with (NA)DES

ChCl-based DESs, were initially selected for polyphenol extraction from CPW since they are reported as the best performing systems in bioactive compound extraction from several biomass materials (plant materials or food wastes) and are by far the most commonly used DESs. Considering the phenolic profile of sweet cherry fruits and pomace which showed high amounts of anthocyanins along with some phenolic acids and flavonoids, three carboxylic acid-based DESs[46–50] and two polyol-based DESs[51–56] were selected. The molar ratio of the prepared (NA)DESs is reported in Table 1. The high viscosity of the acid-based solvents, that could hamper mass transport thus drastically limiting the efficiency of the extraction process, was reduced by adding water at 25 wt%. The beneficial effect of adding water in extraction processes has been extensively studied and reported in the literature.[57,58] To compare the extraction efficiency and the potential selectivity towards specific types of phenols, the extractions were performed under fixed conditions (biomass/solvent 1/10, 1 h and 50 °C). A classical approach using methanol for phenolic acid and flavonoid extraction and acidified methanol for anthocyanin extraction was also carried out and the obtained extracts were used as blank samples. When the methanolic extraction was performed, a subsequent partition between *n*-butanol and water was necessary, to remove undesired materials such as sugars. Instead, when (NA)DESs were used, the extracted phenolic compounds have been separated from the (NA)DES by using a macroporous resin (Amberlite XAD-7) that is able to adsorb the phenols while the (NA)DES was collected with water. Then, desorption was carried out by passing the minimum amount of methanol through the absorbed polyphenol–polymeric resin. This procedure allowed the quantitative recovery of the employed (NA)DES, which can be recycled and tested as extraction media with fresh biomass. For this purpose, all the recovered (NA)DESs were analyzed through ^{1}H-NMR spectroscopy (some examples are reported in Fig. S16†). All the spectra showed the same signals of pure (NA)DESs along with typical signals of sugar based-compounds, especially for acid-based (NA)DESs. This aspect will be discussed in the following sections. In both (NA)DES and classical solvent extraction, the recovered phenols are dried and obtained as a solid material. To qualitatively and quantitatively characterize the extracted phenols, methanolic solutions of each extract were prepared and analyzed as shown in the following sections.

Phenol profiles of cherry pomace waste extracts

All CPW extracts were subjected to UHPLC-HR-Orbitrap/MS/MS analyses to characterize their chemical composition and establish the difference in terms of recovered compounds by both classical and innovative extraction procedures. The attention was focused on phenols reported in cherry fresh fruits to evaluate to which extent cherry industrial waste can be considered a source of high-value substances after juice production. The extracted ion chromatograms obtained by LC-MS showed the phenol profiles of each sample (Fig. 3). Compounds were tentatively identified by a comparison of their elution order and both full and fragmentation HR mass spectra with the literature data (Table 2). A mass error <5 ppm was considered acceptable. All extracts showed a very similar composition, with three main classes of compounds, represented by phenolic acids (peaks **1–3**, **5**, and **6**), flavonoid *C*- and *O*-glycosides (peaks **4**, **7–13**, **15**, **17**, and **18–22**), and flavonoid aglycones (peaks **14**, **16**, **19–21**, **23**, and **24**). Among phenolic acids, the most representative peak in all samples was represented by dihydroxybenzoic acid in two isomeric forms (**1a** and **1b**), followed by hydroxycinnamic acids previously reported in cherry fruits.[59,60] Peaks **3a**/**3b** and **6a**/**6b** were assigned as *p*-coumaroylquinic acid *cis*/*trans* isomers, as deduced by the same deprotonated molecular ion $[M - H]^-$ at *m*/*z* 337.0932. The position of the *p*-coumaroyl moiety on quinic acid was established by MS/MS experiments, showing base ion peaks at *m*/*z* 163.04 and 173.04 for 3-*p*-coumaroylquinic acids

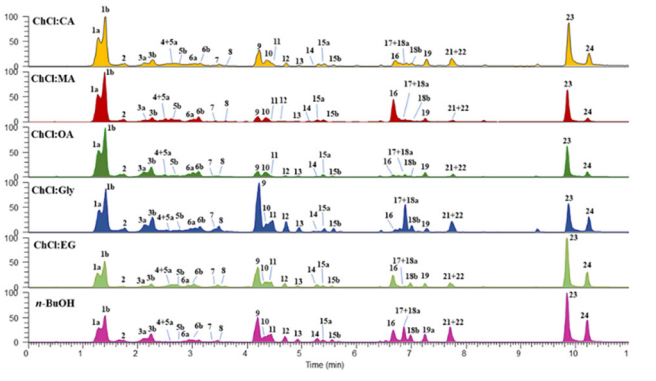

Fig. 3 UHPLC-HR-ESI-Orbitrap/MS/MS phenolic profiles of cherry pomace waste extracts obtained using classical solvents and (NA)DESs (extracted ion chromatograms in negative ionization mode). Peak data are reported in Table 2.

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Table 2 Chromatographic and HR-ESI mass data (full and fragmentation pathway) of chemical constituents identified in cherry pomace waste extracts obtained by classical solvents and (NA)DESs

Peak	Compound	t_R (min)	$[M-H]^-$	HR-ESI-MS/MS ions (m/z)[a]	Molecular formula	Error (ppm)	Ref.
Phenolic acids							
1a, 1b	Dihydroxy benzoic acid (isomers I and II)	1.3, 1.4	153.0188	**109.03**, 91.02	$C_7H_6O_4$	−2.81	60
2	Chlorogenic acid (3-*O*-caffeoylquinic acid)	1.8	353.0881	**191.06**, 179.03	$C_{16}H_{18}O_9$	+0.850	59
3a, 3b	3-*p*-Coumaroylquinic acid (isomers *cis* and *trans*)	2.1, 2.3	337.0932	191.06, **163.04**, 119.05	$C_{16}H_{18}O_8$	+0.890	59
5a, 5b	3-*O*-Feruloylquinic acid (isomers *cis* and *trans*)	2.6, 2.7	367.1034	**193.05**, 173.04, 134.05, 69.76	$C_{17}H_{20}O_9$	−0.272	59
6a, 6b	4-*p*-Coumaroylquinic acid (isomers *cis* and *trans*)	2.8, 3.0	337.0932	**173.04**, 163.04, 119.05	$C_{16}H_{18}O_8$	+0.890	59
Flavonoid glycosides							
4	Kaempferol *O*-rutinoside	2.6	593.1517	**284.03**, 285.04	$C_{27}H_{30}O_{15}$	+0.843	59
7	Quercetin *O*-glucoside *O*-rutinoside	3.4	771.2001	609.15, 463.09, **300.03**, 301.04	$C_{33}H_{40}O_{21}$	+1.43	59
8	Kaempferol *O*-glucoside *O*-rutinoside	3.5	755.2052	593.15, 447.09, **285.04**	$C_{33}H_{40}O_{20}$	+1.59	60
9	Rutin	4.2	609.1470	447.89, 301.04, **300.03**	$C_{27}H_{30}O_{16}$	+1.47	59
10	Apigenin *C*-glucoside	4.4	431.0989	341.07, **311.06**, 283.06, 269.05	$C_{21}H_{20}O_{10}$	+1.15	63
11	Quercetin *O*-glucoside	4.5	463.0888	301.04, **300.03**	$C_{21}H_{19}O_{12}$	−0.431	64
12	Kaempferol *O*-rutinoside isomer	4.8	593.1520	**285.04**, 255.03	$C_{27}H_{30}O_{15}$	+1.35	64
13	Kaempferol *O*-glucoside	5.0	447.0937	285.04, **284.03**, 255.03	$C_{21}H_{20}O_{11}$	+0.895	60
15a, 15b	Naringenin *O*-hexoside (isomers I and II)	5.5, 5.6	433.1144	271.06, 151.00, 119.05	$C_{21}H_{22}O_{10}$	+0.923	60
17	Chrysin *O*-glucoside	6.9	415.1036	**253.05**, 145.03	$C_{21}H_{20}O_9$	+0.241	60
18a, 18b	Naringenin *O*-hexoside (isomers III and IV)	6.9, 7.0	433.1142	**271.06**, 243.07	$C_{21}H_{22}O_{10}$	+0.462	60
22	Dihydrowogonin 7-*O*-glucoside	7.8	447.1298	432.11, 285.08, 269.05, 241.05	$C_{22}H_{24}O_{10}$	+0.291	60
Flavonoid aglycones							
14	Aromadendrin	5.2	287.0561	**259.06**, 269.05, 243.06, 125.02	$C_{15}H_{12}O_6$	−0.349	65
16	Quercetin	6.6	301.0354	273.04, 179.00, 151.00	$C_{15}H_{10}O_7$	0.0	65
19	Naringenin	7.5	271.0613	253.05, 151.00, 119.04	$C_{15}H_{12}O_5$	+0.369	64
20	Apigenin	7.6	269.0457	241.05, 181.06	$C_{15}H_{10}O_5$	+0.743	65
21	Naringenin isomer	7.8	271.0613	253.05, 151.00, 119.04	$C_{15}H_{12}O_5$	+0.369	64
23	Chrysin	9.8	253.0506	**209.06**, 181.06, 151.50	$C_{15}H_{10}O_4$	−0.395	64
24	Dihydrowogonin	10.2	285.0767	**270.05**, 179.00, 166.00	$C_{16}H_{14}O_5$	−0.351	60
Anthocyanins			$[M]^+$				
25	Cyanidin 3-*O*-rutinoside	2.9	595.1647	449.11, **287.05**, 241.05	$C_{27}H_{31}O_{15}^+$	−1.68	59
26	Cyanidin 3-*O*-glucoside	3.3	449.1069	**287.05**, 267.05, 105.04	$C_{21}H_{21}O_{11}^+$	−2.00	60

