Original article
Simple and reliable eco-extraction of bioactive compounds from dark chocolate by Deep Eutectic Solvents. A sustainable study

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Summary   The green solvents and eco-extraction methods are gaining increasing interest in chemical analysis for bioactive compounds in food matrices. Deep Eutectic Solvents (DES) developed as a greener and more sustainable alternative to organic solvents, owing to their non-toxic, highly stable, and biodegradation-friendly nature. DES application for polyphenols and antioxidant compounds extraction in dark chocolate samples has been evaluated in an integrated study for sustainability assessment, based on multivariate analysis and Life Cycle Assessment (LCA) methodology. A green extraction method based on DES was proposed testing different HBA:HBD pairs (ChCl:Fru, ChCl:Teg, Bet:Fru, and Bet:Teg). DES Bet:Fru resulted in the highest extraction yield in terms of both total polyphenols (0.34–3.37 g GAE/100 g) and flavonoids (1.13–8.32 g RUT/100 g), $P < 0.05$. Furthermore, the environmental performances of green and conventional solvents (MeOH:H_2O, H_2O, and MeOH) were evaluated by applying a comparative LCA (c-LCA). The c-LCA study highlighted that conventional extraction for polyphenols in dark chocolate was 60% more impactful than DES. DES pairs analysed quantitatively lowest impacted than conventional methods, considering the macro-categories Human Health ($9.99 \times 10^{-8} \div 1.54 \times 10^{-7}$ DALYs), Ecosystem ($2.29 \times 10^{-10} \div 3.57 \times 10^{-10}$ species.yr), and Resources ($6.57 \times 10^{-3} \div 8.96 \times 10^{-3}$ USD2013).

Keywords   bioactive compounds, dark chocolate, Deep Eutectic Solvents, life cycle assessment, sustainability assessment.

Introduction

The increasing awareness of sustainability and environmental issues has promoted the need to replace conventional solvents with eco-compatible, harmless, and non-toxic extraction methods for bioactive compounds (BCs) in foodstuffs. For the chemical analyses of food matrices, conventional organic solvents (e.g., methanol, ethyl acetate, hexane, etc.) are widespread as an efficient extraction solvent for BCs; nevertheless, its significant impact on the environment, operator safety, and high toxicity is not overlooked. Therefore, in recent years, conventional methods have been joined or replaced by innovative green methods, focusing on both analytical parameters, in terms of extraction yield, purification, and sustainability concerns. Indeed, one of the objectives of Green Chemistry is to develop new analytical methods that reduce or completely replace the use of hazardous substances, thus reducing

the risks to the environment (Ruesgas-Ramón et al., 2017). In this regard, solvents such as supercritical fluids, Ionic Liquids (ILs), Deep Eutectic Solvent (DESs), and supramolecular liquids are used to replace conventional organic solvents (Paiva et al., 2014; Benvenutti et al., 2019; Manuela et al., 2020). According to the principles of Green Chemistry, a process can be considered green or sustainable, when the procedure involves: (i) reduced use of hazardous solvents/reagents; (ii) energy-efficient design for minimised environmental and economic impact; (iii) biodegradation-friendly design for proper disposal of the waste produced (Socas-Rodríguez et al., 2021).

One of the promising green solvents for efficient extraction of polyphenol and antioxidant compounds from food matrices are Deep Eutectic Solvents (DESs). DESs are mixtures of two substances consisting of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) which, when mixed in a specific molar ratio, have a lower melting point than the individual substances, allowing the formation of a clear solution,

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which is used as an extracting solvent (Alañón et al., 2020). The ability of the solvent to form hydrogen bonds with the bioactive compounds allows the analytes to be highly soluble. In addition, DES has a high selectivity; this allows for a decrease in the steps of the extraction procedure, as well as the amount of the solvent to be used and the analysis time (Ijardar et al., 2022). Due to their chemical properties and non-toxicity, DES could be suitable for numerous applications, from cosmetics to pharmaceuticals and food ingredients (Panzella et al., 2020).

This study was aimed at applying different Deep Eutectic Solvents as eco-extraction methods for simple and reliable polyphenol determination in dark chocolate, with an integrated approach for sustainability assessment.

Cocoa and cocoa-based products (i.e., chocolate) have unique sensory characteristics, whose remarkable nutritional properties have been widely exploited by dieticians and food technologists (Indiarto et al., 2021). It is worth noting that cocoa is constituted of about 600 different bioactive compounds, among which polyphenols and alkaloids represent the two main classes, responsible for health and quality-promoting effects on the human organism (Godočiková et al., 2020; Soares et al., 2022). However, these BCs not only determine the quality of the cocoa derivatives, but also are responsible for the psychoactive properties of food products; focusing on polyphenols, these arouse interest for their antioxidant, anti-inflammatory, and antitumor activity, as well as for their ability to inhibit pathological processes (Martini et al., 2018). The main groups of polyphenols in cocoa and its derivatives (i.e., cocoa powder, chocolate bars, etc.) are proanthocyanidins (58% of total dry weight), catechins (37%), anthocyanins (4%), whose content and type may vary depending both on the cocoa cultivar or origin as well as processing of cocoa beans and chocolate manufacturing processes (i.e., fermentation, roasting, conching, alkalinisation, etc.) (Giacometti et al., 2015). In particular, it was well established that alkalinisation might cause a progressive reduction of polyphenols, as well as their antioxidant activity (Miller et al., 2008). The greatest losses have been observed for epicatechins and catechins content, thus highlighting a reduction of 98% and 80% respectively. The changes in content might be ascribed to the oxidation of phenolic components under basic pH conditions (Giacometti et al., 2015; Sioriki et al., 2022).

To the best of our knowledge, there are no references in the literature, focusing on the comparison of eco-extraction methods applied for polyphenols in dark chocolate, by considering the green procedure in terms of both extraction efficiency and sustainability performances. In the present study, DES composed of different HBA (Choline Chloride, ChCl; Betaine, Bet), and HBD (Fructose, Fru; and Triethylene glycol, Teg) at specific molar ratios were tested, ChCl:Teg (1:2), ChCl:Fru (1:1), Bet:Teg (1:2), and Bet:Fru (1:1), thus evaluating different operating conditions in terms of time (50, 40, 30 min), temperature (60 °C, 70 °C, and 80 °C), and water content (10%, 20%, and 30%). DES extraction was also compared with conventional extractants (e.g., methanol/water, water, methanol, etc.), to assess the best extraction solvent for polyphenols in dark chocolate, both from a chemical and sustainable perspective. In addition, to evaluate the sustainability assessment as regards DES extraction considered, a comparative Life Cycle Assessment (c-LCA) was carried out to assess the environmental performances of different polyphenols extraction methods, thus evaluating the most sustainable extraction procedure in terms of impacts on Human Health, Ecosystem, and Resources (Vauchel et al., 2018).

