Sustainable Chemistry and Pharmacy 43 (2025) 101879

Contents lists available at ScienceDirect

Sustainable Chemistry and Pharmacy

journal homepage: www.elsevier.com/locate/scp

Mechanochemical-assisted Natural Deep Eutectic Solvent as a platform for an olive leaves biorefinery: Extraction of bioactive compounds and methane production

Juan Cubero-Cardoso [a,b], Manuel Hernández-Escaño [a,b], Ángeles Trujillo-Reyes [a], Fernando G. Fermoso [a], Ma Ángeles Fernández-Recamales [c], Juan Fernández-Bolaños [a], Guillermo Rodríguez-Gutiérrez [a,**], Juan Urbano [b,*]

[a] Instituto de la Grasa, Spanish National Research Council (CSIC), Campus Universitario Pablo de Olavide, Building 46, Ctra. de Utrera, km. 1, Seville, Spain

[b] Laboratory of Sustainable and Circular Technology, CIDERTA and Chemistry Department, Faculty of Experimental Sciences. Campus de "El Carmen", University of Huelva, 21071, Huelva, Spain

[c] AgriFood Laboratory, Faculty of Experimental Sciences, University of Huelva, 21007, Huelva, Spain

ARTICLE INFO

Handling Editor: Vania Zuin

Keywords:
Agro-waste
Biorefinery
Mechanochemical extraction
Biochemical methane production
Phenolic compound
Lignocellulosic material

ABSTRACT

This study introduces an innovative biorefinery process aimed at maximizing the utilization of olive leaves. The proposed approach seeks to extract valuable phenolic compounds, lignocellulosic material, and biomethane from olive leaves. To achieve this, a combined technique involving mechanochemical extraction and sustainable solvents, specifically natural deep eutectic solvents (NADES), was employed. Unlike conventional methods, NADES were simultaneously formed and utilized for extraction within a ball mill. The optimal conditions for maximum extraction of high-value compounds were 0.5 g of OL using 10 mL of ChCl:Gl (3:1) and 1.1 h in a ball mill. The recovered yields were 14.5 ± 2.9 g/kg OL of lignocellulosic fraction and 1.3 ± 0.3 g gallic acid eq./kg OL of phenolic compounds. OL waste generated after the optimized extraction process was evaluated for methane production, obtaining 142 ± 53 mL CH4/g VS OL waste compared to 126 ± 30 mL CH4/g VS untreated OL. Therefore, NADES represent promising solvents for the biorefinery process and reduce dependence on organic solvents.

1. Introduction

Olive oil production produces massive amounts of by-products, such as olive mill solid waste, mill wastewater, pits, and leaves. Recycling these by-products aligns with global efforts to protect the environment and convert waste into valuable resources for various industries, such as agriculture, chemical, textile, pharmaceutical, and food (Fernández-Prior et al., 2020). Numerous methods are available for repurposing olive oil by-products, most of which involve extracting and recovering bioactive components, particularly

---

* Corresponding author.
** Corresponding author.
E-mail addresses: j.cubero@dqcm.uhu.es (J. Cubero-Cardoso), manuelhdezesca@gmail.com (M. Hernández-Escaño), angeles.trujillo@ugr.es (Á. Trujillo-Reyes), fgfermoso@ig.csci.es (F.G. Fermoso), recamale@uhu.es (M.Á. Fernández-Recamales), j.fb.g@csic.es (J. Fernández-Bolaños), guirogu@ig.csic.es (G. Rodríguez-Gutiérrez), juan.urbano@dqcm.uhu.es (J. Urbano).

https://doi.org/10.1016/j.scp.2024.101879
Received 24 July 2024; Received in revised form 13 September 2024; Accepted 29 November 2024

Abbreviation

DPPH	2,2-Diphenyl-1-Picrylhydrazyl
AD	Anaerobic Digestion
BMP	Biochemical Methane Potential Test
COD	Chemical Oxygen Demand
Chcl	Choline Chloride
DES	Deep Eutectic Solvents
F	Fructose
GC	Gas Chromatography
Gl	Glycerol
HPLC	High-Performance Liquid Chromatography
HBAs	Hydrogen Bond Acceptors
HBDs	Hydrogen Bond Donors
LA	Lactic Acid
MS	Mineral Solids
NADES	Natural Deep Eutectic Solvents
OL	Olive Leaves
OA	Oxalic Acid
DAD	Photodiode Array Detector
RP	Reducing Power
TS	Total Solids
TFA	Trifluoroacetic Acid
U	Urea
VS	Volatile Solids
W	Water

phenolic compounds (Clodoveo et al., 2022). Oleuropein and its derivatives, hydroxytyrosol, and tyrosol, are the principal active constituents of olive leaves. These phenolic compounds can vary depending on the olive tree variety and the season. Recent research has confirmed their notable health benefits, including antiviral, anti-inflammatory, anticarcinogenic, antidiabetic, hypocholesterolaemia, and antihypertensive activities (Palos-Hernández et al., 2022; Talhaoui et al., 2015).