[a] The base ion peak is shown in bold. For peaks **1–24** see Fig. 3.

(**3a** and **3b**) and 4-*p*-coumaroylquinic acids (**6a** and **6b**), respectively, according to the hierarchical scheme proposed by Clifford *et al.*[61] Similarly, peaks **5a**/**5b** ($[M-H]^-$ at m/z 367.1034) were assigned to 3-*O*-feruloylquinic acids (*cis*/*trans* isomers) based on the fragmentation spectra showing a base ion peak at m/z 193.05, due to the loss of the quinoyl moiety. Finally, peak **2** ($[M-H]^-$ at m/z 353.0881) was assigned as chlorogenic acid (3-*O*-caffeoylquinic acid), as deduced by the presence in the MS/MS spectra of a product ion at m/z 191.06 due to the loss of a caffeoyl moiety.[61] Flavonoid glycosides were identified as kaempferol, quercetin, apigenin, naringenin, chrysin, and dihydrowogonin derivatives, as deduced by the detection in the MS/MS experiments of aglycone product ions at m/z 285.04, 301.04, 269.05, 271.06, 253.05, and 285.08, respectively. Saccharide chains were composed of one to three sugar units, represented by hexosyl (−162 u) or deoxyhexosyl (−146 u) moieties linked to the aglycones through an *O*-glycoside bond, except compound **10** that was identified as apigenin *C*-glucoside ($[M-H]^-$ at m/z 431.0989) by the observation in the ESI fragmentation pathway of the diagnostic product ions ($[M-H-90]^-$ at m/z 341.07 and $[M-H-120]^-$ at m/z 311.06) characteristic of flavonoid *C*-glycosides.[62] Flavone *C*-glycosides have not been previously reported in cherry fruits, but they have been recently found in the bark,[63] thus the occurrence of compound **10** could be attributed to woody plant parts in the cherry waste. All aglycones were also detected in the last region of the chromatograms.

The LC-MS analyses of the anthocyanin extracts revealed the occurrence of two cyanidin derivatives, cyanidin 3-*O*-rutinoside (compound **25**) and cyanidin 3-*O*-glucoside (compound **26**) in all samples. According to literature data,[59,60] full MS showed positive molecular ions $[M]^+$ at m/z 595.1647 and 449.1069, for **25** and **26**, respectively, while the base ion peak corresponding to the aglycone cyanidin (m/z 287.05) was generated by the loss of a rutinosyl ($[M-146–162]^+$) and a glucosyl ($[M-162]^+$) moiety, respectively.

Quantitative analysis of phenolic compounds in cherry pomace waste extracts

All analyzed extracts were subjected to a LC-MS quantitative analysis using external standards to calculate the amount of each component, thus of each subclass and total phenols. The

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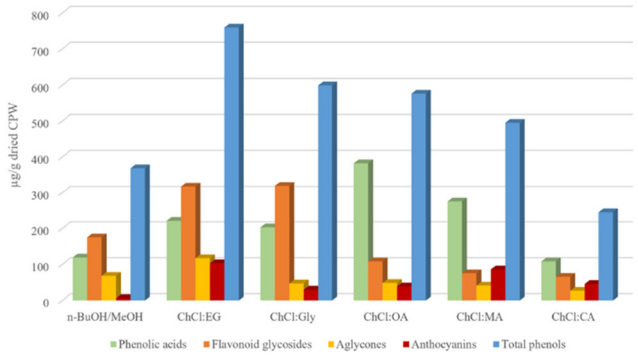

Fig. 4 Distribution of different phenol classes in cherry pomace waste (CPW) extracts (μg g^{-1} of dried CPW) obtained using classical solvents and (NA)DESs.

results (Table S1,† Fig. 4) showed that the amount of total phenol ranged from 245 ± 17 to 759 ± 85 μg g^{-1} of dried CPW, indicating the waste material as a potential source of bioactive agents. Indeed, these values are higher, also for blank samples, than those reported for different varieties of fresh cherries,[59] although in this work dried wastes have been employed and not the frozen biomass which are characterized by a high content of water.

Compared to classical solvents, all ChCl-based (NA)DESs, except ChCl : CA, showed higher phenol extraction capability. However, among them the most promising solvents as extraction media (in terms of total phenolic content) are those obtained combining ChCl with both the investigated polyols (EG and Gly) and OA among the carboxylic acid, with ChCl : EG being the most efficient (total phenol recovered 759 ± 85 μg g^{-1} of dried CPW). Besides the total phenolic content of the extracts, it is worth noting the different selectivity towards the main classes of identified phenols as a function of the employed HBD type. Indeed, opposite selectivity was detected when acids or polyol-based DESs were tested. In detail, acid-based DESs extracted higher amounts of total phenolic acids (values ranging from 108 ± 6.9 to 382 ± 59 μg g^{-1} of dried CPW) compared to flavonoid glycosides (values ranging from 64.9 ± 4.3 to 108 ± 26 μg g^{-1} of dried CPW). Moreover, ChCl : OA, followed by ChCl : MA, showed the highest values of phenolic acids in the extracts (382 ± 59 and 275 ± 41 μg g^{-1} of dried CPW, respectively) in comparison with all the investigated systems. Instead, polyol-based DESs showed higher selectivity towards flavonoid glycosides. The values of 318 ± 32 μg g^{-1} for ChCl : EG and 320 ± 40 μg g^{-1} for ChCl : Gly were the highest obtained among the tested solvents. ChCl : EG displayed also the highest amounts of flavonoid aglycones (117 ± 13 μg g^{-1} of dried CPW) and interestingly of anthocyanins (103 ± 7.1 μg g^{-1} of dried CPW). This latter value is relevant considering that in most of the works reported in the literature on the extraction of anthocyanins from plant material or food industry biomass wastes (especially from wine industry[46,47,66]), the most efficient DESs usually feature a carboxylic acid acting either as a HBD or a HBA. In particular, among them ChCl : oxalic acid[46,49,67] or citric acid-based DESs[47,48,50,68–70] are the most frequently used. The great extraction efficiency was explained with the polarity and the acidity of these systems. Indeed, anthocyanins are polar molecules that at low pH (= 1) are predominantly present in the more stable form of red flavylium cation, while at pH values higher than 7 they may be subjected to degradation.[46,71] Hence, organic acid-based DESs being more polar solvents than polyol or sugar-based ones and showing very low pH values present the most beneficial features and thus are the best performing extraction media for anthocyanins. However, in this work, the best extractant for anthocyanins was the neutral ChCl : EG. For the acid-based DES series, ChCl : MA was the best performing DES (85.8 ± 6.2 μg g^{-1} of total anthocyanins in dried CPW) showing also the highest value for the less abundant cyanidin 3-*O*-glucoside (20.8 ± 2.3 μg g^{-1} of dried CPW). It is worth noting the great improvement in anthocyanin extraction using (NA)DESs compared to that with the classical solvent (5.71 ± 1.3 μg g^{-1} of dried CPW). The classical solvent displayed the same selectivity as polyol-based DESs, showing higher amounts of flavonoid glycosides (175 ± 9.9 μg g^{-1} of dried CPW). Finally, considering the amount of the single extracted phenol, the most abundant compounds in all extracts were dihydroxy benzoic acid (values ranging from 80.2 ± 3.6 to 271 ± 38 μg g^{-1} of dried CPW) and 3-*p*-coumaroylquinic acid (values ranging from 11.0 ± 1.2 to 49.8 ± 9.2 μg g^{-1} of dried CPW, excluding the extract with ChCl : MA) among phenolic acids, rutin (values ranging from 20.9 ± 0.70 to 132 ± 18 μg g^{-1} of dried CPW) among flavonoid glycosides, chrysin (values ranging from 17.1 ± 0.73 to 75.2 ± 8.0 μg g^{-1} of dried CPW) among flavonoid aglycones and cyanidin 3-*O*-rutinoside (values ranging from 29.2 ± to 96.8 ± 6.8 μg g^{-1} of dried CPW, excluding the extract with the classical solvent) among anthocyanins.