Materials and methods

Bioactive compounds determination in dark chocolate samples

Reagents
Methanol (≥99.9%), distilled water, 2-Hydroxyethyl) trimethylammonium chloride (Choline chloride - ChCl), carboxymethyl)trimethylammonium hydrochloride (Betaine - Bet; ≥99%), D-(+)- Fructose (Fru; ≥99.5%) and 1,2-Bis(2-methoxyethoxy)ethane (Triethylene glycol dimethyl ether – Teg; ≥98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin-Ciocâlteu reagent, sodium nitrite ($NaNO_2$; ≥99.9%), sodium ≥98% carbonate (Na_2CO_3; ≥99.5%), Aluminium chloride ($AlCl_3$; ≥99%), Sodium hydroxide (NaOH; ≥98%), 3,4,5-Trihydroxybenzoic acid (gallic acid - GAE; ≥99%) and Quercetin-3-rutinoside trihydrate (Rutin - Rut; ≥99%) standard were purchased from Sigma-Aldrich (Milan, Italy).

Sample
Dark chocolate samples with 70% of cocoa powder were purchased from local supermarkets. The samples had a total fatty acid content of 40 g/100 g of product, carbohydrates: 31 g/100 g of product, simple sugars: 27 g/100 g of product, protein: 8.6 g/100 g of roduct, and fiber: 0.03 g/100 g of product. The samples were previously frozen at T = −18 °C and then grounded. The resulting powder was separated first with a 2 mm diameter filter and then with 0.7 mm. Chocolate powder with a diameter of less than 0.7 mm was used for sample analysis. The samples were then aliquoted and stored at temperatures below T=−18 °C, until the day of analysis.

Table 1 Composition of Deep Eutectic Solvents

HBA	HBD	Water Content (%)	HBA:HBD molar ratio
Choline chloride	Triethylene glycol	10	ChCl:Teg (1:2)
		20	
		30	
	Fructose	10	ChCl:Fru (1:1)
		20	
		30	
Betaine	Betaine	10	Bet:Teg (1:2)
		20	
		30	
	Fructose	10	Bet:Fru (1:1)
		20	
		30	

HBA, Hydrogen Bound Acceptor; HBD, Hydrogen Bound Donor; ChCl, Choline Chloride; Bet, Betaine; Fru, Fructose; Teg, Triethylene-glycol.

Deep Eutectic Solvents preparation

In this study, two different HBAs, Betaine (Bet) and Choline Chloride (ChCl), and two different HBDs, Triethylene glycol (Teg) and Fructose (Fru), were considered in specific molar ratios and different water content (Table 1). Before the ChCl-based DES synthesis, ChCl was dried under vacuum for 6 h at 80 °C (Manuela *et al.*, 2020).

The DES was prepared according to the heating-stirring method (Ruesgas-Ramón *et al.*, 2017): the HBD and HBA were placed in a capped round-bottomed flask and heated at T = 80 °C for 15 min under constant stirring (500 rpm). After the DES formation, no purification step was needed, and the mixtures were kept at room temperature in sealed flasks until their use for analysis.

Ultrasound-assisted solid–liquid polyphenol extraction (UAE)

For the Ultrasound-Assisted solid–liquid Extraction by Deep Eutectic Solvents, polyphenols were extracted according to the method previously described by Manuela *et al.*, 2020 with some modifications. Briefly, 0.1 g of dark chocolate sample and 10 mL of DES solvent were added to a centrifuge tube, and the extraction was conducted by placing the sample in an ultrasonic and thermostatic bath (400 Hz). After, the samples were centrifuged at 3000 rpm for 10 min at room temperature; the supernatant was collected and filled up to volume in a 10-mL flask. After that, the extracts were analysed for spectrophotometric analyses.

For the optimization of the extraction procedure, the samples were treated at different operating conditions, considering solvents, temperature–time, and % water content. Acronyms used for their descriptions were named as follows: CONV refers to Conventional extraction, and DES to green extraction; solvent used for the extraction: MeOH:H_2O, H_2O, and MeOH (_a), Bet:Teg (_b), Bet:Fru (_c), ChCl:Teg (_d), ChCl:Fru (_e); temperature–time conditions were: T 60 °C for 50 min (_1), T = 70 °C for 40 min (_2), and T = 80 °C for 30 min (_3). Percentage (%) of water content was indicated as follows: 0% (_0), 10% (_10), 20% (_20), 30% (_30), 40% (_40), 100% (_100) (% water) (Table S1).

The DES extracts were compared with three different conventional extraction methods, considering the following solvents: (i) MeOH:H_2O (60:40 *v/v*); (ii) MeOH (100%); (iii) H_2O (100%). The conventional extraction procedures were conducted as follows: 0.1 g of sample was extracted with 5 mL of conventional solvent, homogenized in an ultrasonic bath (400 MHz), and centrifuged at 2900 *g* for 10 min. The supernatant was collected in a 10 mL flask, and the extraction procedure was repeated twice. The final volume was adjusted to 10 mL with conventional solvents.

The polyphenol extraction was carried out considering the above-mentioned operating conditions for DES extraction.

Total phenolic content by Folin Ciocâlteu assay

Total phenolic content (TPC) was conducted for all the DES and conventional extracts according to Ciano *et al.*, 2022. Briefly, 0.5 mL of polyphenolic extract was mixed with 0.25 mL of Folin Ciocâlteu reagent in a 10 mL volumetric flask. After 3 min, 0.5 mL of an aqueous solution of sodium carbonate (7.5% *w/v*) was added and the flask was kept in darkness for 30 min. Then, it was filled up to volume with distilled water. The absorbance of the samples was read at λ = 750 nm in a UV–visible spectrophotometer (Jenway, Stone, UK). The results were expressed as grams of gallic acid equivalents per 100 g of dark chocolate extract (g GAE/100 g), and the results were obtained through a calibration curve ranging from 10 to 100 mg/L (R^2 = 0.9997).

Total flavonoid content by aluminum chloride assay

In all the DES and conventional extracts Total Flavonoid Content (TFC) was evaluated according to the Aluminium Chloride method described by Ozbek & Ozmen (2022). Briefly, 0.5 mL of the extract, 2 mL of distilled water, and 150 μL $NaNO_2$ (5%, *w/v*) were added to a volumetric flask. The solution was mixed and incubated in the dark for 5 min; then 150 μL of $AlCl_3$ (10%, *w/v*) was added and the solution was kept in the dark for 5 min. After that, 2 mL of 1 M NaOH was added to the solution and incubated for an additional 15 min, and then filled to a final volume of 5 mL. The absorbance of the extracts was recorded at 510 nm. The TFC results were expressed as grams of

Rutin equivalents per 100 g of dark chocolate extract (g Rut/100 g), by linear regression, ranging between 50 and 1000 mg/L ($R^2 = 0.9995$).

