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Original article

Green extraction of phenolic compounds from strawberry waste based on natural deep eutectic solvents

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Summary The increase in ecological sensitivity and the interest in 'green chemistry' are leading to a decrease in the use of environmentally harmful substances in chemical processes. Replacing traditional solvents with NaDES (Natural Deep Eutectic Solvents) is an excellent green alternative to extract bioactive compounds from agri-food industry wastes, as these solvents bring numerous advantages and are environmentally friendly. In this work, the performance of traditional organic solvents and NaDES were compared for the extraction of phenolic compounds (PCs), with different physicochemical characteristics, from strawberry residues. For this reason, numerous combinations of NaDES have been evaluated, optimising the experimental conditions of the process. The samples were analysed using HPLC–MS/MS, which allowed the identification and quantification of numerous PCs in the samples. The best extraction efficiencies were obtained using choline chloride (ChCl) as hydrogen bond acceptor and lactic acid (LA) as hydrogen bond donor, in a ratio of 1:5 and containing 30% of water. Isoquercetin, hesperidin and catechin were the phenolic compounds most remarkable in samples, with a content of ca. 43%, 26% and 11% of total polyphenols, respectively. Finally, the evaluation of the method through the Analytical GREEness calculator showed how NaDES can satisfactorily replace traditional extractions with a great performance and reduced impact on the environment.

Keywords Circular economy, green chemistry, LC–MS/MS, natural deep eutectic solvents, polyphenols.

Introduction

The development of science and technology allowed to considerably improve the quality of human life, but also led to an increase in the environmental impact, mainly due to the use of dangerous chemicals and to incorrect production and disposal of waste as a result of chemical processes (Dunn, 2012). Recently, the awareness of the environment has significantly increased, redirecting the chemical industry on paths of eco-sustainability. This trend requires chemistry to play a primary role in the conversion of old technologies into new 'clean' processes and the design of new and more eco-friendly products and technologies; these approaches are referred to as 'Green Chemistry' (Anastas & Eghbali, 2010). The main objectives are the reduction of energy consumption, the reduction or elimination of harmful substances for the environment, and, therefore, the development of new processes involving the replacement of conventional organic solvents, such as methanol (MeOH), ethanol (EtOH), acetonitrile (ACN) or hexane (HEX), with eco-compatible and biodegradable ones, without reducing the efficiency of technological processes (Mohammed & Errayes, 2020).

Deep eutectic solvents (DESs) and, especially, natural deep eutectic solvents (NaDESs) are gaining relevance in the field of green solvents for their peculiar characteristics: DESs consist of homogeneous mixtures of two or more components, Lewis or Brønsted acids and bases, and are obtained by hydrogen bond interactions between a hydrogen bond acceptor (HBA),

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usually a quaternary ammonium salt and a metal salt or a hydrogen bond donor (HBD), such as amines, alcohols, carboxylic acids and carbohydrates. The combination of an HBA and an HBH to form DES results in a lower melting point than the individual components, while the viscosity of the solvent is significantly higher than that of most common conventional solvents (Abbasi *et al*., 2021).

Among DESs, the class of NaDESs, with components of natural origin, are more available and eco-compatible (Florindo *et al*., 2019). These solvents, characterised by low vapour pressure, can be customised according to the needs of users, and a wide range of substances are available for their preparation (Smith *et al*., 2014). They can be divided into four groups, based on the HBA and HBD chosen for their preparation: type I (a mixture of organic salts and metal salts), type II (a mixture of organic salts and metal hydrates), type III (a mixture of organic salts and hydrogen bond donor compounds) and type IV (a mixture of chlorides and metal compounds) (Zdanowicz *et al*., 2018). The most popular are type III, as the strength of the hydrogen bond between HBA and HBD is higher than that from the other types, forming a stronger hydrogen bonding network interaction which leads to a remarkable lowering of the melting point and an increase in the viscosity of the mixture (Brett, 2018). Usually, in type III, quaternary salts such as choline chloride (ChCl) are used as HBAs, while amines, carboxylic acids, alcohol and carbohydrates are used as HBD (Pacheco-Fernández & Pino, 2019). The preparation of these solvents is simple, as it is sufficient to mix the various components under stirring and heating at about 100 °C until a homogeneous and transparent solution is obtained. The resulting solvents are very viscous; for this reason, a certain water percentage during the preparation phase is added to reduce the viscosity (Zdanowicz *et al*., 2018). The high availability of HBDs and HBAs gives these solvents a huge versatility and, therefore, allows them to be used in the extraction phase of numerous compounds from foods, biological matrices, etc., obtaining satisfactory yields.

The growing interest in these solvent systems is due to different reasons: high availability in nature of the components, biodegradability, low toxicity, low flammability, renewability and simplicity of synthesis. These features make them particularly interesting for the development of green extraction methods for bioactive compounds, such as phenolic compounds (Chen *et al*., 2019).

Polyphenols are secondary metabolites of plants, mainly known for their antioxidant capacity and particularly present in foods such as phenolic acids, stilbenes, lignans and flavonoids, both free and conjugated forms (Vuolo *et al*., 2018). Thus, in an attempt to reduce waste and by-products within the agrifood industry, the recovery of PCs from these by-products has been extensively studied, initially using conventional technologies and solvents and, nowadays, looking for more sustainable processes (Mir-Cerdà *et al*., 2023). Indeed, in the literature, different DES blends for the extraction of PCs from food matrices are reported. The most used and versatile HBA is ChCl, which is classified as 'type III' of DES. It is relatively cheap, biodegradable, and with very low acute toxicity and is characterised by a strong interaction of the hydrogen bond (Zhekenov *et al*., 2017) which allows it to be combined with many natural compounds and to be used for the extraction of different classes of analytes from food matrices. Thus, ChCl is combined with HBD of various types, including amines, carboxylic acids, alcohol and carbohydrates (Skarpalezos & Detsi, 2019). Usually, for flavonoids, especially for glycosylated forms, DES consists of ChCl and glycerol (Wei *et al*., 2015; Zhao *et al*., 2015; García *et al*., 2016), sugars–for example, fructose, glucose and sucrose (Cui *et al*., 2015; Li *et al*., 2016; Meng *et al*., 2018; Wang *et al*., 2018), or diols–for example, ethylene glycol, propylene glycol, 1,3-butanediol and 1,4-butanediol (Qi *et al*., 2015; Tang *et al*., 2017; Shang *et al*., 2018; Wan Mahmood *et al*., 2019). On the other hand, to enhance the extraction efficiency of catechins, combinations of ChCl with malic acid, citric acid, glucose, or fructose can be used (Nam *et al*., 2015). For phenolic acids, the use of camphor/p-chlorophenol but also ChCl:glycerol or ChCl:ethylene glycol has been reported (Tang *et al*., 2017; Ozturk *et al*., 2018). Therefore, using these solvents is particularly interesting from an extraction point of view as they allow you to work with a huge range of combinations for dealing with molecules with very different physicochemical characteristics.