These results showed the influence of the HBD in the extraction ability of different ChCl-based DESs. To get a further insight into the potential of these media, the influence of the HBAs was also investigated and (NA)DESs with Bet and L-Pro instead of ChCl were prepared in combination with the three best HBDs (ethylene glycol, glycerol and oxalic acid). Betaine-based DESs as well as L-proline-based DESs are, after ChCl-based ones, the most investigated natural DESs,[72–75] although often they showed worse extraction efficiencies compared to the ChCl containing DESs.[76] However, considering the safe profile of these compounds and future potential as DES components, especially in the case of betaine, it was of interest to investigate alternative HBAs.[77] The extractions of phenols were performed using the same procedure under the same operative conditions in terms of temperature, time and biomass/(NA)DES ratio. Quantitative data of recovered phenols in all extracts are shown in Table S2† and Fig. 5. All betaine and L-proline-based DESs did not improve the extraction process compared to the classical solvents, showing a total phenolic content ranging from 167 ± 11 to 267 ± 24 μg g^{-1} of dried CPW, with the lowest values found for the glycerol-based solvents. Obviously, the total phenolic content is also much lower than that obtained by using ChCl-based DESs. However,

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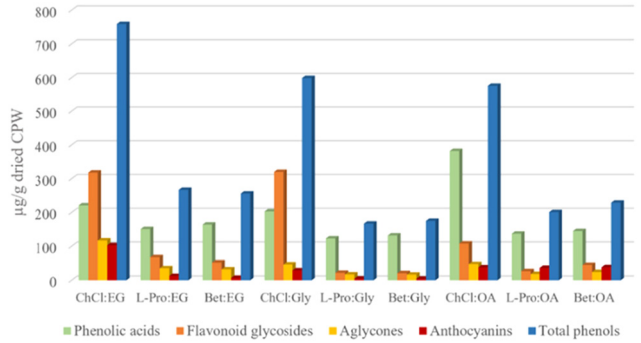

Fig. 5 Distribution of different phenol classes in cherry pomace waste (CPW) extracts obtained using (NA)DES (μg g^{-1} of dried CPW).

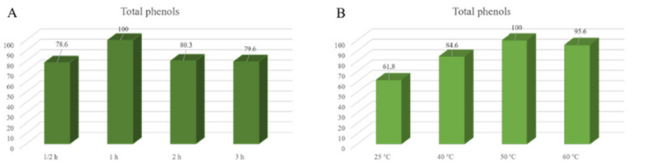

Fig. 6 Percentage variation in quantities of phenolic compound classes at different extraction times (A) or temperatures (B) after extraction with ChCl : EG (choline chloride : ethylene glycol).

it is worth noting that polyol-based betaine and proline DESs showed an inverted selectivity towards phenolic acids and flavonoid glycosides compared to ChCl-based DESs. In particular, for Bet : Gly and L-Pro : Gly the total phenolic content was mainly due to phenolic acids. Indeed, 132 ± 2.4 μg g^{-1} of phenolic acids over 175 ± 4.2 μg g^{-1} of total phenols was detected when Bet : Gly was employed, while 123 ± 8.4 μg g^{-1} of phenolic acids over 167 ± 11 μg g^{-1} of total content was observed for L-Pro : Gly-based extraction. Regarding oxalic acid-based DESs, lower values of all the identified classes of compounds than ChCl : OA were obtained, with the only exception of anthocyanins. For this latter class, similar values were observed, confirming the ability of acid-based DESs to extract thanks to the stabilization of anthocyanins at low pH values. Comparing the amounts of the single phenolic compounds obtained with the betaine- and proline-based DESs, dihydroxy benzoic acid was still the main abundant phenolic acid, showing values significantly higher within this class. Furthermore, chrysin among flavonoid aglycones and cyanidin 3-*O*-rutinoside among the anthocyanins were the most representative compounds of the respective classes. Rutin was still the most abundant flavonoid glycosides, except for Bet : Gly and L-Pro : Gly extracts, showing higher quantities of apigenin *C*-glucoside.

Hence, ChCl : EG was selected as the best performing extraction medium in terms of total phenolic content and total content of both flavonoids glycosides and aglycones, and anthocyanins. It only showed lower amounts of phenolic acids compared to ChCl : carboxylic acid DESs. It is worth noting that the yields of the recovered phenols with ChCl : EG were lower than those obtained with other (NA)DESs, especially the acid-based ones. This result can be correlated to a higher selectivity towards phenolic compounds by this system. Conversely, the other (NA)DESs besides polyphenols simultaneously extract other metabolites.

Subsequently, an optimization of the operative conditions was performed. In particular, extractions with ChCl : EG were repeated either at 50 °C varying the extraction time from 30 min to 3 h or increasing the temperature from 25 to 60 °C keeping the extraction time unchanged (1 h).

A relative estimation of phenols recovered by ChCl : EG at different temperatures and extraction times was done by UHPLC-MS data of extracts and then evaluating the percentage variation compared to the highest one (Fig. 6).

Unsurprisingly, a detrimental effect was noticed on extending the time over an hour. These findings can be correlated to the degradation of phenols when they are maintained at 50 °C for long times. The amount of phenols increases with time up to reaching a maximum value after which it decreases. This behaviour could be explained by Fick's second law of diffusion which establishes that a final equilibrium between the matrix solid and the extractant is achieved after a certain contact time and the extraction efficiency does not improve with prolonged times.[19,52] One hour was selected as the optimal extraction time.

Regarding temperature, it is known that this parameter influences the extraction efficiency since higher temperatures can reduce considerably the viscosity of (NA)DESs enhancing the solubility and diffusion coefficient of the solute thus improving mass transfer. In our study, extractions carried out at different temperatures revealed an increasing recovery of phenols with temperatures up to 50 °C, while at 60 °C a small reduction was observed. Again, this result can be correlated to the thermolability of some phenols. From these preliminary data, 1 h and 50 °C were considered the best extraction conditions. However, further evaluation about the content of anthocyanins is needed and a better comparison will be performed in future studies.

Recovery and reuse of DES

The possibility of recycling the DES was considered for the best performing system applying the optimal operative conditions (ChCl : EG 1 : 2, 1 h at 50 °C). Indeed, the recovery of the solvent, its reuse in a new extraction cycle and the retention of its extraction efficiency (EE) are all aspects that combine to define the real sustainability of an extraction process. Hence, the aqueous fraction recovered after the separation step with the Amberlite XAD-7 resin was evaporated under reduced pressure to collect the ChCl : EG 1 : 2. Before use in further extractions with fresh biomass, the mass of the recovered DES was evaluated, and the molar ratio was checked by ^{1}H-NMR. The entire process was repeated for three consecutive cycles. The data related to DES recovery (expressed as the percentage of mass recovered over mass used) and the EE (assessed by means of LC-MS of the third extract) are reported in Fig. 7 and 8. Recovery of DES was practically quantitative; after the first run a value above 100% was obtained. This result

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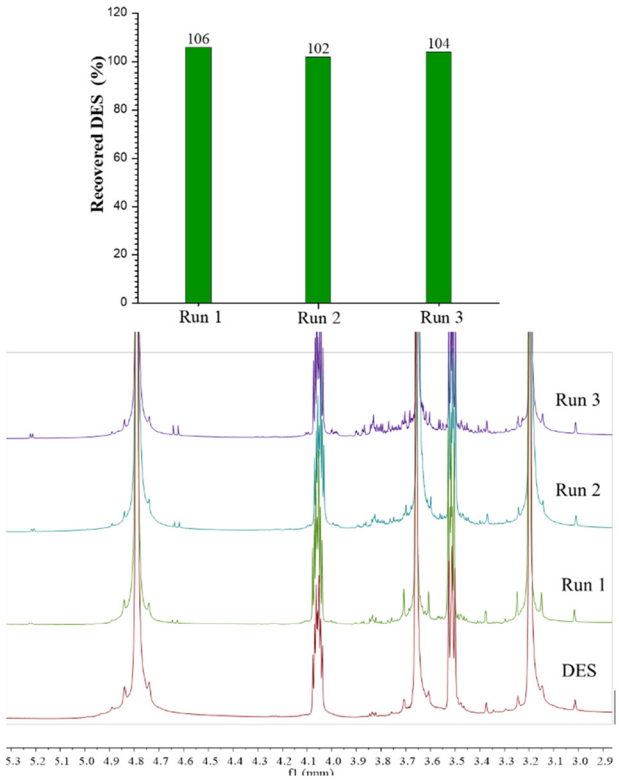

Fig. 7 Yields (top) and ^{1}H-NMR (bottom) of the recovered DES after each cycle.

