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Effect of choline chloride-based deep eutectic solvents on polyphenols extraction from cocoa (*Theobroma cacao* L.) bean shells and antioxidant activity of extracts

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ABSTRACT

The effective extraction of natural compounds from cocoa bean shells using deep eutectic solvents could contribute to the sustainable valorization of this waste material. The objective of this study was to: (1) analyze the extraction kinetics of polyphenols released from cocoa (*Theobroma cacao* L.) bean shells (CBS) by the solid-liquid extraction method using choline chloride-based deep eutectic solvents (ChCl-DES) and their aqueous solutions; (2) investigate the effect of choline chloride-based deep eutectic solvents (ChCl-DES) aqueous solutions on *in-vitro* antioxidant capacity and the main individual compounds of the extracts. ChCl-DES were prepared with lactic acid, glycerol, and ethylene glycol in a 1:2 ratio. Aqueous solutions (30%, 40%, and 50% water) to obtain solvents with different physicochemical properties were performed. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method. The solution of Fick's law model for plate geometry particles was applied to fit the experimental data and calculate the effective diffusivity coefficient (D_e). The antioxidant capacity of the extracts was analyzed by a combination of 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) free radical scavenging capacity and ferric-reducing antioxidant power (FRAP) assays. The main bioactive compounds were quantified by high-performance liquid chromatography. The results showed that the type of hydrogen bond donor influences the total phenolic content, antioxidant activity and the main individual compounds in the extracts. Moreover, the washing/diffusion mechanism adequately depicts the extraction kinetics data for total phenolic content. However, the influence of an additional mechanism that enhanced the extraction capacity of deep eutectic solvents compared with organic solvent was confirmed.

1. Introduction

The cocoa (*Theobroma cacao* L.) bean shell (CBS) as a by-product of the cocoa agro-industrial process is a natural and sustainable source of compounds with biological activity (Martínez et al., 2012; Mazzutti et al., 2018). These compounds mostly include methylxanthines such as theobromine and caffeine and flavanols belonging to the catechin and epicatechin group (Barbosa-Pereira et al., 2018; Mellinas et al., 2020; Okiyama et al., 2017). Theobromine and caffeine have been associated with physiological processes that occur in different systems of the human body, that is, the central nervous, renal, gastrointestinal, and respiratory systems (Carrillo et al., 2014; Li et al., 2012). Among the proven benefits for human health of polyphenols in CBS are their antioxidant (Lecumberri et al., 2006), anti-inflammatory (Cádiz-Gurrea et al., 2017; Rebollo-Hernanz et al., 2019; Rossin et al., 2019), antimicrobial (Nsor-Atindana et al., 2012), anti-cariogenic (Kim et al., 2004), antidiabetic (Rojo-Poveda et al., 2019), anticarcinogenic (Lee et al., 2005), and neuroprotective effects (Arlorio et al., 2005).

Methods for extracting bioactive compounds using emerging solvents have recently become available as an alternative to conventional processes based on organic solvents (Gullón et al., 2020). Deep eutectic solvents (DES) are a new generation of solvents that show excellent properties such as biodegradability, low cost of starting components, easy synthesis, acceptable toxicity for pharmaceutical purposes, low

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volatility and non-flammability. Those qualities make them suitable for industrial-scale applications (Chemat et al., 2019). DES have been defined as "a mixture of pure compounds for which the eutectic point temperature is below that of an ideal liquid mixture" (Martins et al., 2019). The high viscosity of the DES is a disadvantage that acts as a limiting factor during the extraction process. A strategy to decrease viscosity is to dilute the mixture with water (Gullón et al., 2020). Most studies that evaluate the effect of water on the extraction capacity of the DES have added between 10% and 30% water (Rente et al., 2021). However, Pavlović et al. (2020) increased water content by up to 50%, which promoted the extraction of the phenolic compounds from CBS.

Different approaches have been studied to evaluate the efficiency of DES in extracting bioactive compounds (Cvjetko Bubalo et al., 2016; El Kantar et al., 2019; Wei et al., 2015; Wu et al., 2021). To the best of the authors' knowledge, this is the first report analyzing the mass transfer and the effective diffusivity coefficient (D_e) when extracting total phenolic content (TPC) from CBS. These parameters are important when the most effective processing conditions are required for the design of a process. Moreover, the study of those parameters could address a better understanding of the extraction behavior of DES. The model based on Fick's second law has been the most widely applied to represent kinetic data in which the principal extraction mechanisms are washing and diffusion (Perez et al., 2011). Therefore, this study aims to: (1) analyze the extraction kinetics of polyphenols released from cocoa (*Theobroma cacao* L.) bean shells (CBS) by the solid-liquid extraction method using choline chloride-based deep eutectic solvents (ChCl-DES) and their aqueous solutions; (2) investigate the effect of choline chloride-based deep eutectic solvents (ChCl-DES) aqueous solutions on *in-vitro* antioxidant capacity and the main individual compounds of the extracts. ChCl-DES were prepared with lactic acid, glycerol, and ethylene glycol in a 1:2 ratio.

2. Materials and methods

2.1. Reagents

Choline chloride (98%), lactic acid (85%), catechin hydrate (≥98%), epicatechin (≥98%), caffeine (≥98%), theobromine (≥98%), 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium acetate (≥99%), glacial acetic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and iron(III) chloride hexahydrate, ethylene glycol, absolute alcohol (≥99.5%), and gallic acid (99.9%) were purchased Merck KGaA (Darmstadt, Germany). Glycerol (99.5%), anhydrous sodium carbonate (99.5%), and methanol (99.8%) were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India), and Folin-Ciocalteu reagent from Winkler (Santiago, Chile).