In this work, an extraction method for the recovery of PCs from strawberry waste was developed and validated following the principles of green chemistry in the framework of a circular economy (Bebek Markovinović *et al*., 2022), giving value to a product rich in PCs. Strawberry is widely used in the food industries for the production of foods and beverages, so the amount of waste generated worldwide is enormous. In particular, the aim of the work was focused on the selection of the most suitable NaDES solvent for the extraction of these analytes of interest and on the replacement of the classic organic solvents. Different combinations of NaDES and different experimental conditions were then evaluated to obtain the best process conditions. The samples were then analysed using HPLC–MS/MS (Oliva *et al*., 2021, 2022; Sun *et al*., 2022), providing the identification and quantification of numerous PCs in the strawberry waste. Results were analysed statistically to identify the most remarkable variables and to select the best working conditions for the extraction of PCs, showing that green solvents can satisfactorily replace the conventional ones, thus reducing the impact on the environment.

Materials and methods

Chemicals

The standards of selected PCs: gallic acid, caffeic acid, ferulic acid, vanillic acid, ethyl gallate, epicatechin, syringic acid, 4-OH-benzoic acid, *p*-coumaric acid, 2,5-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, apigenin, luteolin-7-glucoside, sinapic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Instead, catechin, rutin, myricetin, 3-OH-tyrosol from TCI (Tokyo, Japan); hesperidin, hesperetin from Glentham (Wiltshire, United Kingdom); quercetin, chlorogenic acid, chrysin, fisetin from Merck (Darmstadt, Germany); (−)-epigallocatechin, naringenin, luteolin from Biosynth Carbosynth (Berkshire, United Kingdom); trans-coutaric acid, caftaric acid from Phytolab (Vestenbergsgreuth, Germany); diosmin from Alfa Aesar (Kandel, Germany); quercetin-3-O-glucoside, kaempferol, trans-cinnamic acid from Fluka (Buchs, Switzerland); pinocembrin from Thermo Fisher Scientific (Waltham, Massachusetts, USA); and naringin from TargetMol (Boston, MA, USA). The working standard mixtures were prepared by appropriate dilution in dimethylsulfoxide (DMSO) and stored at −20°C.

Water (H_2O) (Elix3, Millipore, Bedford, MA, USA), formic acid (HCOOH) (>96%, Merck KGaA, Darmstadt, Germany), ethanol (EtOH) and acetonitrile (ACN) (UHPLC PAI-ACS SuperGradient, Panreac, Castellar del Valles, Barcelona, Spain) and methanol (MeOH) (UHPLC-Supergradient, Panreac ApplyChem, Castellar del Valles, Barcelona, Spain) were used to prepare the solvents and mobile phase of the chromatographic method.

Chemicals for the preparation of NaDES were as follows: choline chloride (ChCl), glycerol (Gly) and urea from Thermofisher (Kandel, Germany), lactic acid (Lac) from Acros organics (Geel, Belgium), glucose and ethylene glycol from Thermo Scientific (Waltham, MA, USA), L-proline from J.T.Baker (Phillipsburg, NJ, USA) and tributylammonium bromide from Fluka (Buchs, Switzerland).

Samples

Fresh strawberries (*Fragaria ananassa*, variety Florida Fortuna from Fresón de Palos), purchased from a local retailer, were used to generate the waste, simulating the working conditions of the agri-food industries dealing with juice and jam processing. Strawberries were mashed with a domestic juicer; the juice was then separated by pressing and the solid part, consisting of pulp, seeds and leaves, was kept as a representative waste. This sample was stored in the freezer at −20 °C until use. The thawed sample waste was thoroughly homogenised with an Ultraturax® IKA Z732346 (Merck KGaA, Darmstadt, Germany).

NaDES preparation

A NaDES composed of a mixture of ChCl:LA with a ratio of 1:5 was used for the extraction of PCs from the residual strawberry sample. This mixture was chosen as the most suitable after a thorough optimization process, in which other donors (e.g. glycerol, glucose and ethylene glycol) and other acceptors (e.g. proline and tributylammonium bromide) were also investigated. The ChCl:LA mixture was placed at 60 °C under constant magnetic stirring until a transparent homogeneous compound was formed, and then an amount of 30% H_2O was added to reduce the viscosity of the solvent. The other NaDES compositions detailed in section 2.4 were prepared analogously.