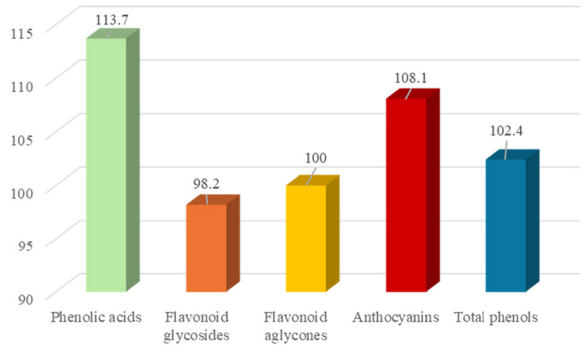

Fig. 8 Percentage variation of phenolic classes at the third cycle compared to the first cycle to which the value of 100% was assigned.

can be ascribed to the concomitant extraction of free sugars and presumably of the pectin part, which remains in the DES (NMR spectra are shown in Fig. 7). After each cycle, a slight increase of the intensity of the characteristic signals of sugar compounds (signals between 3 and 4 ppm and the two signals at 4.6 and 5.2 ppm attributable to the anomeric protons) was observed. However, the presence of these compounds in the DES does not affect the extraction capability of the solvent. In Fig. 8, the relative estimation of phenols recovered at the end of the third cycle were determined by UHPLC-MS and the percentage variation with respect to the first cycle, to which the value of 100% was assigned, was evaluated. The total content of phenols as well as the amount of each subclass was similar to that of the first cycle, which proves the retention of the extraction efficiency of the recycled solvent.

Characterization of the solid residue obtained after polyphenol extraction

The undissolved residues obtained after polyphenol extraction were analyzed to study their composition and further processed to complete the biorefinery process. At first, an evaluation of the amount of the recovered materials was carried out and a clear difference was observed when polyol or acid-based DESs were employed (Fig. 9). For all the tested HBAs, lower amounts of undissolved residue were recovered when the biomass was treated with acid-based DESs. As already discussed in the previous sections, this could be ascribed to the concomitant extraction of free sugars or the pectin fraction with the DES treatment. Indeed, it has been widely reported that acidic media with HCl, H_2SO_4 or citric acid,[78–81] and different NADESs, such as ChCl : MA, ChCl : CA or Bet : CA or different lactic acid-based DESs are able to extract pectin from several biomass materials (*e.g.* pomelo peel, *Averrhoa bilimbi*, dragon fruit peels, mango peel powder, sugar beet pulp, apple pomace).[82–89] As already shown, this hypothesis was confirmed from the ^{1}H-NMR spectra of the recovered (NA)DESs shown in Fig. S15,† where the signals attributable to sugar based-compounds are evident both in the acid-based (NA)DESs, and in the polyol-based DESs. Future studies will be focused on the recovery of the extracted pectin and sugars from the (NA)DESs.

Based on the polyphenol content of the extracts, the subsequent valorization of the undissolved residue was performed on the residue obtained after ChCl : EG treatment (test performed with the optimized extraction conditions: 1 h at 50 °C). The residue was initially characterized by FTIR analysis (Fig. S17†). The FTIR spectrum showed all the characteristic absorption bands of typical dietary fibers which are generally present in wastes of processed fruits and vegetables (cellulose, hemicellulose, pectin, and lignin).[90] The main bands and the corresponding attributions are reported in Table S3.† These

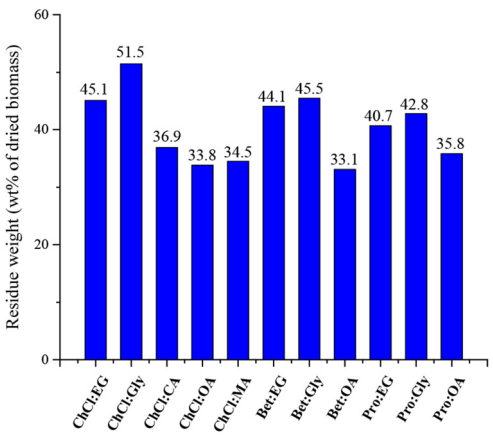

Fig. 9 Amounts of undissolved residues recovered after polyphenol extraction with (NA)DESs.

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data have been confirmed by TG analysis (Fig. S18†) that showed a main degradation step in the temperature range 200–500 °C due to overlapping phenomena. In detail, the sample started to degrade around 150 °C and it displayed a continuous degradation until the end of the analysis at 800 °C, which behaviour is generally observed for lignin extracted from agro-waste biomass.[43,91] Maxima in the derivative curve were around 200–300 °C, which are the typical degradation temperatures of pectin/hemicellulose and cellulose, respectively.

The chemical composition of the residue was ascertained by performing the classical methods reported in the literature (see the ESI†) aimed at the recovery and hence quantification of specific components of a lignocellulosic biomass. This chemical characterization was also performed on the starting material (CPW). At first, the free water-soluble sugar content was obtained. Then, pectin and holocellulose (cellulose and hemicellulose) contents were evaluated leading also to the quantification of lignin content. The obtained results are summarized in Fig. 10, representing the mass balance of the extraction process. The data confirm the hypothesis that the loss of material registered at the end of the process can be related to the concomitant extraction by the DES of free water-soluble sugars and in part of the pectin present in the starting material, along with polyphenols. Conversely, this DES treatment does not practically change the amount of holocellulose and lignin.

Treatment with bio-ILs (ChArg and ChLev) of the undissolved residue obtained after polyphenol extraction: ionogel formation

After the recovery of polyphenols, the full valorization of the cherry pomace was attempted through the formation of a cellulose ionogel. These kinds of functional materials are attracting increasing attention[34,92] since they combine the unique properties of ionic liquids as preferable dispersed liquid phase and cellulose as the solid matrix. Among all the reported ionic liquids for cellulose or lignocellulosic material processing, ChLev has been chosen for its green nature. Indeed, it has been reported as a readily biodegradable and low-toxicity biocompatible ionic liquid.[93] At first, an attempt to directly obtain a gel using the whole residue, characterized by cellulose, hemicellulose, pectin, and lignin was carried out. Unfortunately, this strategy did not lead to complete dissolution of the biomass in ChLev, apart when very low amounts of material not sufficient to start the gelification process were used. This could be ascribed to the presence of lignin and hemicellulose, in agreement with the findings reported by Hopson *et al.* during the formation of an ionogel using EMIM DEP.[94] Therefore, before dissolving the material in ChLev, a pretreatment aimed at obtaining a cellulose enriched material through the selective extraction of hemicellulose and lignin fractions was performed. Pectin was not considered as an interfering factor since pectins occurred in the smallest amounts in all the pomace samples analyzed and especially in cherry pomace.[90] Moreover, as discussed in detail above, the treatment with (NA)DESs can lead to a concomitant extraction of pectin from the biomass, further reducing its amount in the final undissolved residue. Finally, the importance of pectin in the food sector lies in its ability to form gel,[95,96] hence its presence should not influence the gelling process. Therefore, ChArg, a different bio-based ionic liquid able to selectively remove the lignin and hemicellulose from lignocellulosic material[41,42] was selected for the pretreatment step which led to two different fractions. The cellulose enriched material, CRM (35 wt% of dried biomass) was obtained *via* filtration as the undissolved material, while a fraction enriched in lignin and hemicellulose was precipitated from the filtrate acidifying the solution (22 wt% of dried biomass). The two fractions have been analyzed by FTIR spectroscopy and TGA and the obtained spectra and thermograms are compared with the starting material as shown in Fig. 11. Both analyses ascertained the nature of the fractions. Indeed, the FTIR spectrum of CRM (blue line) showed mainly the characteristic bands of cellulose (*i.e.* 1020, 1142, 1316, 1362 cm^{-1}) while all the bands that were ascribable only to lignin or hemicellulose (*i.e.* 1730, 1520, 1225 cm^{-1}) were not present anymore. Instead, these bands were clearly visible in the spectrum of HLRM (red line).