Radical scavenging activity by ABTS and DPPH assays

To assess the radical scavenging activity of the conventional and DES extracts were conducted by DPPH and ABTS assay following the methods described by Ciano *et al.*, 2022. The DPPH radical scavenging assay was conducted as follows: 1.5 mL of DPPH reagent was added to 1 mL of polyphenol extract and kept in darkness for 30 min. The absorbance was read at 517 nm in a UV–visible spectrophotometer. Indeed, the ABTS radical scavenging was determined as followed: 3.6 mL of ABTS reagent was added to 0.4 mL of polyphenol extract and kept in darkness for 15 min. The absorbance was recorded at 734 nm.

The DPPH and ABTS scavenging capacity were expressed as a percentage of inhibition (I %) as in eq.1:

$$I\% = \frac{A_0 - A_f}{A_0} \times 100. \tag{1}$$

where A_0 is the ABTS or DPPH radical cation's initial absorbance, and A_f is the absorbance after the addition of sample extract.

Statistical analysis

The conventional and DES extractions were performed in triplicate as the spectrophotometric analysis. The results were expressed as mean value ± standard deviation ($n = 3$). Pearson's correlation coefficient was used to analyze the linear correlation within the obtained results. As result, the One-Way ANOVA test was applied, followed by the Mann–Whitney parawais posthoc test was possible to identify the significant difference ($P < 0.05$) between variables. Furthermore, multivariate analysis (Principal Component Analysis; PCA, and Cluster Analysis; CA) was performed on the results obtained for the different extraction conditions. Acronyms used for PCA and CA have been previously described (Section 2.1.4.) and were shown in Table S1. The statistical analyses were carried out with XLSTAT software (Addinsoft, 2022).

Sustainability evaluation of polyphenol extraction procedures using life cycle assessment methodology

Materials

The sustainability of conventional and green extraction methods for the evaluation of phenolic content in dark chocolate was assessed by applying the Life Cycle Assessment (LCA) methodology (2015ISO 14040:2006; I2006SO 14044:2006, 2006). The sustainability assessment was performed by using the software SimaPro 9.2.2. (Prè Sustainability B.V.).
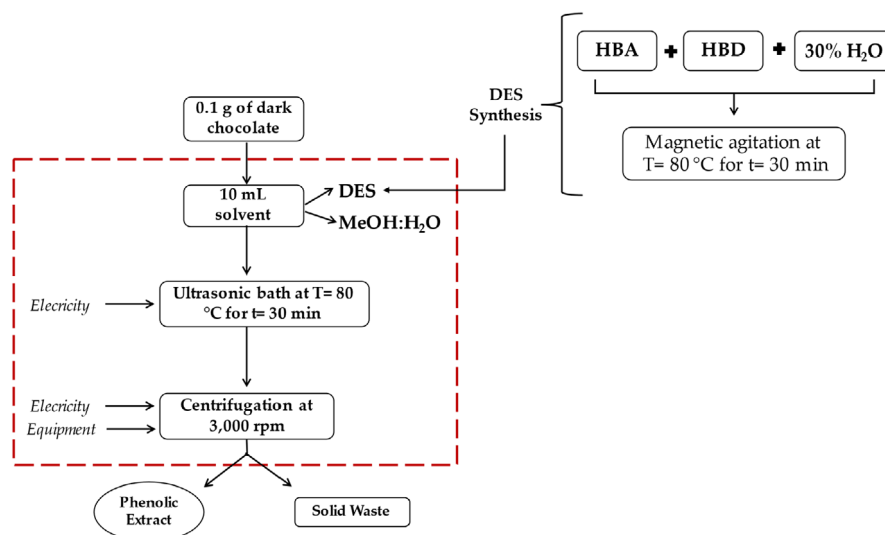
Methods

Life Cycle Assessment was considered a standard tool for environmental impact assessment, and it should involve four phases: (i) Goal and scope definition; (ii) Life Cycle Inventory (LCI); (iii) Life Cycle Impact Assessment (LCIA), and (iv) Interpretation of results.

Goal and scope definition. The aim was to assess the environmental, health, and economic implications of the different polyphenol extraction methods used. In the study, the conventional solvent (MeOH:H$_2$O, 60:40 *v/v*) was compared with 4 DES pairs (Bet:Teg, Bet:Fru, ChCl:Teg, and ChCl:Fru) at 30% of water content, to identify possible hot spots of the polyphenol extraction process, as well as assessing the potential mitigation of environmental, health and economics impacts by applying green solvents. The functional unit (FU) considered was one extraction procedure for both polyphenols' extraction methods. The procedures took into consideration a *gate-to-gate* approach (Fig. 1).

Life Cycle Inventory (LCI). The main inputs and outputs for both polyphenols' extraction methods, conventional and DES, are shown in Table 2. LCI calculations were performed using data from EcoInvent v3.8, and ELCD databases, to model inputs for equipment, solvents, and electricity. Electricity consumption varied depending on operating conditions (i.e., ultrasonic bath, centrifugation, etc.), as detailed in Table 2. In the present work, the same quantity of dark chocolate samples (0.1 g) was used for the five extraction methods; therefore, as a comparative LCA was performed, dark chocolate was not included in the system boundaries.

Life Cycle Impact Assessment (LCIA). The ReCiPe 2016 Endpoint (H) V1.05 method was used for the impact calculations, thus considering the following impact categories: Global warming, Human health (GW, HH); Stratospheric ozone depletion (SOP); Ionising radiation (IR); Ozone formation, Human health (OF, HH); Fine particulate matter formation (FPMF); Human carcinogenic toxicity (HCT); Human non-carcinogenic toxicity (HCNT); Water consumption, Human health (WC, HH); Global warming, Terrestrial ecosystems (GW, TE); Global warming, Freshwater ecosystems (GW, FE); Ozone formation, Terrestrial ecosystems (OF, TE); Terrestrial acidification (TA); Freshwater eutrophication (FE); Marine eutrophication (ME); Terrestrial ecotoxity (TE); Freshwater ecotoxicity (FET); Marine ecotoxicity (MET); Water consumption, Terrestrial ecosystem (WC, TE); Water consumption, Aquatic ecosystems (WC, AE); Land use (LU); Mineral resource scarcity (MRS); Fossil resource scarcity (FRS). These categories are

Figure 1 *Gate-to-gate* system boundaries considered for the polyphenols extraction methods in dark chocolate.