Sample extraction

250 mg of sample were weighed and mixed with 5 mL NaDES or conventional solvents (EtOH, MeOH, ACN and H_2O); the obtained mixture was kept for 15 min at 40 °C under magnetic stirring. The extract was then filtered with 0.45 μm nylon filters (Whatman, Clifton, NJ, USA), diluted 1:1 with H_2O and injected into HPLC-MS/MS for subsequent analysis. Various experimental factors which could have remarkable effects on the recovery of phenolic compounds from the sample were studied to optimise the extraction conditions. The NaDES performance was evaluated by comparing various donors (e.g. lactic acid, urea, glycerol, glucose and ethylene glycol) and acceptors (choline chloride, proline and tributylammonium bromide) using solid–liquid extraction (SLE) assisted by mechanical stirring. Some traditional solvents, including ethanol, methanol, acetonitrile and water, were also investigated for comparison purposes. Once ChCl:LA was chosen as the solvent, other variables were assessed as follows: (i) the ChCl:LA ratio at three levels (2:1, 1:1 and 1:5), (ii) the percentage of water at four levels (0, 10, 30 and 50%), the extraction time at five levels (15, 30, 45, 60 and 120 min), and the extraction temperature at four levels (30, 40, 60 and 80°C). The extraction performance of SLE and ultrasound-assisted extraction (UAE) was evaluated as well. A Branson 5510EMTH ultrasonic cleaner (Sigma-Aldrich, MO, US), working at a frequency of 40 kHz and a power of 130 W, and a water heater (Vpcok Direct, Shenzhen, China) were used for the UAE procedure.

HPLC–MS/MS targeted analysis

Strawberry extracts were analysed using an Agilent 1100 Series HPLC system composed of a degasser (G1322A), a quaternary pump (G1311A), an automatic injection system (G1392A) and a diode array UV–vis detector (G1315B) from Agilent Technologies (Waldbronn, Germany), coupled to an AB Sciex 4000 QTrap mass spectrometer (AB Sciex, Framingham, MA, USA) equipped with an electrospray source (ESI). The PCs were separated using a Kinetex C18 column (150 mm × 2.6 mm ID) from Phenomenex (Torrance, CA, USA) packed with 2.6 μm particles and equipped with a safety guard. The components of the mobile phases were formic acid 0.1% in H_2O (A) and ACN (B). The gradient was set as follows: 0 to 10 min, 3 to 15% ACN; 10 to 20 min, 15 to 45% ACN; 20 to 22 min, 45 to 90% ACN; 22 to 24 min, 90% ACN; (column cleaning); 24.0 to 24.2 min, 90 to 3% ACN; 24.2 to 30 min, 3% ACN (column stabilisation). The flow rate was set at 0.700 mL min^{-1} and the injection volume was set at 10 μL.

The PCs were detected in negative ionisation with a capillary voltage of −4500 V. The ESI source temperature was set at 500 °C. Nitrogen was used as the nebulizer and auxiliary gas and was 10, 20 and 50 arbitrary units for the curtain gas, ion source gas 1 and ion source gas 2, respectively. The instrumental parameters, such as declustering potential (DP), entrance potential (EP), collision energy (CE) and the collision cell exit potential (CXP), were tuned for the targeted analytes by infusion of each single standard methanol solution (1 μg mL^{-1}). The acquisition and quantification of ion currents were performed in multiple reaction monitoring (MRM) mode. The MS/MS parameters and the ion transition pairs are listed in Table S1.

Data analysis

The data generated in the experimental studies were statistically analysed with t-tests and ANOVA using Microsoft Excel. In any case, the significance level considered was $\alpha = 0.05$. Bar plots were used to depict the results, with error bars added to show the standard deviations and letters (a, b, c, etc.) to show the statistical differences (i.e. the same letters for a compound mean no statistical differences and vice versa).

Results and discussion

This work was the first insight into a laboratory-scale study to investigate the most effective working conditions to extract phenolics from strawberry waste. At the same time, a comprehensive characterisation of the composition of the extract was carried out to identify the most abundant compounds. Although the study was designed at a bench scale, the final perspective is the waste revalorization at an industrial level. Hence, the best working conditions were not always those leading to higher extraction yields but achieving a good compromise between recovery and sustainability. In this sense, green solvents and technologies, simplicity of procedures, or saving in process time and costs were important aspects to prioritise, even at the expense of the extraction yield.

For the optimization of extraction conditions, several noticeable factors were selected for investigation based on our previous experience with other agri-food wastes. A sequential approach was applied, meaning that variables were optimised one after the other. The process started with the assessment of the composition of the extraction solvent, including NaDES, conventional green solvents such as ethanol or water, and other organic solvents commonly used for similar purposes (e.g. methanol and acetonitrile). Two extraction techniques viable for scaling were compared, such as solid–liquid extraction (SLE) assisted by mechanical stirring and ultrasound-assisted extraction (UAE). Finally, physical conditions, such as time and temperature, were established.

NaDES selection

PCs are characterised by different structures with different chemical–physical properties, including a wide range of polarity; therefore, there is a need for suitable extraction methods capable of dealing with a broad class of compounds as the PCs. Furthermore, there is an interest in maintaining a green approach both in the method and in the application: the development of a green and biocompatible extraction method and the recovery of bioactive compounds from production waste, in this case, strawberry residues. In order to accomplish these aims, different mixtures of HBD and HBA belonging both to the category of NaDES were tested in various molar ratios. ChCl and L-proline were used as HBA and urea (U), glycerol (Gly), lactic acid (LA) and glucose (Glu) as HBD. Furthermore, tetrabutylammonium bromide (TBAB), belonging to the hydrophobic deep eutectic solvents (HDESs) category, was combined with Gly and ethylene glycol (EG) to investigate the potentiality of hydrophobic interactions in the recovery of antioxidant compounds.