The TGA confirmed these compositions. Indeed, the CRM exhibited a main degradation step at 347 °C, typical behaviour of cellulosic material, while the HLRM showed a main degradation step at 239 °C followed by a continuous mass loss until 800 °C, which are characteristic thermograms of hemicellulose and lignin, respectively.[91] It is worth highlighting that it was possible to recover the bio-IL with satisfactory purity at the end of the treatment (^{1}H-NMR spectra of the recovered bio-IL are shown in Fig. S19†). Finally, the result of the holocellulose content measurement, performed on the CRM, confirmed the enrichment in cellulose of the residue (96.3 ± 2.4% of holocellulose).

Subsequently, in order to prepare the ionogel, the CRM was ground to obtain a fine and more manageable material which was dissolved in ChLev at 2 wt%. The CRM was slowly added to the bio-IL and stirred until complete dissolution (24 h). After dissolution, the hot mixture was poured into a suitable mold and kept at room temperature until gel formation (3 d). The excess of ionic liquid was removed and recovered to further enhance the greenness of the process. The recovered ChLev was analyzed by ^{1}H-NMR spectroscopy to verify its purity (Fig. S20†). The obtained gel (Fig. 12) after saturation

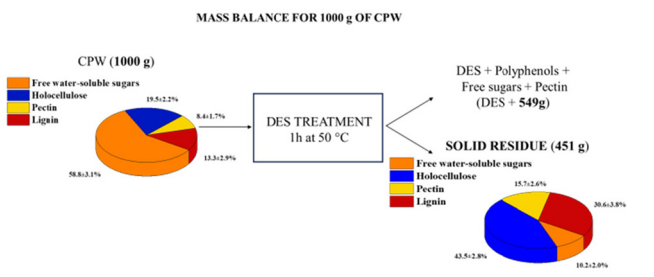

Fig. 10 Mass balance of the DES treatment step.

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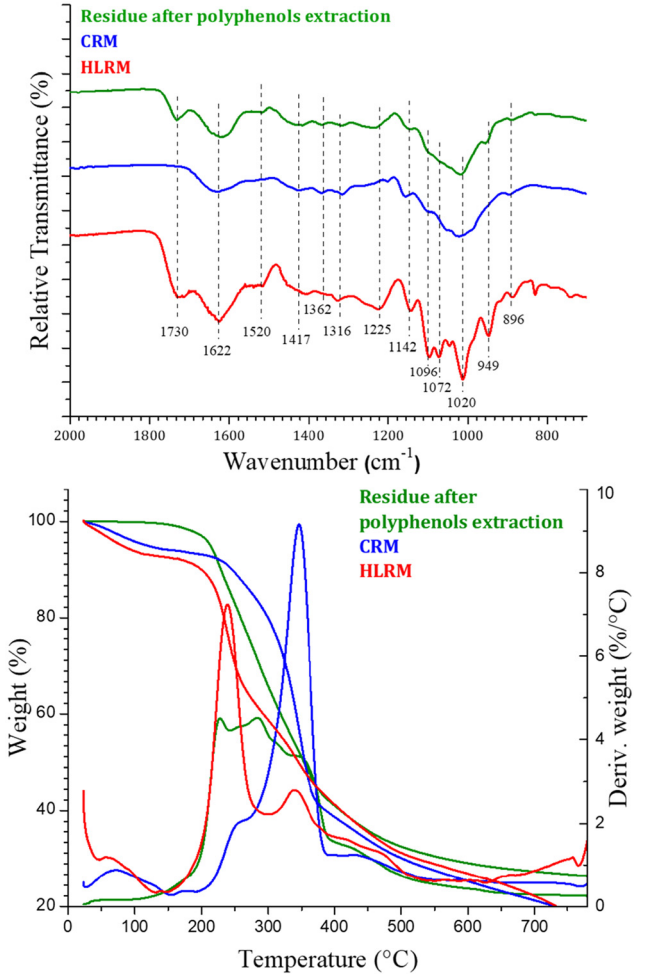

Fig. 11 FTIR spectra (top) and TGA and DTG (bottom) of the two fractions obtained after ChArg (cholinium arginate) treatment compared to the undissolved residue obtained after polyphenol extraction with ChCl : EG (choline chloride : ethylene glycol).

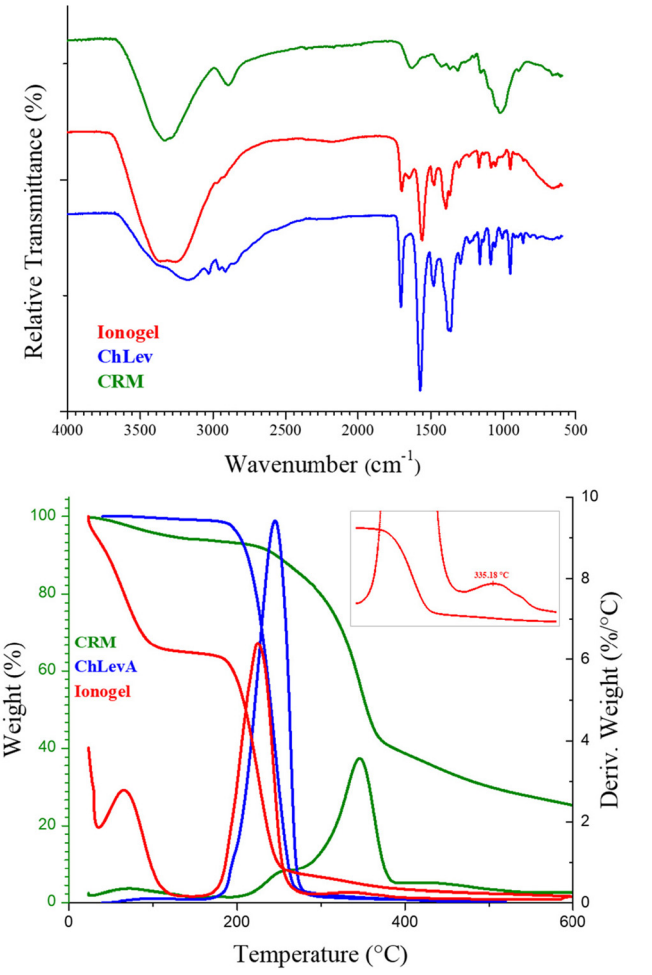

Fig. 13 FTIR spectra (top) and TGA–DTG (bottom) of the obtained ionogel compared to ChLev (cholinium levulinate) and CRM (cellulose enriched material).

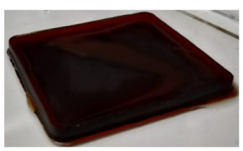

Fig. 12 Image of the obtained ionogel.

with water at 30 °C was instead characterized by FTIR spectroscopy, TGA and rheological analyses.

As expected, the FTIR spectrum of the ionogel (Fig. 13) showed only the bands attributable to the major component of the gel, the ChLev along with the characteristic bands of the absorbed water (the large band between 3000 and 3500 cm^{-1} and the band at 1652 cm^{-1}). The characteristic signals of CRM were covered by the bands of ChLev in the registered spectrum. The thermogram of the ionogel (Fig. 13) exhibited two main degradation steps at 66.9 °C and 225.5 °C, attributable to water evaporation and ChLev degradation, respectively. Moreover, the second mass loss was followed by a shoulder at 335 °C, characteristic of the dissolved cellulosic material, as shown in the inset in Fig. 13. From the thermogram, it was also possible to obtain a rough evaluation of the amount of ChLev and water in the final material: 35% water and 58% ChLev in the final gel were determined from each mass loss.