Although olive leaves contain bioactive compounds, their primary component is lignocellulosic material (Lama-Muñoz et al., 2020). This lignocellulosic material is composed of a complex structure of cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%), with small amounts of other organic and non-organic compound, like pectin, proteins or lipids (Brandt et al., 2013; Sawatdeenarunat et al., 2015). New and innovative methods have been developed to utilize these lignocellulosic materials further. In a study by Reimer et al. (2021), cellulose-based biopolymers were synthesized to manufacture optical fibers, taking advantage of properties such as refractive index dispersion and optical transmission properties of cellulose. Hemicellulose could be used to obtain value-added products, such as xylitol, a polyalcohol with applications as a sweetener in sugar-free products, which is beneficial for people with diabetes and has anticaries properties (Umai et al., 2022).

Recently, new environmentally sustainable techniques have been employed to extract different compounds from lignocellulosic material using green solvents, such as deep eutectic solvents (DES) (Chen et al., 2018). A specific type of deep eutectic solvents (DES) derived from naturally occurring primary metabolites is known as natural deep eutectic solvents (NADES). These metabolites include amino acids, carboxylic acids, sugars, choline chloride, and urea (Santos-Martín et al., 2023). NADES are a field of active research due to their numerous advantages over traditional organic solvents (New et al., 2022). NADES are biodegradable, low-toxicity, cost-effective, and easy to prepare while offering high extraction efficiency across a wide range of compounds (Santana et al., 2019). These attributes make DES suitable solvents for a more sustainable future characterized by a circular economy model (Benvenutti et al., 2020; Cortés-Triviño et al., 2022). However, the use of NADES is still relatively uncommon, and ongoing research aims to explore new types and broader applications for these solvents (Carbonell-Rozas et al., 2021; Ma et al., 2019). Recently, several studies have reported the use of NADES to extract phenolic compounds from olive leaves (Alañón et al., 2020; Mir-Cerdà et al., 2023; Ruesgas-Ramón et al., 2017; Santos-Martín et al., 2023; Siamandoura and Tzia, 2023). However, in the study by Benvenutti et al. (2020), a NADES composed of citric acid, glucose, and water 1:1:3 ratio exhibited a pectin extraction yield of 27.3% from Myrciaria cauliflora. Additionally, in the study by Elgharbawy et al. (2019), different NADES were used to isolate pectin from grapefruit peels, resulting in yields that were 2–11 times higher than those achieved by traditional industrial methods.

Mechanochemistry has emerged as an environmentally friendly method with various applications, including the creation of novel materials, enabling sustainable reactions, and extracting diverse compounds (Douard et al., 2022; Muñoz-Batista et al., 2018). A recent advancement in this field is mechanochemical extraction, which has exhibited excellent extraction efficiency from agro-food compared to conventional solvents in a short extraction time (Hajiali et al., 2022; Mišan et al., 2020). One option to improve the energy efficiency of the extraction process is to simultaneously synthesize NADES and extract lignocellulosic material from olive leaves in a ball mill. Ball mills are commonly used for grinding and blending various materials in industries such as mineral processing, paint manufacturing, ceramics, and even laboratory settings. Additionally, a research study has demonstrated that ball milling can reduce the particle size of cereal grains, increasing the amount of solvent-extractable phenolic compounds. This increase is attributed to the larger specific surface area generated by the reduction in particle size (Wang et al., 2014).

The biomass that remains after the extraction of lignocellulosic material with NADES must undergo further treatment and stabilization to complete the biorefinery process (Fermoso et al., 2018). One option is anaerobic digestion (AD), a process that has been extensively studied with a wide variety of biomasses obtained from agricultural and agro-industrial wastes (Fermoso et al., 2018). According to the literature, pre-treatment is usually necessary to break down and solubilize lignocellulosic material in agricultural waste, thus improving the subsequent AD process due to enhanced biodegradability (Millati et al., 2023; Trujillo-Reyes et al., 2022). Various techniques have been employed to disassemble the complex structure of lignocellulosic biomass, including mechanical milling, steam explosion, hot water washing, acid and alkali pre-treatments, and ammonia fiber expansion. These methods improve the porosity, eliminate lignin and/or hemicellulose, and reduce the overall crystallinity of the biomass structure, making it more readily convertible into bioenergy and biobased products through biological processes (Sawatdeenarunat et al., 2015). The impact of using NADES as a pre-treatment for extracting high-value compounds, followed by anaerobic digestion (AD) to stabilize organic matter remaining from olive leaves, has not been extensively studied. Additionally, few studies have explored the AD process with olive leaves, unless they are pre-treated or utilized in co-digestion (Zhang et al., 2022).