Table 2 LCI of conventional and DES extraction methods (Inputs referred to the FU: No. 1 extraction procedure)

Extraction conditions		Unit	MeOH:H$_2$O (60:40)	Bet/Fru (1:1)	Bet/Teg (1:2)	ChCl/Fru (1:1)	ChCl/Teg (1:2)
Inputs							
Solvents	H$_2$O	kg	0.0040	0.0030	0.0030	0.0030	0.0030
	MeOH	kg	0.0047	/	/	/	/
	Fru	kg	/	0.0035	/	0.0035	/
	Bet	kg	/	0.0023	0.0020	/	/
	Teg	kg	/	/	0.0049	/	0.0047
	ChCl (k)	kg	/	/	/	0.0027	0.0022
Equipment	Glass Pasteur kg pipettes		0.00873 (n. 3)	0.0029 (n. 1)	0.0029 (n. 1)	0.0029 (n. 1)	0.0029 (n. 1)
	Ultrasonic bath	kWh	0.00664	0.04	0.04	0.04	0.04
	Centrifugation	kWh	0.4995	0.04995	0.04995	0.04995	0.04995
	Homogenisation	kWh	0.0664	/	/	/	/
Total electricity		kWh	0.57254	0.08995	0.08995	0.08995	0.08995
Outputs							
Phenolic extract (mL)	10			10	10	10	10

H$_2$O, distilled water; MeOH, Methanol; Fru, Fructose; Bet, Betaine; Teg, Triethylene-glycole; ChCl, Choline Chloride.

considered endpoint indicators showing the environmental impacts on three macro-categories: Human Health (HH), Ecosystems (Es), and Resources (Rs). Results for the macro-category HH are expressed in Disability-adjusted life years (DALYs); for the macro-category Es results are expressed in species lost in a year (species.yr); for the macro-category Rs, results are in US Dollar 2013 (USD2013).

Result and discussion

Aiming to develop an eco-extraction method for dark chocolate polyphenols, different types of DES were tested as an alternative to organic solvent, commonly applied in the extraction of polyphenols from food matrices (Luo *et al.*, 2020). It is worth noting that the selection of DES varies depending on the chemical and physicochemical characteristics of the DES itself and the characteristics of the analytes to be extracted. Therefore, in this process, the highest DES extraction efficiency resulted from harmonisation of several important factors, such as HBD and HBA pairs choice, the molar ratio of DES mixture, temperature, and water content. In particular, this latter is strictly linked to viscosity, pH, polarity, and surface tension of DES mixture used (Zainal-Abidin *et al.*, 2017).

The extraction yield of the proposed solvents was evaluated by spectrophotometric assays (TPC, TFC,

Table 3 Quantitative results for total phenolic content for conventional and DES extraction methods

Solvent			60 °C * 50 min	Extraction conditions	
				70 °C * 40 min	80 °C * 30 min
	MeOH:H$_2$O		0.66 ± 0.07	0.87 ± 0.06	0.92 ± 0.18
Conventional	H$_2$O		0.85 ± 0.12	0.98 ± 0.14	0.99 ± 0.26
	MeOH		0.56 ± 0.09	0.65 ± 0.03	0.74 ± 0.18
		10%	1.06 ± 0.06	1.25 ± 0.18	0.93 ± 0.16
Bet/Teg		20%	1.16 ± 0.08	1.19 ± 0.09	1.06 ± 0.05
		30%	0.99 ± 0.13	1.21 ± 0.11	0.95 ± 0.14
		10%	0.34 ± 0.02	nd	nd
Bet/Fru		20%	2.36 ± 0.15	2.39 ± 0.21	1.08 ± 0.08
Green		30%	1.69 ± 0.11	2.59 ± 0.23	1.76 ± 0.05
		10%	nd	nd	nd
ChCl/Teg		20%	nd	nd	nd
		30%	0.06 ± 0.01	0.20 ± 0.06	0.07 ± 0.01
		10%	1.42 ± 0.17	1.09 ± 0.04	0.07 ± 0.02
ChCl/Fru		20%	0.86 ± 0.13	0.50 ± 0.10	0.15 ± 0.03
		30%	0.15 ± 0.02	2.35 ± 0.21	0.03 ± 0.01

Results are expressed as g GAE/100 g ± standard deviation ($n = 3$).
nd = not detectable.

ABTS, and DPPH assays), which revealed as simple and reliable methods for the analysis of bioactive compounds in food matrices (Yang *et al.*, 2020).

Total polyphenols content in dark chocolate

Table 3 shows the TPC (g GAE/100 g of dark chocolate) obtained for conventional and different DES extracts. In the case of total polyphenols content, the results showed that the DES pairs examined resulted in three times higher extraction efficiency than conventional solvents (MeOH, H$_2$O, and, MeOH:H$_2$O). The amount of extracted polyphenols (0.07–2.59 g/100 g of dark chocolate) differs considerably in green solvents, thus highlighting that the selection of DES pairs is crucial to obtain a higher extraction efficiency in comparison to conventional solvents (Manuela *et al.*, 2020). Among DES, the highest extraction yield was obtained for Bet/Fru at 30% hydration (1.76–2.59 g/100 g of dark chocolate), followed by ChCl/Fru at 10% (1.09–1.42 g/100 g), and Bet/Teg at 10% hydration (1.06–1.25 g/100 g dark chocolate). DES pair composed of ChCl/Teg at both 10% and 20% of water content revealed not applicable for polyphenols extraction in dark chocolate. It is important to note that the composition of the DES can seriously affect the FC assay. Indeed, when ChCl-based DES was used, the formation of a precipitate was observed, which caused interferences with the vertical spectroscopy lecture in the microplate reader. This phenomenon could be ascribed to ion exchanges between ChCl and potassium carbonate, leading to the formation of insoluble salts. Hence, the TPC quantification

of plant biomass extracts by the FC method can lead to misinterpretation (mostly overestimation), so caution must be taken, especially when working with ChCl-based DES as extracting solvent. This could be probably attributable to the fact that DES pairs formed by Choline Chloride and Triethylene glycol gave interference with the Folin-Ciocâlteu assay. Different studies reported that the HBA-HBD pairs could interfere with the binding reaction between the reagent and the extractant (Mahesar *et al.*, 2016; Zheng *et al.*, 2021). When a ChCl-based DES was used, the formation of a precipitate was observed, which caused interference with the spectrophotometric analysis. This phenomenon could be attributed to ion exchanges between ChCl and potassium carbonate, which lead to the formation of insoluble salts (Ruesgas-Ramón *et al.*, 2020).

Several extraction methods have been optimised for phenolic compounds, using DES as extraction solvent. The choice of DES type strictly depends on the phenolic class under consideration (Della Posta *et al.*, 2022). In particular, considering the study of Manuela *et al.* (2020), who analysed cocoa by-products, Betaine-based DES resulted in the case of total polyphenols, with the highest extraction efficiency (15.33–22.82 mg/g dw); as well as it also resulted in the highest extraction efficiency of total procyanidins (0.3–1.41 mg/g dw). While considering ChCl-based DES mixture, Ruesgas-Ramón *et al.* (2020) optimised a procedure for the extraction of chlorogenic acids from coffee and cocoa co-products, thus showing ChCl-based DES as the most effective for the extraction of phenolic acids (i.e., chlorogenic acid, ferulic or

caffeic acids). These results were also confirmed by Khezeli *et al.* (2016), highlighting ChCl:Ethylene glycol (1:2), as the highest extracting solvent of phenolic acids from vegetable oils.