2.2. Characterization of raw material

The raw material used in this study was the residue from processing fermented, dried, and roasted cocoa beans from Peru. This residue is commonly known as cocoa bean shell (CBS). It is well known that the composition of CBS significantly varies according to the origin of the cocoa and the cocoa bean processing conditions. Methodologies proposed by the Association of Official Analytical Chemists (AOAC) were used to determine moisture, ash, fat, and protein contents (N × 6.25) (AOAC, 2005). The soluble and insoluble fiber fractions were determined (AOAC, 2012). The total carbohydrate content was calculated as the difference between the total mass and the sum of the masses of the previously determined components (water, ash, fiber, fat and protein). All the analyses were performed in triplicate and expressed as milligrams per gram of dry matter (mg/g DM). The proximate analysis of the cocoa bean shell is shown in Supplementary Material 1.

Before extraction, the CBS was finely ground in a hammer grinder. Images were taken of the particles (Supplementary Material 2) with a scanning electron microscope (SU3500, Hitachi, Krefeld, Germany). Then, particle length was calculated by averaging the length of 150 particles measured with the Matlab R2021a Image Processing Toolbox (Mathworks Inc., Natick, MA, USA).

2.3. Preparation of deep eutectic solvents

The choline chloride-based deep eutectic solvents (ChCl-DES) were prepared with choline chloride as a hydrogen bond acceptor (HBA) combined in a binary mixture with different hydrogen bond donor (HBD) substances such as lactic acid, glycerol, and ethylene glycol referred to as ChLa, ChGly, and ChEt, respectively. The components of each compound were mixed at a molar ratio of 1:2. The mixture was heated at 80 °C with constant agitation at 500 rpm to obtain a homogeneous liquid. All solvents were diluted with distilled water to standardize water content as shown in Table 1. Distilled water was used with electrical conductivity of 0.04 ± 0.01 mS/cm, and pH = 5.8 at 25 °C.

2.4. Determination of physical properties of deep eutectic solvents

2.4.1. Dynamic viscosity

The rheological analysis was performed with a rheometer (Physica MCR 300, Anton Paar, Filderstadt, Germany) with parallel plate geometry (25 mm diameter) and a 1 mm space was maintained between plates at a constant temperature (25 °C). Samples were subjected to deformation speeds between 0.1 and 100 s^{-1} at 25 °C. The reported value was the viscosity mean of seven analyses.

2.4.2. Density and pH

The pH was measured with a pH meter (Orion Star A214, Thermo Fisher Scientific, Waltham, MA, USA). Density was determined with an automatic density meter (DDM 2911 plus, Rudolph Research Analytical, Hackettstown, NJ, USA).

2.4.3. Fourier-transform infrared spectroscopy

The Fourier-transform infrared spectroscopy (FTIR) spectrum of the samples was determined with an infrared spectrophotometer (IR-Prestige 21, Shimadzu Corporation Pte. Ltd., Kyoto, Japan) with a 2 cm^{-1} resolution and 128 scans per sample in the 4000 to 650 cm^{-1} range.

2.5. Extraction procedure

Samples of 0.5 g CBS were weighed in 50 mL Falcon centrifuge tubes and 10 mL ChCl-DES was added, which was prepared according to

Table 1
Nomenclature and composition of choline chloride-based deep eutectic solvents (ChCl-DES) and their aqueous solutions.

Nomenclature ChCl-DES	Hydrogen bond acceptor (HBA)	Hydrogen bond donor (HBD)	Molar ratio HBA:HBD	Water content (%)
ChLa	Choline chloride	Lactic acid	1:2	15.08*
ChLa30				30
ChLa40				40
ChLa50				50
ChGly	Choline chloride	Glycerol	1:2	1.63*
ChGly30				30
ChGly40				40
ChGly50				50
ChEt	Choline chloride	Ethylene glycol	1:2	1.41*
ChEt30				30
ChEt40				40
ChEt50				50

Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). 30, 40 and 50 means water percentage in the solvent. *Initial moisture content calculated by moisture content provided by each reagent.

section 2.2. The sample suspended in the solvent was immersed in a temperature-controlled water bath at 30 °C and magnetically stirred for 0–140 min time intervals. The extract was centrifuged for 15 min at 4000 rpm, filtered, and stored at −80 °C until further analyses. A reference sample was treated in the same way using ethanol/water at a 70:30 ratio (v/v). Each sample was analyzed in triplicate.

The yield of TPC at equilibrium (M_∞) was experimentally determined for each ChCl-DES by applying the previously described procedure for 240 min. This was a conservative value to prevent the degradation of the bioactive compounds. The residue from the extraction with each solvent at the 140 min retention time was washed twice with 20 mL of distilled water, oven-dried at 30 °C for 24 h, and stored in plastic bags at room temperature until further use.