Firstly, for each HBD:HBA combination, a 1:1 ratio was tested. Different amounts of each component were weighed, mixed and placed under magnetic stirring at 60 °C until a homogeneous and transparent phase was obtained as reported in the literature (de Almeida Pontes *et al.*, 2021). The preparation time varies from 30 min to 2 h depending on the NaDES components

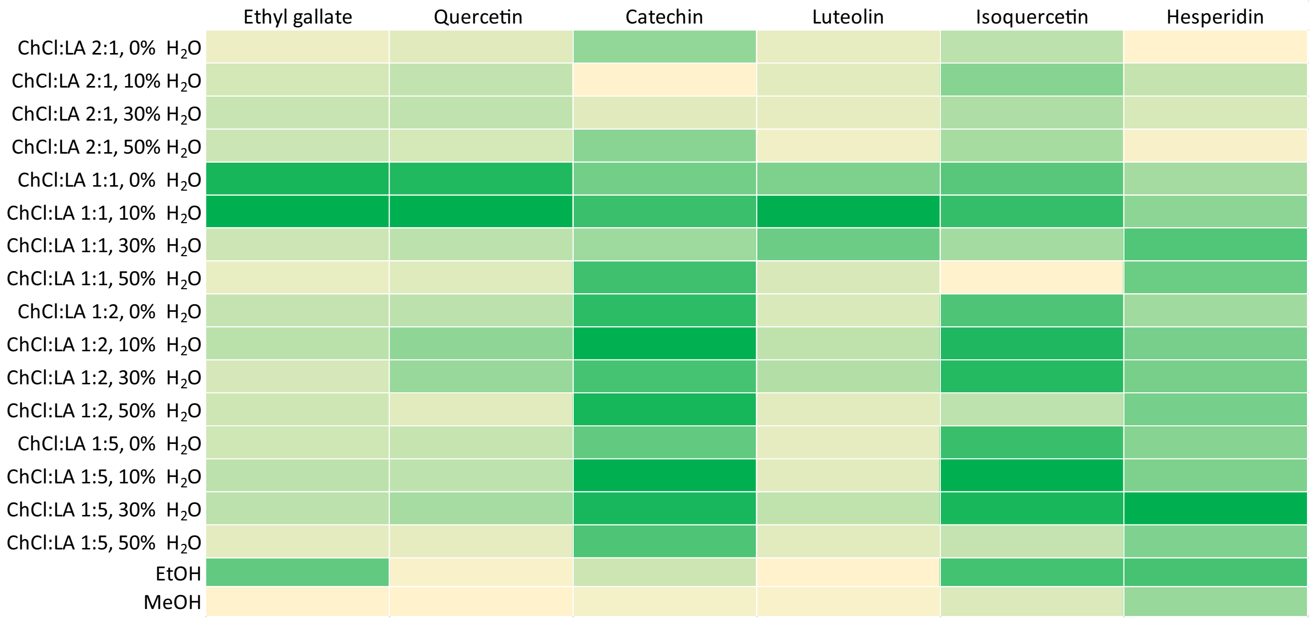

Figure 1 Heat map depicting the recovery of the most abundant PCs at different ChCl:LA ratios and H_2O percentages. Ethanol and methanol results are also included for comparative purposes. The colour-grading indicates the extraction efficiency according to the normalised peak area of the corresponding compound; the intensity of the green colour denotes the best extraction while the pale yellow tone implies low extraction.

chosen. However, as the hydrogen bonding mechanism of DESs directly affects its properties, a strong hydrogen bond interaction between HBA and HBD results in a decrease in the melting point but an increase in viscosity (Han *et al.*, 2022). The addition of H_2O (0%, 10%, 30% and 50%) to each NaDES combination was evaluated to overcome this issue.

To assess the performance of the different NaDES, strawberry waste was extracted at 60 °C for 1 h under magnetic stirring; the results were then compared with those obtained from extraction with conventional solvents (EtOH, MeOH, ACN and H_2O) under the same conditions.

The best recovery values were obtained with the ChCl:LA mixture, which showed an extraction of phenolic compounds analogous to that provided by traditional solvents, also allowing the extraction of 2,5-dihydroxybenzoic acid, not detected in any of the classical solvent extracts.

The next step involved the evaluation of different ratios between the selected HBA and HBD, ChCl and LA, respectively, along with the water content. These are essential aspects that affect the properties of the extraction solvent itself and therefore its performance; in particular, the ratios 2:1, 1:1, 1:2 and 1:5 and the percentages of water of 10%, 30% and 50% were tested.

Fig. 1 shows a heatmap of representative compounds extracted from the strawberry waste matrix using different extraction media. Since the concentrations of these compounds notably differ in magnitude (e.g. hesperidin or isoquercetin occur at concentrations ca. tenfold higher than some others), normalised concentrations were used to visualise more efficiently the best global extraction (for each analyte, the concentration of each condition was divided by the largest value in the series). In this way, the intense green colour indicates the experiment(s) that provide a comparatively greater extraction of a given compound regardless of its absolute concentration, while the pale-yellow colours correspond to low compound extraction. As can be seen, the best extraction conditions in terms of overall performance correspond to ChCl:LA 1:1 with 0% or 10% water. However, the NaDES mixtures generated for the two compositions are very viscous and difficult to handle. Hence, for practical reasons, they were not recommended despite their superior performance. If we dismiss them, the next medium that provided a high performance is ChCl:LA 1:5 with 30% water. Thus, this composition was selected for further studies as a compromise between extraction efficiency and ease of use, resulting in an excellent opportunity to address the extraction of phenolic compounds from strawberry waste.

Extraction set-up

For the extraction of PCs from the strawberry residue sample, different extraction steps were optimised to obtain the maximum yield for all analytes; then, the

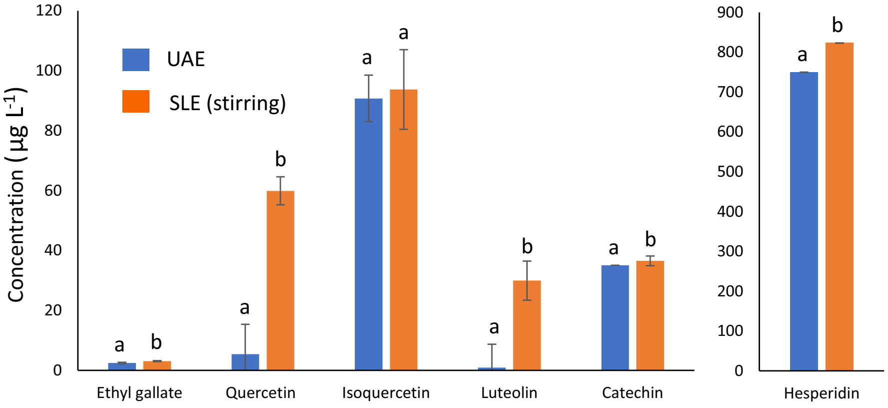

Figure 2 Composition of the most abundant PCs in the extracts obtained by UAE and SLE with mechanical stirring using the ChCl:LA (1:5) 30% water as the solvent. Error bars indicate the standard deviation; the same letters for a compound mean no statistical differences.