Finally, the rheology of the formulated ionogel was studied to get an insight into its internal structure and gel characteristics. Indeed, from dynamic frequency sweeps it is possible to determine if the ionogel is a gel type material or not and also whether it is a physical or a chemical ionogel.[34] In our case, the formation of a physical gel is expected since the interactions between the cellulosic material and the ionic liquid are non-covalent (mainly hydrogen bonds). Initially, the amplitude sweep test was performed between 0.1 and 1% at 1 Hz in order to select the correct value of the shear strain within the LVR (linear viscoelastic region). The elastic modulus G' is almost constant during strain variation suggesting that the internal structure/network of the material does not change with the

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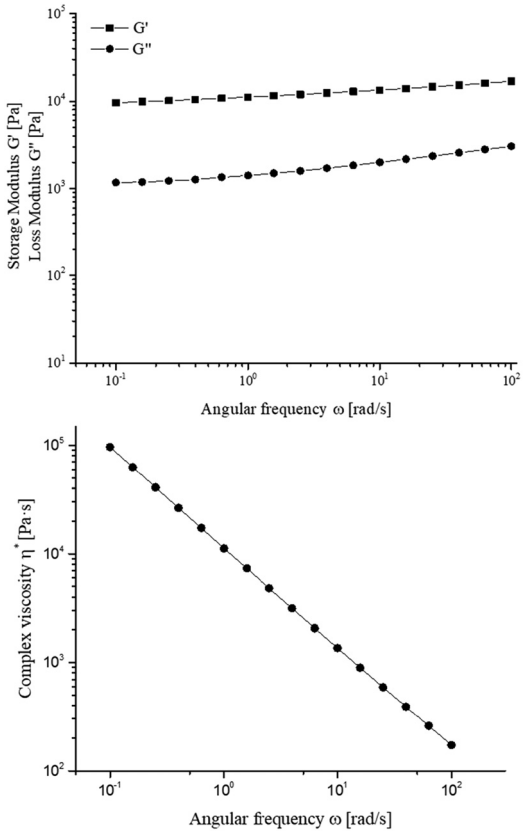

Fig. 14 Rheological characterization of the formulated ionogel.

applied strain. Subsequently, a frequency sweep test was performed at 0.1% (Fig. 14 and Table S4†).

As observed from the graph, the elastic modulus G' was higher than the loss modulus G'' within the plateau region. Indeed, G' remained constant with the frequency, which is a typical behaviour of a gel like material. These data are similar to that obtained by Mar Villar-Chavero *et al.*[35] for the ionogel prepared dissolving 2 wt% microcrystalline cellulose in cholinium lysinate (ChLys) or to the ionogel obtained by Hopson dissolving 1.7 wt% cellulose rich-solid derived from a biorefinery approach in EMIM DEP.[94] Then, to determine the strength of the formulated ionogel and hence to classify it as physical or chemical gel, the dependence of the elastic modulus was calculated applying eqn (1) (see rheological properties in the ESI†). Since the parameter a was >0 (Table 3), the expected physical nature of the gel, due to the hydrogen bonds formed between ChLev and the –OH groups of cellulosic enriched material was confirmed. The nature of the interaction forces was ensured also by FTIR spectrum previously shown, where

Table 3 Values of a, G_0 and R^2 for the correlation of $G' \sim \omega^a$, and the relation between G' and G'' mean values for the formulated ionogel

	a	G_0 (Pa)	R^2	$(G'/G'')_{mean}$
Ionogel	0.08173 ± 0.00189	11 298 ± 1	0.99254	7.2

Table 4 Parameters of the power-law model applied to complex viscosity for the formulated ionogel

	q (Pa s^p)	p	R^2
Ionogel	11 378 ± 1	0.08297 ± 0.00201	0.99993

no modification or new bands were found along with the characteristic bands of the components. Regarding the strength of the ionogel (G_0), a similar value to that of the ionogel based on cellulose and ChLys was obtained.[35] Moreover, according to the criterion based on the mean relation G'/G'', the formulated ionogel showing a ratio <10 (Table 3) was classified as weak physical gel[97] in contrast to all the strong ionogels composed of EMIM DEP and different concentrations of cellulose rich-solids.[94] However, it showed a higher strength than the cellulosic bionogel with ChLys, which showed values ranging from 2.6 to 6.5 at 4 Hz and from 1.2 to 3.4 at 45 Hz.[35]

Finally, the complex viscosity η^* of the formulated ionogel was evaluated through the data obtained in the frequency sweep test. The results of η^*, reported in Fig. 14, showed that the material exhibited a shear-thinning or pseudoplastic behaviour due to the decrease of η^* when the frequency was increased. To further ascertain this behaviour, the complex viscosity was fitted according to eqn (3) (see rheological properties in the ESI†). The obtained data are reported in Table 4. The "p" value was less than the unit which corroborated the shear-thinning behaviour. The parameter q, which is a measure of the consistency of the material, was higher than the values obtained for the bionogel composed of cellulose and ChLys mentioned before[35] and more similar to the value obtained from chitosan reinforced cellulosic bionogel prepared by the same authors with ChLys as the ionic liquid.[98] However, as widely reported in the literature, this parameter is strongly influenced by the amount and type of cellulose, biopolymer load and post-gelation time. Hence, an in-depth study on the preparation of different ionogels with different CRM loads will be performed in a dedicated work.

Conclusions

The influence of both HBA and HBD components of different (NA)DESs on the extraction efficiency and the selectivity towards phenolic compounds contained in cherry pomace wastes obtained after juice production was evaluated. The extractions were performed under fixed conditions (biomass/solvents of 1/10, extraction time of 1 h and temperature of 50 °C) in order to compare the obtained results. UHPLC-HR-ESI-MS was performed to initially qualitatively identify and then quantify the extracted phenols. More than 25 compounds, classifiable in four main subclasses (phenolic acids, flavonoid glycosides, flavonoid aglycones, and anthocyanins) were detected. The best performing system in terms of total phenolic content was identified as ChCl : EG. The influ-

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ence of the HBD (carboxylic acids or polyols) on the selectivity performance and then also of the HBA (choline chloride, betaine or L-proline) was evident especially towards phenolic acids and flavonoid glycoside subclasses. All ChCl-based DESs, except ChCl : CA, improved the total phenol extraction compared to classical solvents. Conversely, all Bet and L-Pro-based DESs displayed lower total phenolic content than classical solvents. Optimization studies of the working conditions for the best performing system suggested 1 h and 50 °C as most promising. The DES recyclability was investigated for ChCl : EG 1 : 2. The solvent was quantitatively recovered, and the extraction efficiency was successfully retained over 3 cycles. Finally, valorization of the undissolved lignocellulosic residue obtained after polyphenol extraction with ChCl : EG was carried out by employing two different bio-based ILs endowed with complementary dissolution properties towards lignocellulosic components. At first, ChArg known to dissolve selectively lignin over cellulose was used to obtain a cellulose enriched material. Then, this was dissolved in ChLev to prepare a weak physical ionogel, a functional biomaterial with potential application in medical and pharmaceutical sectors. The proposed biorefinery approach showcases the valorization of all main components of cherry pomace waste.

Author contributions

Lorenzo Guazzelli: conceptualization; project administration; supervision; funding acquisition; and writing – review and editing. Marinella De Leo: conceptualization; funding acquisition; supervision; methodology; writing – original draft; and writing – review and editing. Alessandra Braca: conceptualization; resources; methodology; and writing – review and editing. Andrea Mezzetta: conceptualization; methodology; data curation; writing – original draft; and writing – review and editing. Angelica Mero: investigation; methodology; data curation; and writing – original draft.

Conflicts of interest

There are no conflicts to declare.