Moreover, temperature is reported to be the parameter that has the greatest influence on extraction yield. According to the literature, as the extraction time increases, the extraction yield tends to increase (Della Posta *et al.*, 2022). Nevertheless, high temperatures can degrade the analytes of interest causing the opposite effect. This factor can be controlled by operating on the time–temperature interaction. Based on studies in the literature, working at 60 °C for 1 h for polyphenols extraction from cocoa products (Ruesgas-Ramón *et al.*, 2017; Manuela *et al.*, 2020), we observed a particular extraction efficiency, increasing the temperature to 70 °C and reducing the extraction time to 40 min. This is probably because high temperatures affect the viscosity and surface tension of DES by enhancing the interaction between the green solvent and the target molecules (Chu *et al.*, 2022).

It was also found that the polyphenols extraction yield from 70% dark chocolate varied according to water content, which might affect the viscosity of the DES mixture and its polarity, thus playing a major role in the extraction of these molecules from food matrices (Ruesgas-Ramón *et al.*, 2017). The moisture content and the rheology of the solvents are considered, in fact, crucial factors in determining their extraction efficiency. In terms of physical–chemical properties, it is desirable that the solvent does not present high viscosity, since the lower the viscosity, the greater its diffusivity and therefore the stronger the efficiency of solubilization and extraction of the analyte of interest in the matrix (Cunha & Fernandes, 2018). There are several studies investigating the relationship between solvent rheology and extraction efficiency. Santana *et al.* (2019) characterized, by thermogravimetric analysis (TGA), infrared spectroscopy, and rheometry measurements, natural Deep Eutectic Solvents (NADES) prepared according to three different methods: controlled heating and stirring, ultrasound-assisted synthesis (UAS), and microwave-assisted synthesis (MAS). The results indicated that the solvent synthesised by ultrasound-assisted technique provided a lower viscosity value than other samples. Then, UAS was recognizedas fast and efficient synthesis technique, showing promise as new methods for the synthesis of NADES. Dai *et al.* (2015) investigated the effect of water content on the structure and characteristics of NADES. The authors found that the physicochemical properties can be tailored in a controllable way when diluted with water. The addition of water weakened the hydrogen bonding interactions between the solvent's components, lowering the viscosity and increasing the extraction ability. The "best"

results were detected for 5% (v/v) of water dilution where NADES reached the highest solubilization capacity. Besides, the water content represents also the main factor for the best selectivity of extracting compounds (Ruesgas-Ramón *et al.*, 2017). According to Zainal-Abidin *et al.* (2017), DES containing high water content (about 25%) have a significant tendency to extract highly-polar compounds; on the contrary, DES with low percentage of water (<10%) showed high selectivity for less-polar compounds. Other researchers ascertained that the application of external stresses, including heating (Savi *et al.*, 2019) or physical forces (stirring or microwave radiations) (Dai *et al.*, 2015) improved the extraction efficiency. Specifically, higher temperature increases the entropy of the solvent system (increasing molecular motion) and consequently reduces the viscosity because of the weakening of the intermolecular bonds in the solvent. The above-reviewed literature survey investigating the effect of the physicochemical properties of solvents concerning their extraction efficiency will be considered as a basis on which to build future experimentation on our solvents. In this regard, thermal (e.g., TGA) and rheological analysis will be performed to study how synthesis parameters (e.g., water content) as well as process variables can maximize the extraction capacity of our solvents.

Total flavonoids content

To assess the different interactions of DES solvents with phenolic compounds, the total flavonoids (TFC) assay was performed using the Aluminum Chloride method and subsequent detection at $\lambda = 510$ nm. Table 4 shows the results of the TFC for the different DES pairs and conventional solvents under different extraction conditions.

The study showed that the DES examined were able to extract flavonoids from the 70% dark chocolate sample in different yields, thus highlighting a different interaction of DES pairs with molecules belonging to the flavonoid class (Zheng *et al.*, 2021). It was found that the HBA-HBD pair composed of Betaine and Triethylene glycol at 10% and 20% hydration, respectively had the best extraction yield of flavonoids for all three extraction conditions, which was about 3.5 times higher than the conventional extraction with MeOH: H2O at 60:40 *v/v*. Furthermore, it was seen that the extraction yield differs based on the hydration content of the DES, this being likely because the water content influences the viscosity and hydrogen bond formation between HBA and HBD by interacting differently with the target molecules (Panzella *et al.*, 2020). Concerning the three extraction conditions adopted in the study (60 °C × 50 min; 70 °C × 40 min and 80 °C × 30 min), they resulted in different extraction

Table 4 Total flavonoid content for conventional and DES extraction methods

| | Solvent | Water content (%) | Extraction conditions | | |
			60 °C ˟ 50 min	70 °C ˟ 40 min	80 °C ˟ 30 min
Conventional	MeOH:H$_2$O		3.73 ± 0.43	4.07 ± 0.22	4.35 ± 0.26
	H$_2$O		5.83 ± 0.62	6.08 ± 0.62	6.29 ± 0.44
	MeOH		2.47 ± 0.35	3.05 ± 0.41	3.57 ± 0.12
Green	Bet/Teg	10%	12.83 ± 1.09	12.40 ± 1.21	12.05 ± 0.98
		20%	13.12 ± 1.25	13.64 ± 1.23	10.43 ± 1.14
		30%	7.73 ± 0.45	6.82 ± 0.97	5.43 ± 0.34
	Bet/Fru	10%	2.63 ± 0.32	4.63 ± 0.65	1.13 ± 0.22
		20%	8.35 ± 0.74	5.79 ± 0.52	13.18 ± 1.11
		30%	4.14 ± 0.23	6.88 ± 0.67	5.26 ± 0.36
	ChCl/Teg	10%	4.88 ± 0.39	10.03 ± 1.03	4.92 ± 0.13
		20%	6.18 ± 0.84	4.14 ± 0.56	8.92 ± 0.47
		30%	7.87 ± 0.93	6.37 ± 0.63	7.57 ± 0.68
	ChCl/Fru	10%	1.90 ± 0.23	4.70 ± 0.21	2.35 ± 0.17
		20%	9.50 ± 1.05	5.05 ± 0.33	4.25 ± 0.21
		30%	3.17 ± 0.37	4.55 ± 0.25	5.81 ± 0.13

Results are expressed as g Rut/100 g ± standard deviation ($n = 3$).

H$_2$O, distilled water; MeOH, Methanol; Fru, Fructose; Bet, Betaine; Teg, Triethylene-glycole; ChCl, Choline Chloride.

yields among the DES pairs considered, and the increase in flavonoid extraction yield from dark chocolate to 70% did not follow the increase in extraction temperature or extraction time. The highest yield was obtained for extraction at 80 °C × 30 min with the DES consisting of Betaine and Triethylene glycol hydrated at 20% (13.64 ± 1.34 g RUT/100 g).

This result highlighted the importance of selecting the right HBA-HBD pair to extract the target compounds from food matrices; in fact, the literature showed that using Triethylene glycol as the HBD and varying the HBA between Betaine and Choline Chloride resulted in a different interaction between polar and non-polar phenolic compounds, affecting the extractive yield of TFC (Ruesgas-Ramón et al., 2017). The composition of the DES greatly influences its polarity and thus its interaction with the target analytes. As was also found in the case of phenolic compounds, polar DES (i.e., DES based on organic acids) extract polar analytes better, while DES with medium or low polarity (i.e., DES based on polyalcohols) extract less polar analytes more efficiently (Della Posta et al., 2022).