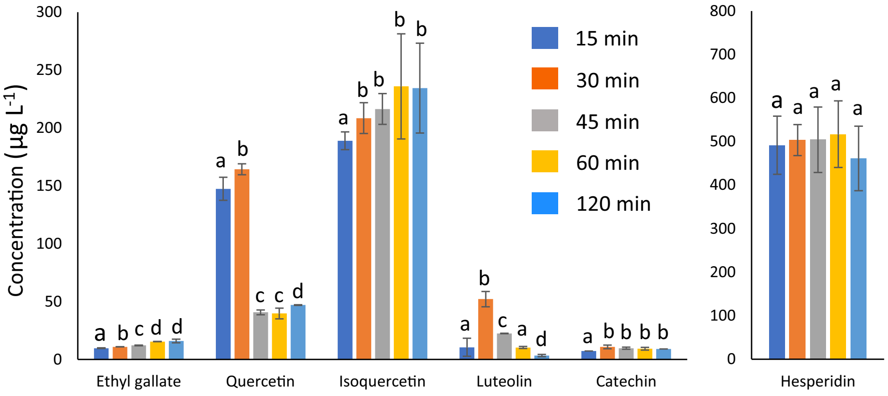

Figure 3 Composition of the most abundant PCs in the extracts obtained at different extraction times using the ChCl:LA (1:5) 30% water as the solvent. Error bars indicate the standard deviation; the same letters for a compound mean no statistical differences.

results obtained were compared with the classical extraction using ethanol as the solvent.

First, the extraction mechanism was evaluated; for such a purpose, the extraction yield was compared by using the ultrasonic bath and by simple magnetic stirring (Fig. 2). Therefore, 250 mg of strawberry residue samples were weighed, in triplicate, treated with 5 mL of solvent (ChCl:LA), and extracted with the different devices at 60 °C for 1 h. The samples were then filtered, diluted with a ratio of 1:1 with H_2O, and analysed by HPLC–MS/MS. As an example, Fig. 2 shows the concentrations of the most relevant analytes in the extracts from SLE with magnetic stirring and UAE. In addition, the data were evaluated by statistical analysis with a *t*-test, which confirms that, in general, significant differences (P-value <0.05) exist between both extraction mechanisms for compounds such as hesperidin, luteolin, or quercetin, with magnetic stirring being the one providing the best extraction yields. Therefore, it was selected for further experiments. Furthermore, from the point of view of procedure scaling, the option of SLE by magnetic stirring is also more recommended for both simplicity and cost.

Afterwards, the extraction time was evaluated as may be an essential factor to obtain a satisfactory extraction yield while avoiding the occurrence of unwanted thermal degradation reactions. Extraction times between 15 and 120 min were studied in an SLE procedure with magnetic stirring that used ChCl:LA 1:5 (30% water) at 30°C. The results reported in Fig. 3 show that the behaviour of the compounds is different. Hesperidin does not show significant differences in the recovered concentration with time. Other phenolics, such as isoquercetin and ethyl gallate, increased slightly the yields by increasing the extraction time. Finally, compounds such as quercetin or luteolin achieved better recoveries at times of 15 or 30 min. However, thinking about the future scaling of

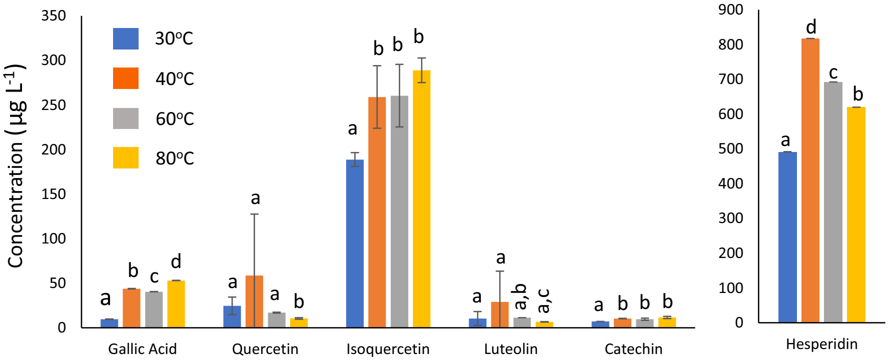

Figure 4 Composition of the most abundant PCs in the extracts obtained at different extraction temperatures using the ChCl:LA (1:5) 30% water as the solvent. Error bars indicate the standard deviation; the same letters for a compound mean no statistical differences.

the process, an extraction time of 15 min was selected to speed up the procedure without a significant loss of extraction capacity.

Temperature was subsequently tested as may have a remarkable influence on the process, simultaneously promoting solubility and mass transfer rate by reducing the viscosity and surface tension of solvents, thus contributing to greater extraction yields. Too low temperatures may not be sufficient for the efficient mass transfer of the analytes from the samples to the solvent while a high temperature could favour unwanted oxidation or degradation of the PCs. The extraction was then tested at different process temperatures (30, 40, 60 and 80 °C). The results depicted in Fig. 4 showed, in general, an improvement in extraction yield with increasing temperature from 30 to 40°C. Next, depending on the analyte, the rise in temperature led to different situations, with a slight increase in the extraction performance (e.g. gallic acid), no significant changes (e.g. isoquercetin or catechin) or even decays in the recovered concentration (e.g. hesperidin, quercetin or luteolin). The decreases in the extract concentrations could be attributed to the degradation of thermolabile compounds, while the increases could be associated with higher solubilities or in-situ compound generation from related analytes, for example from hydrolysis reactions. With this range of behaviours, 40°C was finally selected as the consensus temperature for the analyte extraction from the strawberry waste.