2.6. Phenols extraction kinetics

Phenols extraction kinetics was simulated by Eq. (1), which represents the solution to Fick's law second equation. This equation mathematically describes solute diffusion in planar particles in a non-steady state. The equation can be applied and simplified under some basic assumptions (Chan et al., 2014).

$$\frac{M_t}{M_\infty} = 1 + \frac{8}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\frac{(2n+1)^2\pi^2 Dt}{4L^2}\right] \quad (1)$$

where M_t and M_∞ are the yields of total phenolic compounds (TPC) at any time t (seconds) and at equilibrium, respectively, expressed as mg/g D.M. For long extraction times, Eq. (1) reduces to Eq. (2) where the model coefficients are A and B. The effective diffusion coefficient (D_e) of the system was calculated by Eq. (3), which considers particle geometry.

$$\frac{M_t}{M_\infty} = 1 - A * \exp(-Bt) \quad (2)$$

$$B = \frac{D_e\pi^2}{4L^2} \quad (3)$$

2.7. Determination of total phenolic content

The TPC in the samples was determined by the Folin-Ciocalteu colorimetric method described by Chanioti and Tzia (2018) with some modifications. A 0.125 mL aliquot of sample was diluted in 15 mL of distilled water, 1.25 mL of Folin-Ciocalteu reagent was immediately added and allowed to stand for 5 min, and 2.5 mL of 20% sodium carbonate aqueous solution was added. Finally, the mixture was brought to 25 mL with distilled water, shaken vigorously, and left to stand for 2 h protected from light. The sample was centrifuged for 1 min at 4000 rpm before reading the absorbance at 765 nm with a spectrophotometer (UV-Visible T-70, PG Instruments Ltd., Leicestershire, UK). The calibration curves were prepared with gallic acid standard solutions, and the results were expressed as milligrams of gallic acid equivalent per gram of CBS dry matter (mg GAE/g DM).

2.8. Antioxidant capacity

2.8.1. 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay

The DPPH radical scavenging capacity was determined according to the detailed assay described by Thaipong et al. (2006) with slight modifications. Thus, 0.1 mL of the adequately diluted sample was mixed with 2.9 mL of methanolic DPPH solution. The mixture was incubated for 30 min at ambient temperature in the dark. Absorbance was then measured at 515 nm with a spectrophotometer (UV-Visible T-70, PG Instruments Ltd., Leicestershire, UK). The Trolox reagent was used as a standard, and the results were expressed as milligrams of Trolox equivalent per gram of dry matter (mg TE/g DM).

2.8.2. Ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) assay was performed according to Mellinas et al. (2020) with slight modifications. Fresh FRAP reagent was prepared by mixing 25 mL acetate buffer at pH = 3.6, 2.5 mL of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) at a 10 mM concentration, and 2.5 mL iron chloride at a 20 mM concentration. The solution was incubated at 37 °C and 2850 μL of the solution was then mixed with 150 μL of the sample diluted with distilled water (1 mL/10 mL). Tubes were incubated for 30 min in the dark. Absorbance was measured at 593 nm with a spectrophotometer (UV-Visible T-70, PG Instruments Ltd., Leicestershire, UK). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard to construct the calibration curve. Results were expressed as milligrams of Trolox equivalent per gram of dry matter (mg TE/g DM).

2.9. High-performance liquid chromatography analysis

The chromatographic analysis was carried out using Merck Hitachi high-performance liquid chromatography (HPLC) equipment that consisted of an autosampler L-7250, pump L-7100, UV detector L7400, and column oven L-7350 (Merck KGaA, Darmstadt, Germany). The procedure described by Barbosa-Pereira et al. (2018) was used as a reference. Samples were diluted (1mL/50 mL) and filtered before the analysis with 25 mm × 0.45 μm MCM (mixed cellulose membrane: acetate cellulose and nitrate cellulose) filters (Everest Scientific, Newark, DE, USA). Compounds were separated in a 250 mm × 4.6 mm × 5 μm reverse phase column (Inertsil ODS-3, C18, GL Sciences, Torrance, CA, USA). The column oven temperature was set at 35 °C and the mobile phase flow at 1.0 mL/min, while the injection volume was 10 μL. A gradient elution method was applied using 0.1% formic acid (pH = 3.0) as solvent A and 100% methanol as solvent B. The elution conditions were programmed as 0–0.5 min, 90% A and 10% B; 0.5–3 min, 10%–30% B; 3–8 min, 30–35% B; 8–11 min, 35%–40% B; 11–30 min, 40%–80% B; 30–31 min, 80%–10% B; and finally, a conditioning 1 min cycle under the initial conditions to prepare the column for the next analysis. The spectra were recorded at wavelengths from 200 to 400 nm, and the separated compounds were monitored at 278 nm. The compounds were identified in the sample by comparing them with the spectral data of the standards. Linear regression data to quantify theobromine, caffeine, catechin, and epicatechin in the sample are shown in Supplementary Material 3. All the analyses were performed in triplicate.

2.10. Scanning electron microscopy (SEM)

The effect of solvents on the microstructure surface of CBS residue and its basic composition were investigated by scanning electron microscope (SU3500, Hitachi, Krefeld, Germany). The sample was placed on metal slides on conductive carbon glue (Pelco, Ted Pella Inc., Redding, CA, USA).

2.11. Statistical analysis

Results were reported as the mean of three analyses ± standard deviation. Experimental data were fitted to the mathematical model (Eq. (2)) by nonlinear regression using the Matlab R2021a Curve Fitting tool. The one-way analysis of variance (ANOVA) was applied to estimate the statistical significance of the data at a 95% confidence level with the StatGraphic Centurion XVI.I version 16.1.03 program (StatPoint Technologies, Inc., The Plains, VA, USA). Fisher's least significant difference (LSD) test was applied to determine significant differences ($p < 0.05$) between means.

3. Results and discussion

3.1. Characterization of deep eutectic solvents

The type of HBD and increased water content influenced the physical properties of the ChCl-DES under study. The pH of the ChLa eutectic mixture decreased when water content increased, whereas the contrary occurred with the ChGly and ChEt mixtures (Table 2). Water content had a slight effect on solvent density. According to Hansen et al. (2021), the density of DES can provide information regarding their molecular interactions. A drastic decrease in viscosity occurred when the water content increased in all the ChCl-DES. Physicochemical properties of a large number of deep eutectic solvents have been reported in the literature. However, most of the literature data do not provide information about their aqueous solutions. Since aqueous solutions of DES enhance extraction processes, this study provides a characterization of pH, density, and viscosity values, properties that could influence metabolite extraction.