References

1 United States Department of Agriculture Foreign Agricultural Service, *Usda*, 2021, **https://public.govdelivery.com/accounts/USDAFAS/subscriber/new**.
2 S. Chockchaisawasdee, J. B. Golding, Q. V. Vuong, K. Papoutsis and C. E. Stathopoulos, *Trends Food Sci. Technol.*, 2016, **55**, 72–83.
3 M. Serrano, F. Guillén, D. Martínez-Romero, S. Castillo and D. Valero, *J. Agric. Food Chem.*, 2005, **53**, 2741–2745.
4 B. Mozetic, P. Trebse and J. Hribar, *Food Technol. Biotechnol.*, 2002, **40**, 207–212.
5 D. Średnicka-Tober, A. Ponder, E. Hallmann, A. Głowacka and E. Rozpara, *Antioxidants*, 2019, **8**, 534.
6 V. Usenik, F. Stampar, M. M. Petkovsek and D. Kastelec, *J. Food Compos. Anal.*, 2015, **38**, 121–130.
7 G. Domínguez-Rodríguez, M. L. Marina and M. Plaza, *Food Chem.*, 2021, **339**, 128086.
8 S. Pathania and N. Kaur, *Bioact. Carbohydr. Diet. Fibre*, 2022, **27**, 100295.
9 F. M. Yılmaz, M. Karaaslan and H. Vardin, *J. Food Sci. Technol.*, 2015, **52**, 2851–2859.
10 K. Dziadek, A. Kopeć and M. Tabaszewska, *Eur. Food Res. Technol.*, 2019, **245**, 763–772.
11 S. Hosseini, K. Parastouei and F. Khodaiyan, *Int. J. Biol. Macromol.*, 2020, **158**, 911–921.
12 A. Chaovanalikit and R. E. Wrolstad, *J. Food Sci.*, 2004, **69**, FCT73–FCT83.
13 M. A. R. Martins, S. P. Pinho and J. A. P. Coutinho, *J. Solution Chem.*, 2019, **48**, 962–982.
14 T. El Achkar, H. Greige-Gerges and S. Fourmentin, *Environ. Chem. Lett.*, 2021, **19**, 3397–3408.
15 S. P. Ijardar, V. Singh and R. L. Gardas, *Molecules*, 2022, **27**, 1368.
16 S. J. Bryant, A. J. Christofferson, T. L. Greaves, C. F. McConville, G. Bryant and A. Elbourne, *J. Colloid Interface Sci.*, 2022, **608**, 2430–2454.
17 Y. H. Choi, J. van Spronsen, Y. Dai, M. Verberne, F. Hollmann, I. W. C. E. Arends, G.-J. Witkamp and R. Verpoorte, *Plant Physiol.*, 2011, **156**, 1701–1705.
18 M. Vieira Sanches, R. Freitas, M. Oliva, A. Mero, L. De Marchi, A. Cuccaro, G. Fumagalli, A. Mezzetta, G. Colombo Dugoni, M. Ferro, A. Mele, L. Guazzelli and C. Pretti, *Environ. Sci. Pollut. Res.*, 2023, **30**, 17268–17279.
19 P. Gullón, B. Gullón, A. Romaní, G. Rocchetti and J. M. Lorenzo, *Trends Food Sci. Technol.*, 2020, **101**, 182–197.
20 A. Mišan, J. Nađpal, A. Stupar, M. Pojić, A. Mandić, R. Verpoorte and Y. H. Choi, *Crit. Rev. Food Sci. Nutr.*, 2020, **60**, 2564–2592.
21 M. Jablonský, A. Škulcová, A. Malvis and J. Šima, *J. Biotechnol.*, 2018, **282**, 46–66.
22 J. González-Rivera, A. Mero, E. Husanu, A. Mezzetta, C. Ferrari, F. D'Andrea, E. Bramanti, C. S. Pomelli and L. Guazzelli, *Green Chem.*, 2021, **23**, 10101–10115.
23 H. Moni Bottu, A. Mero, E. Husanu, S. Tavernier, C. S. Pomelli, A. Dewaele, N. Bernaert, L. Guazzelli and L. Brennan, *Food Chem.*, 2022, **386**, 132717.
24 F. S. Costa, L. S. Moreira, A. M. Silva, R. J. Silva, M. P. dos Santos, E. G. P. da Silva, M. T. Grassi, M. H. Gonzalez and C. D. B. Amaral, *J. Food Compos. Anal.*, 2022, **109**, 104510.
25 B. M. Popovic, N. Micic, A. Potkonjak, B. Blagojevic, K. Pavlovic, D. Milanov and T. Juric, *Food Chem.*, 2022, **366**, 130562.
26 G. Kaur, H. Kumar and M. Singla, *J. Mol. Liq.*, 2022, **351**, 118556.

Published on 12 Abrëll 2024. Downloaded on 04.02.26 10:25:48.

27 Q. Hou, M. Ju, W. Li, L. Liu, Y. Chen and Q. Yang, *Molecules*, 2017, **22**, 490.
28 I. Hasanov, M. Raud and T. Kikas, *Energies*, 2020, **13**, 1–24.
29 J. Zhang, J. Wu, J. Yu, X. Zhang, J. He and J. Zhang, *Mater. Chem. Front.*, 2017, **1**, 1273–1290.
30 P. C. Marr and A. C. Marr, *Green Chem.*, 2016, **18**, 105–128.
31 C. Manasa, V. Basavanna and S. Ningaiah, *Biointerface Res. Appl. Chem.*, 2022, **13**, 391.
32 X. Fan, S. Liu, Z. Jia, J. J. Koh, J. C. C. Yeo, C.-G. Wang, N. E. Surat'man, X. J. Loh, J. Le Bideau, C. He, Z. Li and T.-P. Loh, *Chem. Soc. Rev.*, 2023, **52**, 2497–2527.
33 Z. Zhang, R. Zhao, S. Wang and J. Meng, *Front. Bioeng. Biotechnol.*, 2022, **11**, 1117944.
34 C. Hopson, M. M. Villar-Chavero, J. C. Domínguez, M. V. Alonso, M. Oliet and F. Rodriguez, *Carbohydr. Polym.*, 2021, **274**, 118663.
35 M. M. Villar-Chavero, J. C. Domínguez, M. V. Alonso, V. Rigual, M. Oliet and F. Rodriguez, *Int. J. Biol. Macromol.*, 2019, **133**, 262–269.
36 M. M. Villar-Chavero, J. C. Domínguez, M. V. Alonso, M. Oliet and F. Rodriguez, *Carbohydr. Polym.*, 2019, **207**, 775–781.
37 P. Fan, Y. Yuan, J. Ren, B. Yuan, Q. He, G. Xia, F. Chen and R. Song, *Carbohydr. Polym.*, 2017, **162**, 108–114.
38 R. M. Kalinoski and J. Shi, *Ind. Crops Prod.*, 2019, **128**, 323–330.
39 P. Jia, X. Ji, B. Zheng, C. Wang, W. Hao, W. Han, J. Zhang, G. Xia, X. Ji and J. Zhang, *Polymers*, 2022, **14**, 1589.
40 T. Q. To, K. Procter, B. A. Simmons, S. Subashchandrabose and R. Atkin, *Faraday Discuss.*, 2018, **206**, 93–112.
41 A. Mero, N. R. Moody, E. Husanu, A. Mezzetta, F. D'Andrea, C. S. Pomelli, N. Bernaert, F. Paradisi and L. Guazzelli, *Front. Chem.*, 2023, **11**, 1270221.
42 Y. X. An, M. H. Zong, H. Wu and N. Li, *Bioresour. Technol.*, 2015, **192**, 165–171.
43 E. Husanu, A. Mero, J. G. Rivera, A. Mezzetta, J. C. Ruiz, F. D'Andrea, C. S. Pomelli and L. Guazzelli, *ACS Sustainable Chem. Eng.*, 2020, **8**, 18386–18399.
44 X.-D. Hou, J. Xu, N. Li and M.-H. Zong, *Biotechnol. Bioeng.*, 2015, **112**, 65–73.
45 A. Mezzetta, S. Becherini, C. Pretti, G. Monni, V. Casu, C. Chiappe and L. Guazzelli, *New J. Chem.*, 2019, **43**, 13010–13019.
46 K. Radošević, N. Ćurko, V. Gaurina Srček, M. Cvjetko Bubalo, M. Tomašević, K. Kovačević Ganić and I. Radojčić Redovniković, *LWT–Food Sci. Technol.*, 2016, **73**, 45–51.
47 K. M. Jeong, J. Zhao, Y. Jin, S. R. Heo, S. Y. Han, D. E. Yoo and J. Lee, *Arch. Pharmacal Res.*, 2015, **38**, 2143–2152.
48 D. Aslan Türker and M. Doğan, *LWT–Food Sci. Technol.*, 2021, **138**, 110775.
49 X. Fu, D. Wang, T. Belwal, J. Xie, Y. Xu, L. Li, L. Zou, L. Zhang and Z. Luo, *LWT–Food Sci. Technol.*, 2021, **144**, 111220.
50 M. Panić, V. Gunjević, G. Cravotto and I. Radojčić Redovniković, *Food Chem.*, 2019, **300**, 125185.
51 M. Z. Gao, Q. Cui, L. T. Wang, Y. Meng, L. Yu, Y. Y. Li and Y. J. Fu, *Microchem. J.*, 2020, **154**, 104598.
52 B. Ozturk, C. Parkinson and M. Gonzalez-Miquel, *Sep. Purif. Technol.*, 2018, **206**, 1–13.
53 N. Guo, Y. P. Zou, H. K. Li, P. Kou, Z. M. Liu and Y. J. Fu, *Microchem. J.*, 2020, **159**, 105586.
54 M. E. Alañón, M. Ivanović, A. M. Gómez-Caravaca, D. Arráez-Román and A. Segura-Carretero, *Arabian J. Chem.*, 2020, **13**, 1685–1701.
55 L. Chen, Y. Y. Yang, R. R. Zhou, L. Z. Fang, D. Zhao, P. Cai, R. Yu, S. H. Zhang and J. H. Huang, *Anal. Methods*, 2021, **13**, 1226–1231.
56 J. C. López-Linares, V. Campillo, M. Coca, S. Lucas and M. T. García-Cubero, *J. Chem. Technol. Biotechnol.*, 2021, **96**, 481–490.
57 X. Liu, N. Fu, Q. Zhang, S. Cai, Q. Wang, D. Han and B. Tang, *J. Chromatogr. Sci.*, 2019, **57**, 272–278.
58 A. Ali Redha, *J. Agric. Food Chem.*, 2021, **69**, 878–912.
59 M. De Leo, A. M. Iannuzzi, M. P. Germanò, V. D'Angelo, F. Camangi, F. Sevi, G. Diretto, N. De Tommasi and A. Braca, *Food Chem.*, 2021, **360**, 129999.
60 S. Martini, A. Conte and D. Tagliazucchi, *Food Res. Int.*, 2017, **97**, 15–26.
61 M. N. Clifford, K. L. Johnston, S. Knight and N. Kuhnert, *J. Agric. Food Chem.*, 2003, **51**, 2900–2911.
62 J. Cao, C. Yin, Y. Qin, Z. Cheng and D. Chen, *J. Mass Spectrom.*, 2014, **49**, 1010–1024.
63 M. Beszterda and R. Frański, *Eur. J. Mass Spectrom.*, 2020, **26**, 369–375.
64 L. Agulló-Chazarra, I. Borrás-Linares, J. Lozano-Sánchez, A. Segura-Carretero, V. Micol, M. Herranz-López and E. Barrajón-Catalán, *Antioxidants*, 2020, **9**, 418.
65 M. Sanz, E. Cadahía, E. Esteruelas, A. M. Muñoz, B. Fernández De Simón, T. Hernández and I. Estrella, *J. Agric. Food Chem.*, 2010, **58**, 4907–4914.
66 T. Bosiljkov, F. Dujmić, M. Cvjetko Bubalo, J. Hribar, R. Vidrih, M. Brnčić, E. Zlatic, I. Radojčić Redovniković and S. Jokić, *Food Bioprod. Process.*, 2017, **102**, 195–203.
67 L. Loarce, R. Oliver-Simancas, L. Marchante, M. C. Díaz-Maroto and M. E. Alañón, *LWT–Food Sci. Technol.*, 2021, **149**, 111889.
68 E. Kurtulbaş, A. G. Pekel, M. Bilgin, D. P. Makris and S. Şahin, *Biomass Convers. Biorefin.*, 2022, **12**, 351–360.
69 A. M. G. Maclean, Y. P. A. Silva, G. Jiao and M. S. Brooks, *Food Technol. Biotechnol.*, 2021, **59**, 56–62.
70 N. Guo, Ping-Kou, Y. W. Jiang, L. T. Wang, L. J. Niu, Z. M. Liu and Y. J. Fu, *Food Chem.*, 2019, **296**, 78–85.
71 A. Castañeda-Ovando, M. de L. Pacheco-Hernández, M. E. Páez-Hernández, J. A. Rodríguez and C. A. Galán-Vidal, *Food Chem.*, 2009, **113**, 859–871.
72 L. Wu, L. Li, S. Chen, L. Wang and X. Lin, *Sep. Purif. Technol.*, 2020, **247**, 117014.
73 G. Li, Q. Xie, Q. Liu, J. Liu, C. Wan, D. Liang and H. Zhang, *Asia-Pac. J. Chem. Eng.*, 2020, **15**, 1–10.
74 Y. Dai, J. van Spronsen, G. J. Witkamp, R. Verpoorte and Y. H. Choi, *Anal. Chim. Acta*, 2013, **766**, 61–68.