Different studies in literature highlighted the great efficiency of DES composed of organic acids as HBD (i.e., triethylene glycol) for flavonoids extraction from food matrices (Zhao et al., 2015; Mansur et al., 2019). Zhao et al. (2015) optimised a green extraction of Rutin from Sophora japonica flower buds, and based on 20 different DES, consisting of organic acids, sugars, polyalcohols and amines such as HBD, good results were obtained in terms of extraction efficiency using ChCl-triethylene glycol-based DES as extraction

solvent. The worst results were obtained with sugar-based DES, probably due to their high viscosity. Mansur et al. (2019) selected ChCl-triethylene glycol-based DES as the most promising solvent for the extraction of several flavonoid classes in common buckwheat sprouts, thus revealing DES composed of organic acids such as HBD, were found to be unsuitable for flavonoid extraction. This could be explained by the low polarity of the target analytes, which are more to DES based on polyalcohols than those based on organic acids.

Radical scavenging activity by ABTS and DPPH assays

In addition to the determination of the total polyphenol and flavonoid content, the extraction capacity of the different DES on antioxidant compounds was studied by means of ABTS and DPPH assays.

For the ABTS assay, the results showed a different extraction yield of antioxidant compounds between the different DES (Table 5). Particularly, the highest anti-radical scavenging was determined in the 70% dark chocolate extracts obtained with the ChCl:Teg (91.33%–97.40% ABTS radical inhibition %, respectively). For this DES, no remarkable inhibition percentage changed between the different hydration rates of DES or the three conditions under which the extraction was conducted were revealed. Furthermore, the Bet/Teg pair also showed good anti-radical capacities, between 90.42% and 94.66% inhibition of the ABTS radical, for the 30% hydration, showing similar extraction capacities with the conventional solvent MeOH:H2O. The data also show that the formation

Table 5 ABTS assay result as inhibition percentage (I%) of ABTS radical $\pm$ standard deviation ($n = 3$)

Solvents	MeOH:H$_2$O		Extraction Conditions		
			60 °C * 50 min 99.76 $\pm$ 0.12	70 °C * 40 min 98.76 $\pm$ 0.41	80 °C * 30 min 98.54 $\pm$ 0.36
Conventional	H2O		81.16 $\pm$ 0.34	83.16 $\pm$ 0.33	86.16 $\pm$ 0.14
	MeOH		40.77 $\pm$ 0.34	43.77 $\pm$ 0.11	45.77 $\pm$ 0.09
		10%	70.80 $\pm$ 0.31	76.13 $\pm$ 0.2 7	76.56 $\pm$ 0.28
	Bet/Teg	20%	75.45 $\pm$ 0.46	75.55 $\pm$ 0.39	76.21 $\pm$ 0.11
		30%	90.42 $\pm$ 0.32	94.66 $\pm$ 0.33	94.25 $\pm$ 0.51
		10%	3.48 $\pm$ 0.31	nd	nd
	Bet/Fru	20%	58.95 $\pm$ 0.21	64.08 $\pm$ 0.12	65.54 $\pm$ 0.36
Green		30%	79.75 $\pm$ 0.33	85.52 $\pm$ 0.24	65.96 $\pm$ 0.33
		10%	96.49 $\pm$ 0.32	95.79 $\pm$ 0.14	95.20 $\pm$ 0.25
	ChCl/Teg	20%	93.98 $\pm$ 0.41	92.17 $\pm$ 0.033	91.33 $\pm$ 0.36
		30%	97.40 $\pm$ 0.38	95.79 $\pm$ 0.21	96.28 $\pm$ 0.23
		10%	nd	10.05 $\pm$ 0.09	63.23 $\pm$ 0.11
	ChCl/Fru	20%	17.64 $\pm$ 0.12	54.14 $\pm$ 0.36	84.23 $\pm$ 0.07
		30%	75.68 $\pm$ 0.23	83.96 $\pm$ 0.07	86.36 $\pm$ 0.40

nd, not detectable.

of Deep Eutectic Solvents affects the extraction capabilities of antioxidant compounds (Hassani et al., 2019; Trombino et al., 2022).

As regards the scavenging activity of the extracts by DPPH radical assay, differences were found between extractions with conventional solvents and DES (Table 6). However, extracts obtained with DES showed a lower anti-radical capacity than conventional solvents MeOH:H$_2$O, and MeOH. Among the DES, ChCl/Fru at 10% (67.78%) and ChCl/Fru at 30%

(61.74%) ChCl/Teg at 30% (51.00%), and Bet/Teg at 30% (42.00%) showed a higher percentage of DPPH radical inhibition. The extraction condition, which led to a better result in the anti-radical capacity for DES extracts, is 60 °C for 50 min.

In addition, for some DES pairs at different hydration rates, it was not possible to determine the antioxidant capacity because the components of the DES pair (HBA-HBD) may in turn interact with the assay reagent leading to the formation of the precipitate that

Table 6 DPPH assay result in inhibition percentage (I%) of DPPH radical $\pm$ standard deviation ($n = 3$)

Solvents			Extraction Conditions		
			60 °C * 50 min	70 °C * 40 min	80 °C * 30 min
	MeOH:H2O		73.99 $\pm$ 0.18	83.99 $\pm$ 0.34	93.99 $\pm$ 0.58
Conventional	H$_2$O		11.53 $\pm$ 0.07	15.53 $\pm$ 0.08	31.53 $\pm$ 0.27
	MeOH		67.13 $\pm$ 0.23	77.13 $\pm$ 0.53	87.13 $\pm$ 0.38
		10%	nd	nd	nd
	Bet/Teg	20%	nd	nd	nd
		30%	42.02 $\pm$ 0.16	37.80 $\pm$ 0.09	35.40 $\pm$ 0.08
		10%	nd	nd	nd
	Bet/Fru	20%	nd	nd	nd
Green		30%	nd	nd	nd
		10%	21.40 $\pm$ 0.08	21.00 $\pm$ 0.08	23.01 $\pm$ 0.07
	ChCl/Teg	20%	25.50 $\pm$ 0.05	31.80 $\pm$ 0.22	26.30 $\pm$ 0.14
		30%	51.00 $\pm$ 0.12	24.10 $\pm$ 0.22	19.40 $\pm$ 0.09
		10%	67.78 $\pm$ 0.35	60.72 $\pm$ 0.24	46.16 $\pm$ 0.11
	ChCl/Fru	20%	nd	nd	nd
		30%	61.74 $\pm$ 0.38	45.81 $\pm$ 0.12	nd

n.d., not detectable.

alters the data reading (Chen *et al.*, 2021; Zheng *et al.*, 2021). Indeed, the different results between the ABTS and DPPH assays are partly due to analytes to which the reagents bind, respectively ABTS with hydrophilic and lipophilic antioxidants while DPPH with only lipophilic. Furthermore, the DPPH radical does not react with phenolic acids and therefore the anti-radical capacity between ABTS and DPPH is partially different (Godočiková *et al.*, 2020). In addition, the differences in anti-radical capacity in the two assays could be attributed not only to the extraction capacity of the target molecules by DES, but also to the interference between the reagents and other molecules present in the chocolate samples that are co-extracted from the test matrix, such as methylxanthines and pigments (Pierre *et al.*, 2015).