The type of molecular interaction, functional groups involved in the studied ChCl-DES, and the effect of adding water were investigated by FTIR. The spectra of ChLa, ChGly, and ChEt and their 50% aqueous solutions are shown in Fig. 1. A stretching vibration absorption band of the hydroxyl group (O-H) is evident, which is represented by a broad and intense band. In all the spectra, adding water produced "deeper valleys" in the transmittance mode, since ChCl-DES are hydrophilic solvents and interact with water. For ChLa, the band of the hydroxyl group (O-H) shifted from 3335 to 3393 cm^{-1} when the water content increased. The same occurred with ChEt, which shifted from 3300 to 3384 cm^{-1}, whereas for ChGly the band shifted from 3312 to 3317 cm^{-1}. In particular, the O-H bands shifted to higher frequencies indicate a weakening of the hydrogen bonds between the HBD and the HBA (Gabriele et al., 2019). The band between approximately 2850 and 2990 cm^{-1} in all spectra was the characteristic band of the alkyl (C-H) group. The stretching vibration absorption band of the carboxyl (C = O) group was found at 1737 and 1720 cm^{-1} for ChLa and ChLa50, respectively. The same functional group was estimated at 1746 and 1650 cm^{-1} for ChGly and ChGly50, and at 1699 and 1649 cm^{-1} for ChEt and ChEt50, respectively. Wavenumber values lower than 1400 cm^{-1} represented the fingerprint of each compound.

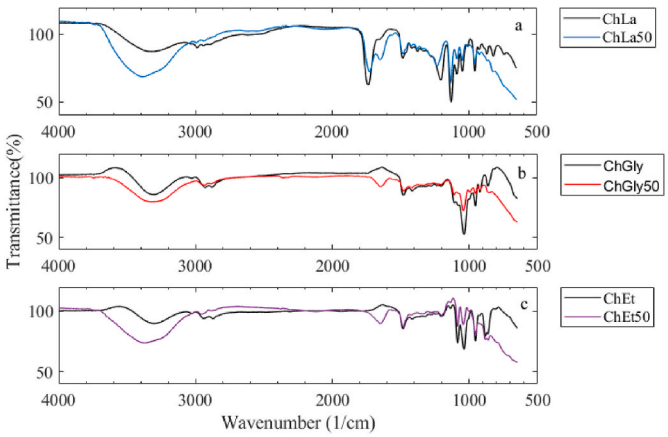

Fig. 1. Infrared spectrum of choline chloride-based deep eutectic solvents: choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly) and choline chloride-ethylene glycol (ChEt) and their 50% aqueous solutions (ChLa50, ChGly50, ChEt50).

3.2. Effect of hydrogen bond donor and water content in the performance of deep eutectic solvents

Three types of HBD compounds were used to form the ChCl-DES: lactic acid, glycerol, and ethylene glycol. The TPC extracted at equilibrium ranged from 8.09 to 14.33 mg GAE/g DM for ChLa and their aqueous solutions, 6.46–11.71 mg GAE/g DM for ChGly and their aqueous solutions, and 6.81–11.62 mg GAE/g DM for ChEt and their aqueous solutions. The type of HBD used to form the eutectic mixture had a significant ($p < 0.05$) influence on TPC (Table 3). ChLa was the most efficient in extracting polyphenols from CBS compared with ChGly and ChEt in which the HBD belongs to the polyol group (glycerol and ethylene glycol). The highest TPC was obtained with the ChLa50, which was significantly higher than the TPC for the ChGly50 and ChEt50. ChGly50 and ChEt50 extracts showed equal TPC values ($p > 0.05$), thus showing similar behavior between the ChCl-DES that contained polyols. This result is consistent with the findings by Saha et al. (2019) and Cvjetko Bubalo et al. (2016). Authors stated that DES based on organic acids are more suitable for extracting polar compounds, such as polyphenols, than those based on polyols. A specific study on extracting polyphenols from CBS with different DES showed that a choline chloride-lactic acid combination used to form the eutectic mixture

Table 2
Mean values ± standard deviations of pH, density, and viscosity of choline chloride-based deep eutectic solvents (ChCl-DES) and their aqueous solutions.

ChCl-DES	pH, 25 °C	Density (kg/m^3), 20 °C	Viscosity × 10^{-3} (mPa × s), 25 °C
ChLa	0.34 ± 0.02	1177.36 ± 0.01	222.00 ± 3.06
ChLa30	0.66 ± 0.06	1157.00 ± 0.01	32.79 ± 2.00
ChLa40	1.05 ± 0.27	1137.22 ± 0.02	12.50 ± 0.22
ChLa50	1.28 ± 0.25	1111.09 ± 0.01	5.10 ± 0.25
ChGly	4.43 ± 0.21	1193.06 ± 0.01	297.40 ± 2.70
ChGly30	3.75 ± 0.06	1147.03 ± 0.01	13.73 ± 0.77
ChGly40	3.78 ± 0.01	1128.27 ± 0.01	7.34 ± 0.33
ChGly50	3.75 ± 0.06	1109.97 ± 0.02	4.49 ± 0.26
ChEt	4.49 ± 0.14	1119.01 ± 0.01	34.92 ± 2.67
ChEt30	4.04 ± 0.18	1093.16 ± 0.01	6.97 ± 0.39
ChEt40	3.92 ± 0.17	1081.18 ± 0.01	4.71 ± 0.23
ChEt50	3.89 ± 0.17	1068.85 ± 0.01	3.46 ± 0.06

Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). 30, 40 and 50 means water percentage in the solvent.