Comparison of NaDES and ethanol extracts

In preliminary studies (see Section 3.1), several conventional solvents such as ethanol, methanol, acetonitrile, or water were evaluated. Among them, ethanol provided the best outcomes in quantitative performance. Besides, along with water, they both are green options compatible with food applications. For these reasons, ethanol was selected for comparison with NaDES in terms of extraction efficiency to assess whether NaDES could be an effective alternative to conventional organic green solvents such as ethanol.

The comparison of the results obtained with the selected NaDES composition and ethanol shows a spectacular improvement in the extracted amounts (see Table 1). The concentrations of the main compounds, such as isoquercetin or hesperidin, are ca. 20 to 60% higher while levels of other molecules are up to 6-fold richer. It is also encountered that some minor compounds, such as 2,5-dihydroxybenzoic or ferulic acid, are only detected in NaDES extracts.

As an example, Fig. 5 shows the MRM chromatograms of some compounds present in a NaDES extract from strawberry waste. Several representative peaks are highlighted. As shown in Table 1, the major polyphenols in the strawberry waste is quercetin-hexoside (isoquecetin), which corresponds to 50% of the phenolic content of the targeted analytes, followed by hesperidin (accounting for ca. 27%). Other molecules, such as 4-hydroxybenzoic acid, quercetin, catechin, luteolin and gallic acid, are also quantitatively noticeable. All of them are candidates to be recovered and purified from the matrix studied to proceed with its revalorization.

Furthermore, the proposed method was evaluated with the Analytical GREEnness (Pena-Pereira *et al.*, 2020) calculator, which employs several criteria, based on the 12 principles of 'Green Chemistry' to assess the sustainability of the analytical methodology. The outcome is a normalised value between 0 and 1 that estimates the general green character of the process, the closer to 1 being better. The result obtained using NaDES was 0.74 (Fig. 6) despite using an analytical instrument with high energy consumption, such as the mass spectrometer. The value for ethanol option is the same, since it is also considered a green solvent and compatible with agri-food or nutraceutical applications. On the other hand, with other solvents that

Table 1 Polyphenol concentrations in the NaDES and ethanolic extracts. Data are reported as μg/g of strawberry waste

	NaDES		**Ethanol**		
Compounds	**Concentration (μg/g)**	**RSD (%)**	**Concentration (μg/g)**	**RSD (%)**	**Yield improvement (%)**
Hesperidin	1433	4	961	7	49,1
Isoquercetin	2348	1	1850	1	26,9
Catechin	621	3	474	4	31,0
Quercetin	593	6	85.3	5	595,2
4-Hydroxybenzoic acid	189	6	116	11	62,9
Luteolin	169	7	79.8	3	111,8
Gallic acid	49.4	4	41.5	6	19,0
p-Coumaric acid	37.8	2	18.8	3	101,1
Diosmin	34.7	1	26.6	1	30,5
Chlorogenic acid	11.3	1	9.17	3	23,2
Apigenin	11.3	4	3.61	8	213,0
Ethyl gallate	11.2	2	1.62	2	591,4
Hesperitin	10.7	7	5.44	9	96,7
Caffeic acid	9.87	1	9.11	1	8,3
Hydroxytyrosol	9.25	1	2.15	10	330,2
Caftaric acid	7.6	2	3.86	5	96,9
Rutin	6.07	7	2.28	7	166,2
2,5-Dihydroxybenzoic acid	5.13	3	<LOQ	–	>99
Naringenin	4.84	2	1.14	2	324,6
Ferulic acid	3.16	2	<LOQ	–	>99

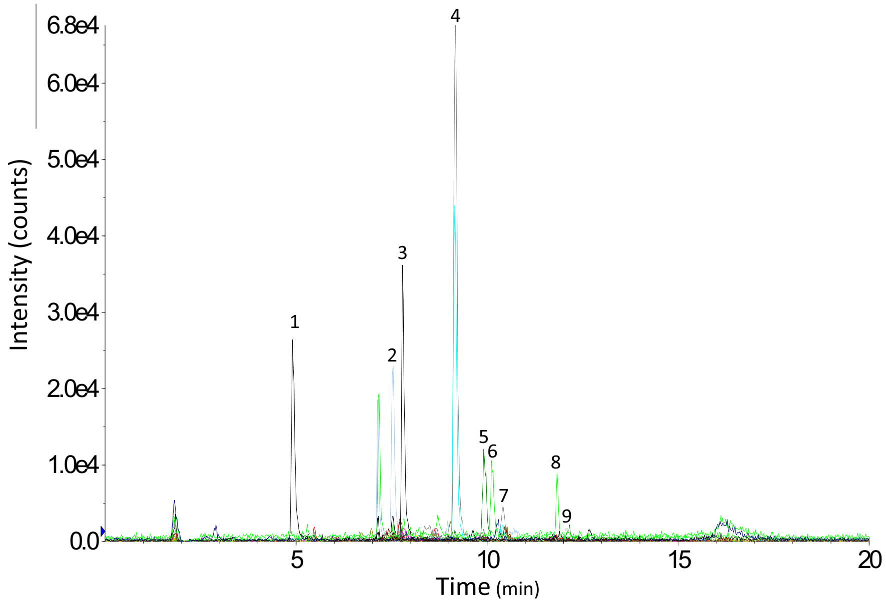

Figure 5 HPLC-MS/MS chromatogram of the NaDES extract with the MRM signals of selected analytes (NaDES: ChCl:LA 1:5, 30% water; temperature: 40°C; extraction time: 15 min). Peak assignment: 1, gallic acid; 2, catechin; 4, 4-hydroxybenzoic acid; 5, Isoquercetin; 6, *p*-coumaric acid; 7, diosmin; 8, luteolin; 9, quercetin.