Statistical analysis

The Pearson correlation coefficient (p) between the TPC, TFC, ABTS, and DPPH assays methods was calculated from the results obtained for the three-extraction condition considered. Table S2 showed the results of the Pearson correlation for all the extraction conditions. Considering the three different extraction conditions, the results of TPC, TFC, ABTS, and DPPH were positively correlated ($P < 0.05$). Specifically, for the extraction at T = 60 °C ˣ 50 min, TPC was closely correlated to the ABTS ($r = 0.902$, $P < 0.05$) and DPPH ($r = 0.856$, $P < 0.05$) assays, as well as for the extraction at T = 70 °C ˣ 40 min. In contrast, TFC correlated strongly with ABTS assay ($r = 0.991$, $P < 0.05$) for extraction conditions at T = 80 °C ˣ 30 min. Furthermore, the results were subjected to the ANOVA One-way test, which showed that there were significant differences between the data averages for all the extraction conditions T = 60 °C ˣ 50 min, ($P < 0.001$); T = 70 °C ˣ 40 min ($P < 0.001$), and T = 80 °C ˣ 30 min ($P < 0.001$). Therefore, the Mann–Whitney parawais *post hoc* test was subsequently performed to assess the significance of the different variables. The significant differences ($P < 0.05$) between the several variables are shown in Table S3. For the extraction at T = 60 °C ˣ 50 min, the significant difference results for DPPH-ABTS ($P = 0.0298$, $P < 0.05$), TFC-ABTS ($P = 0.0051$, $P < 0.05$) and TFC-TPC ($P = 0.0098$, $P < 0.05$). Instead, for extraction at all differences between variables were found to be statistically significant ($P < 0.05$). Instead, for extraction at T = 80 °C ˣ 30 min, the statistically significant variables ($P < 0.05$) are DPPH-ABTS ($P = 0.0154$), TFC-ABTS ($P = 0.0051$), TFC-DPPH ($P = 0.0001$) and TFC-TPC ($P = 0.0157$). To better understand the results obtained and to investigate the correlation between the spectrophotometric assays and the

extraction under different operating conditions of the bioactive compounds with conventional extraction procedures and DES, PCA was applied. The analysis was performed on the matrix of observations for all extractions using Pearson's correlation. Furthermore, as some extractions present missing data, these were estimated by the software algorithm based on the arithmetic mean. Figure 2 shows the results of the PCA.

Comparing both graphically and *via* Kaiser's rule (*eigenvalue* > 1) the principal components resulting from the analysis, the cumulative variability obtained for the following model is 76.4%, which is explained by the first three components PC1, PC2, and PC3. The results showed that the samples were evenly distributed along the principal components. Despite this, three groupings can be identified. However, the grouping of variables in the third quadrant is negatively correlated with both PC1 and PC2. In the first quadrant, grouping is positively correlated with both PC1 and PC2. Finally, the grouping in the fourth quadrant is positively correlated with PC1 and negatively correlated with PC2.

Subsequently, a clustering analysis (CA) was performed on the results obtained from the PCA, based on the agglomeration method (weighted pair-group average) while the proximity type is based on similarity using Pearson's correlation coefficient. The results of the CA are shown in Fig. 3. The CA confirmed the PCA data by showing three different clusters. The dendrogram was divided based on the similarity principle, around values of 0.7118. Within Cluster 1 (C1), the element of similarity is represented by lower extraction temperature (60 °C) for a long time (50 min). In contrast, Cluster 3 (C3) is grouped according to high extraction temperature (80 °C) for short time (30 min). Within Cluster 2 (C2), dissimilar statistical units result, and therefore the variable points cluster to the closest cluster for low similarity value.

Sustainability analysis

In recent years, the application of DES solvents has been widely studied for the extraction of bioactive compounds from food matrices as they are regarded as "green" solvents with numerous advantages in their application (Ang *et al.*, 2021). These compounds are classified as green solvents because they are non-toxic and biodegradable, but to date, there has been no comprehensive analysis of their real impact (Vauchel *et al.*, 2018). Table 7 reported the impact results for all the eighteen damage impact categories studied for conventional and green polyphenols extraction.

For the LCIA, the ReCiPe 2016 Endpoint, with the 100-year perspective (H) V1.05 method was used for the impact calculations. The results showed that

Figure 2 Biplot of Principal Component Analysis: Group in quadrant 1 (blue trace); Group in quadrant 3 (red trace); Group in quadrant 4 (green trace).

Figure 3 Dendrogram of Cluster analysis: C1 (red trace); C2 (blue trace); C3 (green trace).

conventional extraction (MeOH:H2O) had the highest impact for all eighteen categories considered, except for "Marine Eutrophication" category where the extraction method with the highest impact is attributed to the HBA-HBD composed by Bet/Fru. This is probably since both Betaine and Fructose are compounds of plant origin and therefore their production involves all those inputs typical of crops (such as fertilisers, insecticides, herbicides, etc.) that affect marine eutrophication, due to the accumulation in the soil and subsequent release into the sea from rivers and coastal settlements of nitrogen compounds,

phosphorus and other phytostimulants (Kralisch *et al.*, 2015; Murugan *et al.*, 2021).

These results showed that DES extraction has a lower damage impact than conventional solvent, however, to better understand the different impacts on the "Human Health", "Ecosystem" and "Resources", the eighteen damage impact categories were grouped into three macro-categories to identify DES with a lower impact (Fig. 4). The results showed that the green extraction methods have a lower impact, ranging between 65% and 80%, than conventional extraction, however, among DES the HBA-HBD pair composed

Table 7 Impact of DES and conventional extraction for all the 18 damage impact categories