Table 3
Total phenolic content at 140 min (M_t) and 240 min (M_∞) extraction times in choline chloride-based deep eutectic solvents aqueous solutions and 70% ethanol aqueous solution.

Solvent	$M_{t\ 140\ min}$ (mg GAE/g DM)	M_∞ (mg GAE/g DM)
70% Ethanol	9.45 ± 0.26^{aA}	9.46 ± 0.50^{A}
ChLa30	8.09 ± 0.16^{bA}	9.02 ± 0.09^{B}
ChLa40	11.45 ± 0.23^{cA}	11.97 ± 0.17^{B}
ChLa50	14.33 ± 0.23^{dA}	14.44 ± 0.31^{A}
ChGly30	6.46 ± 0.12^{eA}	6.70 ± 0.12^{A}
ChGly40	10.12 ± 0.22^{fA}	10.18 ± 0.03^{A}
ChGly50	11.71 ± 0.08^{cA}	11.60 ± 0.07^{A}
ChEt30	6.81 ± 0.12^{gA}	6.96 ± 0.10^{A}
ChEt40	10.07 ± 0.11^{fA}	9.36 ± 0.03^{B}
ChEt50	11.62 ± 0.08^{cA}	11.44 ± 0.19^{A}

Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). 30, 40 and 50 means water percentage in the solvent. Different lowercase letters in the same column and different uppercase letters in the same row indicate significant differences ($p < 0.05$).

proved to be the best (Ruesgas-Ramon et al., 2020).

Significant differences ($p < 0.05$) were observed between M_∞ and M_t values reached at 140 min for ChLa30, ChLa40, and ChEt40 (Table 3).

When considering the TPC concentration with 70% ethanol solvent (9.45 ± 0.26 mg GAE/g DM), the improvement in the extraction was 1.52, 1.24, and 1.23 times for ChLa50, ChGly50 and ChEt50, respectively. Results reported in the literature confirm the increased efficiency of the polyphenol extraction process from different food matrices when using DES. For example, the DES composed of choline chloride-oxalic acid increased anthocyanin extraction yield from grape skins by a factor of 5 and 2 than water and methanol, respectively (Cvjetko Bubalo et al., 2016). Grapefruit polyphenol extraction was 1.3 times higher in lactic acid-glucose than in water (El Kantar et al., 2019).

Adding water to the mixture appeared to have different effects on the ChCl-DES. Several authors have mentioned that excessive water content can weaken the molecular interactions of DES and decrease their extraction capacity (Gullón et al., 2020; Saha et al., 2019). However, our results showed that increasing the water percentage in the ChCl-DES promoted the extraction of TPC. This behavior concurs with a study by Pavlović et al. (2020) in which the extraction of CBS secondary metabolites was higher when 50% water was added to different DES.

3.3. Extraction kinetics

Extraction kinetics is illustrated in Fig. 2 and shows a rapid increase in the TPC concentration at the beginning of extraction. This is typical of the washing stage in which compounds on the particle surface are easily transferred to the solvent. After this stage, the predominant phenomenon is metabolite diffusion from the interior of the particle, which leads to a decreased degree of extraction (Chan et al., 2013).

The proposed model is adequate to represent the behavior of the experimental data; the coefficient of determination (R^2) was greater than 0.92 for all the studied solvents. Model-fitting curves and averages of experimental values are presented in the Supplementary Material 4. The model fit was better for the extraction kinetics of TPC in ChCl-DES with higher water percentages. Table 4 shows the model fit coefficients (A and B) and D_e. L was considered for calculating D_e and had a value of 64.7 ± 57.6 μm. The D_e ranged from 9.8 to 29.0 × 10^{-12} m^2/s. These values are similar to those reported by Amrouche et al. (2020), who studied the polyphenol extraction kinetics of sticky fleabane (*Inula viscosa* (L.) Aiton) leaves (2.25–31.44 × 10^{-12} m^2/s). Results were lower than those indicated by Ben Amor and Allaf (2009) in which anthocyanin diffusivity of Malaysian roselle (*Hibiscus sabdariffa*) was intensified because of pretreatment with thermo-mechanical instant controlled pressure drop. Similar values were also reported (23–29 × 10^{-12} m^2/s) in polyphenol extraction from grapefruit peel when high voltage electrical discharges, deep eutectic solvents and aqueous glycerol were applied (El Kantar et al., 2019). To our knowledge, extraction kinetics of CBS secondary metabolites (specifically flavonoids) has only been studied by Okiyama et al. (2018), who did not mention D_e values because they used the Peleg model to simulate the experimental data. Therefore, D_e values in our study could not be adequately compared with scientific experiments conducted with the same plant matrix.

Table 4
Coefficient of determination (R^2), model fit coefficients (A and B), and effective diffusion coefficient (D_e) for the extraction kinetics of polyphenols from cocoa bean shells in choline chloride-based deep eutectic solvents aqueous solutions and 70% ethanol aqueous solution.

Solvent	R^2	A	B	D_e (10^{-12} (m^2/s)
70% Ethanol	0.966	0.991	0.067	27.8
ChLa30	0.933	0.923	0.024	9.8
ChLa40	0.962	0.978	0.049	20.2
ChLa50	0.982	0.994	0.070	29.0
ChGly30	0.921	0.939	0.032	13.3
ChGly40	0.978	0.971	0.035	14.5
ChGly50	0.981	0.992	0.063	26.4
ChEt30	0.952	0.964	0.037	15.4
ChEt40	0.971	0.978	0.045	18.6
ChEt50	0.990	0.992	0.059	24.5

Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). 30, 40 and 50 means water percentage in the solvent.