 This journal is © The Royal Society of Chemistry 2024

Published on 12 Abrëll 2024. Downloaded on 04.02.26 10:25:48.

75 J. Bentley, E. K. Olsen, J. P. Moore and J. M. Farrant, *Phytochemistry*, 2020, **173**, 112323.
76 M. Ruesgas-Ramón, M. L. Suárez-Quiroz, O. González-Ríos, B. Baréa, G. Cazals, M. C. Figueroa-Espinoza and E. Durand, *J. Sci. Food Agric.*, 2020, **100**, 81–91.
77 D. O. Abranches, L. P. Silva, M. A. R. Martins, S. P. Pinho and J. A. P. Coutinho, *ChemSusChem*, 2020, **13**, 4916–4921.
78 M. C. Roy, M. Alam, A. Saeid, B. C. Das, M. B. Mia, M. A. Rahman, J. B. Eun and M. Ahmed, *J. Food Process. Preserv.*, 2018, **42**, 1–9.
79 E. Hassan, S. Fadel, W. Abou-Elseoud, M. Mahmoud and M. Hassan, *Polymers*, 2022, **14**, 4605.
80 T. Mada, R. Duraisamy and F. Guesh, *Food Sci. Nutr.*, 2022, **10**, 1222–1238.
81 S. Valdivia-Rivera, I. E. Herrera-Pool, T. Ayora-Talavera, M. A. Lizardi-Jiménez, U. García-Cruz, J. C. Cuevas-Bernardino, J. M. Cervantes-Uc and N. Pacheco, *Foods*, 2021, **10**, 2093.
82 A. Elgharbawy, A. Hayyan, M. Hayyan, M. Mirghani, H. Salleh, S. Rashid, G. Ngoh, S. Liew, M. Nor, M. Y. Z. bin Mohd Yusoff and Y. Alias, *Processes*, 2019, **7**, 416.
83 S. Chen, L. Xiao, S. Li, T. Meng, L. Wang and W. Zhang, *Ultrason. Sonochem.*, 2022, **86**, 106045.
84 M. H. Shafie and C. Y. Gan, *Int. J. Biol. Macromol.*, 2020, **149**, 835–843.
85 N. N. T. Tien, N. L. Le, T. T. Khoi and A. Richel, *J. Food Process. Preserv.*, 2022, **46**, 1–12.
86 M. H. Shafie, R. Yusof and C. Y. Gan, *Carbohydr. Polym.*, 2019, **216**, 303–311.
87 S. Q. Liew, G. C. Ngoh, R. Yusoff and W. H. Teoh, *Biocatal. Agric. Biotechnol.*, 2018, **13**, 1–11.
88 M. Chen and M. Lahaye, *Food Hydrocolloids*, 2021, **115**, 106601.
89 M. Chen, X. Falourd and M. Lahaye, *Carbohydr. Polym.*, 2021, **266**, 118113.
90 A. Nawirska and M. Kwaśniewska, *Food Chem.*, 2005, **91**, 221–225.
91 P. Manara, A. Zabaniotou, C. Vanderghem and A. Richel, *Catal. Today*, 2014, **223**, 25–34.
92 Z. Luo, W. Li, J. Yan and J. Sun, *Adv. Funct. Mater.*, 2022, **32**, 2203988.
93 M. Markiewicz, J. Maszkowska, V. Nardello-Rataj and S. Stolte, *RSC Adv.*, 2016, **6**, 87325–87331.
94 C. Hopson, V. Rigual, J. C. Domínguez, M. V. Alonso, M. Oliet and F. Rodríguez, *Ind. Crops Prod.*, 2022, **186**, 115230.
95 B. R. Thakur, R. K. Singh, A. K. Handa and M. A. Rao, *Crit. Rev. Food Sci. Nutr.*, 1997, **37**, 47–73.
96 W. G. Willats, J. P. Knox and J. D. Mikkelsen, *Trends Food Sci. Technol.*, 2006, **17**, 97–104.
97 S. B. Ross-Murphy and K. P. Shatwell, *Biorheology*, 1993, **30**, 217–227.
98 M. M. Villar-Chavero, J. C. Domínguez, M. V. Alonso, M. Oliet and F. Rodriguez, *Carbohydr. Polym.*, 2020, **229**, 115569.