present toxicity (e.g. acetonitrile or methanol), the score will decrease. Examining the individual criteria, the less green points of the procedure refer to the use of a high-performance analytical technique such as LC–MS/MS. A thorough characterisation of the sample is necessary at the beginning but, once its

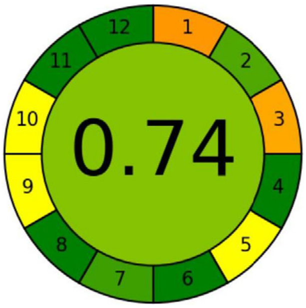

Figure 6 The result evaluated with the Analytical GREEnness calculator (Pena-Pereira *et al.*, 2020).

composition is known, the extract analysis can be addressed with simpler techniques such as HPLC-UV or spectrophotometry, improving the GREEnness index. In any case, the selected option involving NaDES with UAE combines a notable greenness and superior performance (Table 1), so we considered this proposal to be a good option for the recovery of phenolic compounds. This choice was also sound for scaling the procedure.

Once demonstrated that NaDES dramatically improved the quantitative results compared to ethanol as the conventional green solvent of reference, it is speculating on the uses of the extracts, especially for dealing with chemical, nutraceutical or food industry applications. An adsorption/desorption approach with polymeric sorbents can be applied when the recovery and purification of phenolics from the NaDES is required. This process can be implemented in batch or column chromatography arrangements, with the latter setup being more suitable for scaling up. Phenolic compounds are thus retained in polymeric stationary phases and the NADES is recovered after the process for its next use. The analytes are then eluted, resulting in a product with notable antioxidant capacity and high polyphenolic purity of interest in food supplementation or nutraceuticals. More interestingly, the NADES extract can be used directly without any solvent removal. An illustrative case deals with the preparation of films for packaging, in which the bioactive compounds provide antioxidative and antibacterial capacity to the film, making it very suitable for food preservation. Furthermore, in the case of waste rich in pigments, such as the strawberry matrix, anthocyanins would give coloured material films that may act as a pH sensor.

Conclusions

The growing interest in protecting the environment has led to an evolution of increasingly 'clean' and environmentally friendly technological processes, leading to the continuous search for procedures aligned with the concept of 'Green Chemistry'. The optimization of the main process steps aims, of course, to achieve the best recovery of bioactive compounds, but also considers other essential aspects such as lowering process time, energy consumption and waste generation, while avoiding toxic or dangerous chemicals for the environment and human health. NaDES emerges as a substitute for conventional organic solvents that satisfy some of the required principles, helping to develop more eco-friendly process methods while providing satisfactory yields.

This study has shown how conventional organic solvents, such as ethanol, can be replaced by NaDES for the recovery of bioactive substances from agri-food industry by-products, leading to improved extraction performance. A method for extracting phenolics from food matrices, such as strawberry waste, has been developed. The choice of the ChCl:LA combination allowed phenolics extraction with satisfactory yields, in a simple and fast way. In our opinion, the proposed method is a viable alternative to traditional extractions. Indeed, similar strategies aimed at identifying optimal extraction conditions can be applied to other case studies.

Author contributions

Eleonora Oliva: Investigation; writing – original draft; methodology. **Aina Mir-Cerdà:** Investigation; methodology. **Manuel Sergi:** Writing – review and editing; supervision. **Mercè Granados:** Conceptualization; funding acquisition; supervision. **Sonia Sentellas:** Conceptualization; writing – review and editing; supervision. **Javier Saurina:** Conceptualization; funding acquisition; writing – review and editing; supervision.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics statement

Ethics approval was not required for this research.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

Abbasi, N.M., Farooq, M.Q. & Anderson, J.L. (2021). Modulating solvation interactions of deep eutectic solvents formed by ammonium salts and carboxylic acids through varying the molar ratio of hydrogen bond donor and acceptor. *Journal of Chromatography A*, **1643**, 462011.

Anastas, P. & Eghbali, N. (2010). Green chemistry: principles and practice. *Chemical Society Reviews*, **39**, 301–312.

Nowadays moving to green chemistry is crucial to face the current demand for more sustainable methodologies.

Bebek Markovinović, A., Brčić Karačonji, I., Jurica, K. *et al.* (2022). Strawberry tree fruits and leaves (Arbutus unedo L.) as raw material for sustainable functional food processing: a review. *Horticulturae*, **8**, 881.

Brett, C.M.A. (2018). Deep eutectic solvents and applications in electrochemical sensing. *Current Opinion in Electrochemistry*, **10**, 143–148.

Chen, J., Li, Y., Wang, X. & Liu, W. (2019). Application of deep eutectic solvents in food analysis: a review. *Molecules*, **24**, 1–12.

In this manuscript, the use of DES as a green alternative for enhancing the extraction efficiency of several compounds, including polyphenols, from food is reported.

Cui, Q., Peng, X., Yao, X.H. *et al.* (2015). Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots. *Separation and Purification Technology*, **150**, 63–72.

de Almeida Pontes, P.V., Ayumi Shiwaku, I., Maximo, G.J. & Caldas Batista, E.A. (2021). Choline chloride-based deep eutectic solvents as potential solvent for extraction of phenolic compounds from olive leaves: extraction optimization and solvent characterization. *Food Chemistry*, **352**, 129346.

Dunn, P.J. (2012). The importance of green chemistry in process research and development. *Chemical Society Reviews*, **41**, 1452–1461.

Florindo, C., Branco, L.C. & Marrucho, I.M. (2019). Quest for green-solvent design: from hydrophilic to hydrophobic (deep) eutectic solvents. *ChemSusChem*, **12**, 1549–1559.

García, A., Rodríguez-Juan, E., Rodríguez-Gutiérrez, G., Rios, J.J. & Fernández-Bolaños, J. (2016). Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs). *Food Chemistry*, **197**, 554–561.

Han, P., Nie, W., Zhao, G. & Gao, P. (2022). Theoretical investigation on the structure and physicochemical properties of choline chloride-based deep eutectic solvents. *Journal of Molecular Liquids*, **366**, 120243.