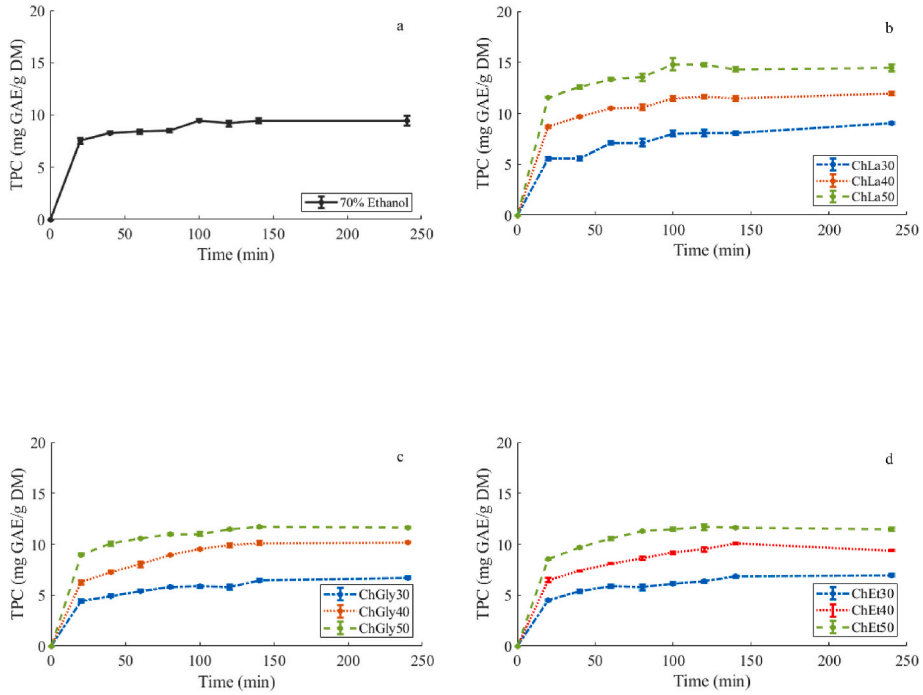

Fig. 2. Experimental extraction kinetic curves for total phenolic content (TPC) using a: 70% ethanol, b: choline chloride-lactic acid (ChLa), c: choline chloride-glycerol (ChGly) and d: choline chloride-ethylene glycol (ChEt). The water percentage in the solvents is denoted by 30,40 and 50. Points and bars represent the mean and standard deviation.

As expected, D_e increased with increasing water content in the ChCl-DES due to a decrease in viscosity that enabled greater mass transfer from the solute to the solvent. However, the similarity between D_e values for 70% ethanol and ChLa50, ChGly50, and ChEt50 solvents suggests that other mechanisms besides washing/diffusion influence the mass transfer rate.

3.4. Antioxidant activity

The antioxidant properties of the extracts obtained at 140 min are hereby described using a combination of the DPPH and FRAP methods. Fig. 3 *A* and *B* show that the antioxidant activity of the CBS extract varies significantly ($p < 0.05$) with the different solvents and is markedly higher when ChCl-DES are used compared with the control sample (70% ethanol). The extract from the ChLa50 solvent had the highest DPPH free radical scavenging capacity and FRAP values, which concur with the high TPC of this extract. Contrary to expectations, the DPPH free radical scavenging capacity of the extract from ChGly50 and ChEt50 exhibited significant differences, although there were no significant differences in the TPC of both extracts. The same occurs with FRAP values of the extracts obtained with the solvents mentioned before. Results were lower than those reported by Mellinas et al. (2020) in a study in which microwave-assisted extraction was optimized to increase the recovery of CBS polyphenols.

Antioxidant activity depends on several factors such as sample preparation, extraction method, type of solvent, temperature, pH, antioxidant compound concentration, profile of individual phytochemical compounds, and chemical structure of antioxidant molecules (Lessa et al., 2018; Macedo et al., 2011; Md Yusof et al., 2019; Oracz et al., 2014). According to Radošević et al. (2018), the compounds used to prepare DES maintain their functional properties once the eutectic mixture is formed (e.g., antioxidant power of organic acids). Therefore, further studies are required for a better understanding of the contribution of DES to the antioxidant capacity of the extracts.

3.5. Quantification of methylxanthines (theobromine, caffeine) and flavonoids (catechins, epicatechins)

Table 5 provides the quantification results for theobromine, caffeine, catechins, and epicatechins by HPLC. The results showed significant differences ($p < 0.05$) between the ChCl-DES extracts and the control sample. Theobromine was the compound with the highest concentration, followed by caffeine; methylxanthines were the predominant bioactive compounds in the CBS extracts. The theobromine and caffeine concentrations ranged from 2.59 to 5.80 mg/g DM and 0.87–2.08 mg/g DM, respectively. Okiyama et al. (2018) mentioned similar theobromine (3.68–6.70 mg/g) and caffeine (0.51–1.91 mg/g) concentrations for the

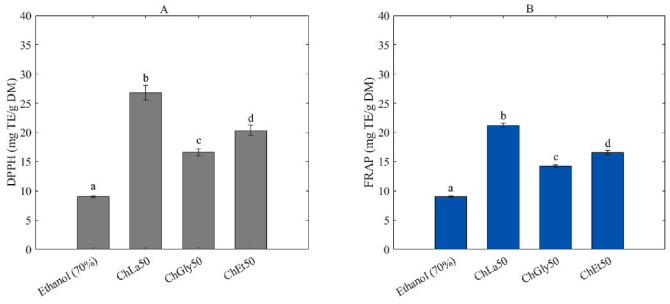

Fig. 3. DPPH radical scavenging capacity (A) and ferric reducing/antioxidant power (FRAP, B) using 70% ethanol, choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly) and choline chloride-ethylene glycol (ChEt). The water percentage in the solvents is denoted by 30, 40 and 50. Error bars indicate standard deviations and a, b and c indicate significant differences ($p < 0.05$).