Damages impact category	Unit	MeOH:H^{2}O	Bet/Fru	Bet/Teg	ChCl/Fru	ChCl/Teg
Human health						
Global warming, Human health		2.34 E-07	7.25 E-08	6.13 E-08	5.17 E-08	5.14 E-08
Water consumption, Human health		5.8 E-09	2.32 E-09	1.81 E-09	1.33 E-09	1.29 E-09
Human carcinogenic toxicity		1.28 E-08	5.3 E-09	4.37 E-09	3.51 E-09	3.53 E-09
Human non-carcinogenic toxicity	DALY	2.11 E-08	6.74 E-09	6.31 E-09	4.08 E-09	5.01 E-09
Ionising radiation		2.24 E-10	5.18 E-11	5.13 E-11	4 E-11	4.47 E-11
Ozone formation, Human health		4.34 E-10	1.59 E-10	1.3 E-10	1.08 E-10	1.05 E-10
Fine particulate matter formation		1.68 E-07	6.68 E-08	5.1 E-08	4.21 E-08	3.85 E-08
Ecosystem						
Ozone formation, Terrestrial ecosystems		6.28 E-11	2.34 E-11	1.91 E-11	1.58 E-11	1.54 E-11
Terrestrial acidification		1.66 E-10	5.82 E-11	4.42 E-11	4.19 E-11	3.63 E-11
Freshwater eutrophication		3.63 E-11	1.44 E-11	1.16 E-11	9.33 E-12	9.23 E-12
Marine eutrophication		6.87 E-15	1.48 E-14	5.04 E-15	1.2 E-14	4.29 E-15
Terrestrial ecotoxicity		1.57 E-12	8.37 E-13	5.69 E-13	4.83 E-13	3.85 E-13
Freshwater ecotoxicity	species.yr	1.36 E-12	5.43 E-13	4.36 E-13	3.73 E-13	3.55 E-13
Marine ecotoxicity		2.88 E-13	1.13 E-13	9.21 E-14	7.63 E-14	7.47 E-14
Global warming, Terrestrial ecosystems		7.06 E-10	2.19 E-10	1.85 E-10	1.56 E-10	1.55 E-10
Global warming, Freshwater ecosystems		1.93 E-14	5.98 E-15	5.05 E-15	4.26 E-15	4.24 E-15
Land use		3.74 E-11	2.95 E-11	8.19 E-12	2.74 E-11	7.12 E-12
Water consumption, Terrestrial ecosystem		1.51 E-11	1.12 E-11	7.89 E-12	5.04 E-12	4.69 E-12
Water consumption, Aquatic ecosystems		9.78 E-16	7.47 E-16	5.02 E-16	3.47 E-16	2.95 E-16
Resources						
Mineral resource scarcity	USD2013	1.69 E-05	8.03 E-06	6.13 E-06	6.74 E-06	5.78 E-06
Fossil resource scarcity		2.51 E-02	8.95 E-03	8.00 E-03	5.79 E-03	6.57 E-03
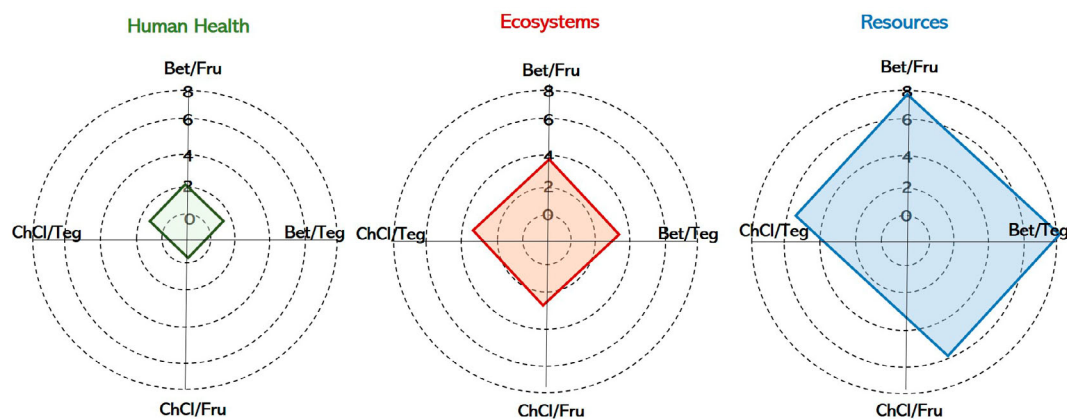
Figure 4 Macro-categories results for all the DES extraction methods.

of Bet/Fru is the most impactful for all the three macro-categories. For the "Human Health" macro-categories, Bet/Fru has about 35% higher impact than the DES composed of ChCl as HBA and 19% higher than Bet/Teg. Indeed, the lower impact for the "Ecosystem" macro-categories was obtained with ChCl/Teg, which has 36% lower impact than Bet/Fru. For the macro-categories "Resources" a lower impact than Bet/Fru was obtained for the DES composed of ChCl/Fru (−35%). This is probably due to the

process's synthesis of the molecules of which DES is composed (Kralisch et al., 2015). These could be attributed to the different origins of these molecules; furthermore, chemical LCA studies have some limitations attributable to the absence of some chemical inputs (Parvatker & Eckelman, 2018). In fact, to consider all HBA-HBD pairs in the LCA study, the inputs, following the reaction stoichiometry, for the synthesis of Chlorine Chloride and Betaine were reconstructed, as they are absent within the software

databases. However, the creation of these new chemical 'processes' may result in estimated missing data in the life cycle inventory phase, leading to greater variability in the output data (Kralisch *et al.*, 2015; Parvatker & Eckelman, 2018).

Conclusions

In this work, the application of DES revealed as a sustainable and greener alternative for the extraction of bioactive compounds from dark chocolate samples. The study highlighted DES dual advantages in terms of both extraction yield and environmental assessment. Based on the results obtained, both DES pairs consisting of betaine and choline chloride as HBA exhibited, on average, a 35% higher extraction yield than conventional solvents. The impact of different operating conditions in terms of time, temperature, and water content significantly influenced the extraction performance of DESs, as multivariate analyses confirmed. Herein, the sustainability assessment of solvents for polyphenols eco-extraction from chocolate was carried out for the first time. The comparative-LCA study highlighted the conventional solvent as 60% quantitatively more impactful than DES on the eighteen damage impact categories considered, especially in terms of mineral and fossil resources availability. Furthermore, results revealed DES pairs of natural origin (ChCl: Fru, and Bet:Fru) as the best extractive solvents both in terms of extraction efficiency and environmental performance. Due to their plant-based synthetic nature, these green compounds could be directly used in the formulation of foods, and additives as well as in cosmetics and pharmaceutical preparations. Therefore, this work presents a valuable step towards the application of simple and reliable eco-extraction methods for bioactive compound determination in food matrices, thus promoting their direct adoption in the agro-industrial application and ready-to-use extracts for functional food.

Future experiments will be aimed at optimising the efficiency of the synthesised solvents, analytically studying the influence of viscosity (and therefore water content as well as process parameters) on their extraction performance.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Giuliana Vinci: Conceptualization (lead); data curation (lead); supervision (lead); validation (lead); writing – original draft (lead); writing – review and editing (lead). **Lucia Maddaloni:** Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); software (equal); writing – original draft (equal). **Sabrina Antonia Prencipe:** Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); software (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Eleonora Orlandini:** Formal analysis (equal); investigation (equal); methodology (equal). **Matteo Sambucci:** Conceptualization (equal); data curation (equal); writing – review and editing (equal).

Ethical approval

Ethical approval was not applicable to the present study.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

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

Table S1 Acronyms used for Principal Component and Cluster Analyses.

Table S2. Person correlation ($P < 0.05$) for all the extraction conditions.

Table S3. Significance difference ($P < 0.05$) between variables of all the extraction condition.