Li, L., Liu, J.Z., Luo, M. *et al.* (2016). Efficient extraction and preparative separation of four main isoflavonoids from Dalbergia odorifera T. Chen leaves by deep eutectic solvents-based negative pressure cavitation extraction followed by macroporous resin column chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1033–1034**, 40–48.

Meng, Z., Zhao, J., Duan, H., Guan, Y. & Zhao, L. (2018). Green and efficient extraction of four bioactive flavonoids from pollen Typhae by ultrasound-assisted deep eutectic solvents extraction. *Journal of Pharmaceutical and Biomedical Analysis*, **161**, 246–253.

Mir-Cerdà, A., Nuñez, O., Granados, M., Sentellas, S. & Saurina, J. (2023). An overview of the extraction and characterization of bioactive phenolic compounds from agri-food waste within the framework of circular bioeconomy. *TrAC, Trends in Analytical Chemistry*, **161**, 116994.

Mohammed, W. & Errayes, A. (2020). Green chemistry: principles, applications, and disadvantages. *Chemical Methodologies*, **4**, 408–423.

Nam, M.W., Zhao, J., Lee, M.S., Jeong, J.H. & Lee, J. (2015). Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: application to flavonoid extraction from Flos sophorae. *Green Chemistry*, **17**, 1718–1727.

Oliva, E., Fanti, F., Palmieri, S. *et al.* (2022). Predictive multi experiment approach for the determination of conjugated phenolic compounds in vegetal matrices by means of LC-MS/MS. *Molecules*, **27**, 3089. https://doi.org/10.3390/molecules27103089

Oliva, E., Viteritti, E., Fanti, F. *et al.* (2021). Targeted and semi-untargeted determination of phenolic compounds in plant matrices by high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, **1651**, 462315.

Ozturk, B., Parkinson, C. & Gonzalez-Miquel, M. (2018). Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. *Separation and Purification Technology*, **206**, 1–13.

Pacheco-Fernández, I. & Pino, V. (2019). Green solvents in analytical chemistry. *Current Opinion in Green and Sustainable Chemistry*, **18**, 42–50.

Pena-Pereira, F., Wojnowski, W. & Tobiszewski, M. (2020). AGREE–analytical GREEnness metric approach and software. *Analytical Chemistry*, **92**, 10076–10082.

In this manuscript, a method for evaluating the greenness of a method is proposed.

Qi, X.L., Peng, X., Huang, Y.Y. *et al.* (2015). Green and efficient extraction of bioactive flavonoids from Equisetum palustre L. by deep eutectic solvents-based negative pressure cavitation method combined with macroporous resin enrichment. *Industrial Crops and Products*, **70**, 142–148.

Shang, X., Tan, J.N., Du, Y., Liu, X. & Zhang, Z. (2018). Environmentally-friendly extraction of flavonoids from cyclocarya paliurus (batal.) iljinskaja leaves with deep eutectic solvents and evaluation of their antioxidant activities. *Molecules*, **23**, 2110.

Skarpalezos, D. & Detsi, A. (2019). Deep eutectic solvents as extraction media for valuable flavonoids from natural sources. *Applied Sciences (Switzerland)*, **9**, 4169.

Smith, E.L., Abbott, A.P. & Ryder, K.S. (2014). Deep eutectic solvents (DESs) and their applications. *Chemical Reviews*, **114**, 11060–11082.

Sun, M.F., Jiang, C.L., Kong, Y.S., Luo, J.L., Yin, P. & Guo, G.Y. (2022). Recent advances in analytical methods for determination of polyphenols in tea: a comprehensive review. *Food*, **11**, 1425.

Tang, W., Li, G., Chen, B., Zhu, T. & Row, K.H. (2017). Evaluating ternary deep eutectic solvents as novel media for extraction of flavonoids from *Ginkgo biloba*. *Separation Science and Technology (Philadelphia)*, **52**, 91–99. https://doi.org/10.1080/01496395.2016.1247864

Vuolo, M.M., Lima, V.S. & Maróstica Junior, M.R. (2018). Phenolic compounds: structure, classification, and antioxidant power. *Bioactive Compounds: Health Benefits and Potential Applications*, 33–50. https://doi.org/10.1016/B978-0-12-814774-0.00002-5

In this chapter, an overview of the structural diversity of polyphenols in several vegetal matrices is given. Also, their mechanisms of action against oxidative stress are discussed.

Wan Mahmood, W.M.A., Lorwirachsutee, A., Theodoropoulos, C. & Gonzalez-Miquel, M. (2019). Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from Chlorella vulgaris [research-article]. *ACS Sustainable Chemistry and Engineering*, **7**, 5018–5026.

Wang, H., Ma, X., Cheng, Q., Wang, L. & Zhang, L. (2018). Deep eutectic solvent-based ultrahigh pressure extraction of Baicalin from Scutellaria Baicalensis Georgi. *Molecules*, **23**, 3233.

Wei, Z.F., Wang, X.Q., Peng, X. *et al*. (2015). Fast and green extraction and separation of main bioactive flavonoids from radix Scutellariae. *Industrial Crops and Products*, **63**, 175–181.

Zdanowicz, M., Wilpiszewska, K. & Spychaj, T. (2018). Deep eutectic solvents for polysaccharides processing. A review. *Carbohydrate Polymers*, **200**, 361–380.

Zhao, B.Y., Xu, P., Yang, F.X., Wu, H., Zong, M.H. & Lou, W.Y. (2015). Biocompatible deep eutectic solvents based on choline chloride: characterization and application to the extraction of Rutin from Sophora japonica. *ACS Sustainable Chemistry and Engineering*, **3**, 2746–2755.

Zhekenov, T., Toksanbayev, N., Kazakbayeva, Z., Shah, D. & Mjalli, F.S. (2017). Formation of type III deep eutectic solvents and effect of water on their intermolecular interactions. *Fluid Phase Equilibria*, **441**, 43–48.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. MS/MS parameters of the selected analytes for the MRM acquisition.