Table 5
Quantification of methylxanthines (theobromine and caffeine) and polyphenols (catechins and epicatechins) in cocoa bean shell extracts from solid-liquid extraction using choline chloride-based deep eutectic solvents aqueous solutions and 70% ethanol aqueous solution.

Solvent	Theobromine	Caffeine	Catechins	Epicatechins
	mg/g DM			
70% Ethanol	3.29 ± 0.02^a	1.17 ± 0.02^a	0.38 ± 0.01^a	0.68 ± 0.03^a
ChLa50	5.80 ± 0.07^b	2.08 ± 0.08^b	0.92 ± 0.02^b	0.91 ± 0.05^b
ChGly50	2.59 ± 0.01^c	0.87 ± 0.03^c	0.38 ± 0.01^a	0.79 ± 0.05^c
ChEt50	3.24 ± 0.02^a	1.14 ± 0.07^a	0.58 ± 0.03^c	0.93 ± 0.01^b

Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). 50 mean water percentage in the solvent. Different letters in the same column indicate significant differences ($p > 0.05$).

extraction with pressurized liquid at 60–90 °C for a 5 min retention time. The ChLa50 solvent exhibited the best performance in methylxanthine extraction, while ChEt50 did not improve its extraction compared with the control extract performed in 70% ethanol.

As expected, the highest catechin and epicatechin concentrations occurred with the ChLa50 solvent with values of 0.92 mg/g DM and 0.91 mg/g DM, respectively. Although the extracts derived from ChGly50 and ChEt50 showed no significant difference ($p > 0.05$) in the ANOVA performed to evaluate TPC, there was a significant difference ($p < 0.05$) in the catechin and epicatechin contents. The extract from ChEt50 had a higher concentration of catechins and epicatechins; this can explain its greater antioxidant activity compared with the antioxidant capacity of the extract from ChGly50. This demonstrated that DES are selective in extracting specific molecules. In addition, an important number of unknown molecules were detected but not quantified, which were found mostly in the extract from ChGly50.

Results differed from the findings by Pavlović et al. (2020), who evaluated the extraction capacity of CBS compounds in 16 different DES, including the 3 evaluated in the present study. Although the extraction conditions applied by Pavlović et al. (2020) (60 min agitation and 50 °C) were different than those developed in the present study (140 min agitation and 30 °C), the water content in the DES was the same in both studies. The magnitude of the theobromine, caffeine, catechin, and epicatechin values in each study were different. It is known that the composition of CBS can vary according to factors such as the origin of the cocoa, species, cocoa bean processing conditions, CBS particle size, and extraction method. In the study by Pavlović et al. (2020), the order in the extraction capacity of DES also varied; the choline chloride-glycerol combination performed better than the choline chloride-lactic acid and choline chloride-ethylene glycol DES preparations. This is likely because the molar ratio used to prepare the DES was different than the one applied in our study. Before designing the experiments for the present investigation, the 1:2 ratio was defined as the best molar ratio to prepare the different ChCl-DES under study. The molar ratio used in this study was based on results reported by different researchers who evaluated the performance of several DES when extracting secondary metabolites (Bajkacz and Adamek, 2018; Chanioti and Tzia, 2018; C. H. C. H. Pan et al., 2021; Radošević et al., 2016).

3.6. Chemical composition and surface morphology of the residue

Micrographs of CBS residues after the extraction process are illustrated in Fig. 4. The surface morphology of the residues treated with ChCl-DES is shown in Fig. 4b–d, respectively. Surfaces treated with ChLa50, ChGly50, and ChEt50 are damaged compared with the surface of the residue treated with 70% ethanol (Fig. 4a).

Previous studies have indicated that the CBS surface is composed of lignin, hemicellulose, and polysaccharides (Fioresi et al., 2017). The elemental composition analysis for surface topography (Table 6) showed

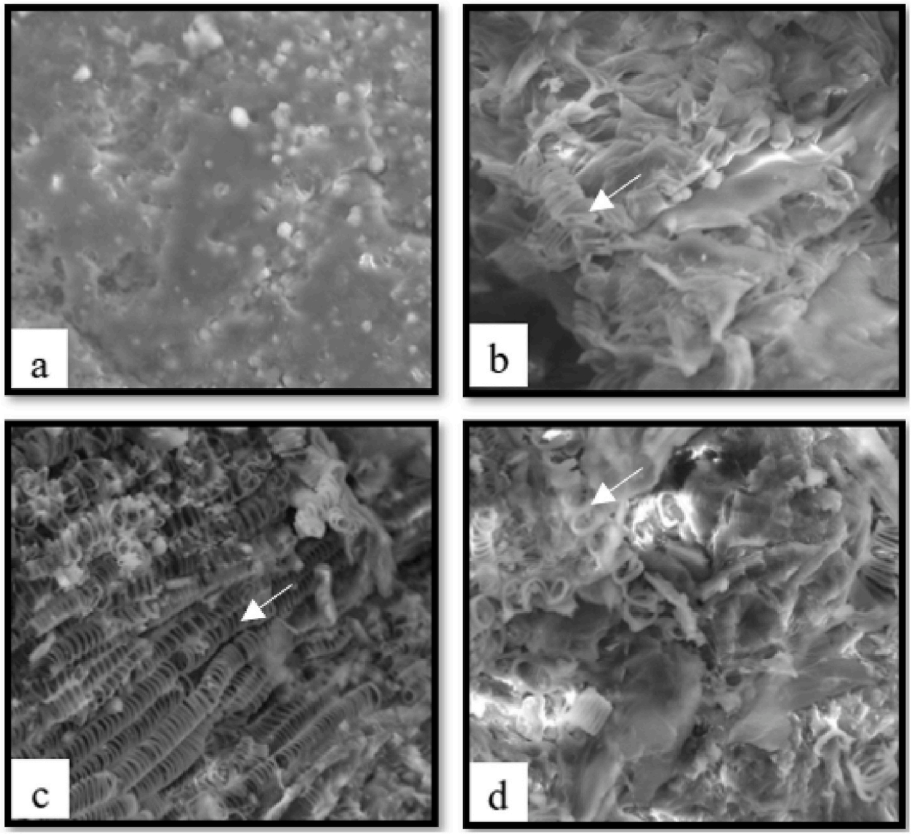

Fig. 4. Surface morphology of residue at 140 min retention time and different solvents: a) 70% ethanol, b) choline chloride-lactic acid (ChLa), c) choline chloride-glycerol (ChGly) and d) choline chloride-ethylene glycol (ChEt) containing 50% of water.

Table 6
Elemental composition of cocoa bean shell (CBS) material and the residues of the extraction with aqueous deep eutectic solvents and aqueous ethanol after 140 min treatment.

Sample	W_{carbon} %	W_{oxygen}%	$W_{nitrogen}$%	W_{others} %
CBS	55.07-54.37	39.63-40.14	0	5.3-5.49
R-70% Ethanol	56.46-57.03	38.84-40.48	0	3.06-4.13
R-ChLa50	52.16-52.59	37.43-38.88	6.76-7.47	2.20-2.51
R-ChGly50	52.85-54.84	35.51-40.08	5.53-7.15	1.54-2.50
R-ChEt50	51.32-53.52	33.78-39.09	5.13-9.20	2.26-5.70

Weight percent (*W*), Residue (*R*), Choline chloride-lactic acid (ChLa), choline chloride-glycerol (ChGly), and choline chloride-ethylene glycol (ChEt). Results are expressed as minimum and maximum values of three analyses. 30, 40 and 50 means water percentage in the solvent.

a significant change in the residues obtained after the extraction with ChLa50, ChGly50, and ChEt50 compared with the raw material (CBS) and the residue from 70% ethanol extraction. Nitrogen element was not detected in the CBS or in the residue treated with 70% ethanol. As mentioned in the literature reviewed, DES can selectively separate lignin and cellulose (Alam et al., 2021; Francisco et al., 2012; Z. Z. Pan et al., 2021; Wu et al., 2021). When cell wall molecules are destroyed, other compounds are exposed, some of them containing nitrogen, as shown in Table 6. Organic compounds consist of carbon and hydrogen and may also contain nitrogen. These nitrogen-containing compounds can be seen as derivatives of ammonia, where one or more hydrogen atoms are replaced with hydrocarbon groups. One specific subgroup within the nitrogen-containing compounds category are α-amino acids and their peptide and protein derivatives. It is possible, therefore, that the nitrogen content in the residues from ChCl-DES extraction could be closely related to the protein content, whose value is 15.65 mg/g for dry matter cocoa bean shell (Supplementary Material 1).

In this paper, the elemental composition of the plant surface and residues are analyzed as a way to demonstrate quantitatively the cell wall damage. Therefore, subjective visual interpretation of an image can be avoided. Variations of this technique for other plant sources with a different composition should be explored in future research.

As mentioned in section 3.3, extraction kinetics is influenced by other mechanisms in addition to washing/diffusion. This section demonstrated that there was at least one other mechanism that consisted of destroying the outer layer of the plant material particle. This explains why DES performed better in bioactive compound extraction compared with conventional organic solvents. The breakdown of the plant structure is also a characteristic mechanism of emerging technologies, which have been applied to the extraction process of bioactive compounds. Emerging technologies have also been combined with DES in previous studies to improve extraction efficiency (Gullón et al., 2020). Given that DES works with a mechanism similar to that of emerging technologies, the extraction of bioactive compounds using only DES could be considered an easy and economical method compared with the higher acquisition and operation costs of technologies and equipment.

4. Conclusions

The data from the present study have indicated that DES should be appropriately designed to modify their physicochemical properties and benefit the extraction process. The ChLa50 showed the best extraction performance and antioxidant activity of cocoa bean shell extract. Additionally, the extraction of the principal bioactive molecules in cocoa bean shells is intensified by using the best-designed ChCl-DES as compared with the conventional solvent. The quantification of methylxanthines and flavonoids by high-performance liquid chromatography demonstrated that ChCl-DES were selective when extracting specific

molecules. Fick's second law model, which describes the washing/diffusion mechanisms, adequately depicts the extraction kinetics data for total phenolic content. However, the present study confirmed that the destruction of the outer layer of the plant particle is another mechanism that influences polyphenol extraction when using DES. In that sense, future research could be addressed to compare the performance of DES and emerging technologies, which work similarly in the extraction of natural compounds. Researchers should consider not only efficiency in the extraction but also environmental and economic impact.

CRediT authorship contribution statement

Elaine Benítez-Correa: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **José Miguel Bastías-Montes:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing, Supervision, Validation. **Sergio Acuña-Nelson:** Supervision, Writing – review & editing, Validation. **Ociel Muñoz-Fariña:** Formal analysis, Methodology, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100614.

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