The main objective of this study was to evaluate the extraction of high-value compounds from olive leaves using mechanochemical extraction with NADES and improve its valorization with biomethane production from the generated biomass. Furthermore, the suitability of NADES as a candidate feedstock for the AD process was examined. Due to their origin from natural sources, the integration of NADES has the potential to impact the degradation pathways within the AD process.

2. Experimental section

2.1. Regents

The reagents used to carry out all the experiments are:

Ammonium Hydroxide, Anhydrous citric acid, Crystalline urea, D(+)-Anhydrous Glucose, Ethanol 96%(v/v), Folin-Ciocalteu reagent, Lactic acid, Methanol, n-Hexane, Sodium Carbonate Sodium Hydroxide, Sodium Tetraborate 10-Hydrate, Trichloroacetic acid, Oxalic acid 2-hydrate (*PanReac AppliChem ITW Reagents, Barcelona, Spain*); 1-methylimidazole, 2,2-Diphenyl-1-picrylhydrazyl, 2,2-Bipyridyl, 3-Hydroxybiphenyl 90%, Antrone, D(−)-Fructose, D-(+)-Galacturonic Acid Monohydrate, Dichloromethane, Inositol, Iron (III) chloride, Sodium Borohydride, Trifluoroacetic acid, Trolox, Gallic acid (*Sigma Aldrich, Steinheim, Germany*); Glacial Acetic Acid, Potassium Hydroxide (*Scharlab, Barcelona, Spain*); Choline chloride 98% (*ALGRY Química S.L, Huelva, Spain*); Acetic Anhydride (*HoneyWell, Fluka, Germany*); Acetone (*Brenntag, Seville, Spain*);Glycerin 99% (*Labkem, Barcelona, Spain*); Sulfuric Acid 96% (*Carlo Erba Reagents, Barcelona, Spain*).

2.2. Olive leaves

Olive leaves (OL) of the Picual variety were obtained from the olive mill at the Instituto de la Grasa (CSIC) facilities in Seville. The leaves were air-dried for three days before being milled in a Thermomix® TM5 (Vorwerk, Germany) to a particle size less than 1 cm. The OL were minced at maximum speed, i.e., 1200 rpm, for 3 min. The physicochemical characterization of the OL included the following parameters: total solids (TS): 810 ± 4 g/kg of OL; mineral solids (MS): 61 ± 3 g/kg of OL; volatile solids (VS): 749 ± 33 g/kg of OL; moisture: 19.0 ± 0.4%; total phenolic compounds: 485 ± 16 g in eq. gallic acid/kg of OL; and the lignocellulosic materials express in percentage on a dry weight basis: cellulose: 6,98 ± 0.13 %; hemicellulose: 5.69 ± 0.11 %, and lignin: 16,08 ± 0.69 % (Lama-Muñoz et al., 2020).

2.3. High-added-value compounds mechanochemical extraction

The novelty of this work consists of forming the NADES at the same time as the milling of the extractable sample is carried out through the mechanochemical treatment, saving the previous stage of formation of the NADES. Prior to the main experiment, a test was conducted to determine the optimal conditions for NADES formation, including the ratios of NADES components, processing durations, and milling speeds. The optimal conditions were determined to be those that resulted in a homogeneous liquid (Table S1). The selection of NADES was based on previous research on the extraction lignocellulosic material from various biomass sources (Ünlü, 2021; Zurob et al., 2020). Choline chloride (ChCl) was selected as the hydrogen bond acceptor due to its low cost, extensive industrial use, minimal toxicity, low volatility, low melting point (remaining liquid at temperatures below 20 °C), broad polarity range, and strong solubilizing capacity for a variety of compounds (Satija et al., 2024). Oxalic acid (OA), urea (U), fructose (F), water (W), lactic acid (LA), and glycerol (Gl) were used as hydrogen bond donors, as they are known to form NADES when combined with ChCl. Six NADES were finally used along of experiment with the next molar ratio: ChCl:OA(1:1), ChCl:U(1:2), ChCl:F:W(5:2:5), ChCl:LA(1:2), ChCl: OA(2:1), ChCl:Gl(3:1).

The natural components used to synthesize NADES were mixed with milled OL in a 50 mL stone grinding vessel containing four balls of the same material measuring 1 cm in diameter. Mechanochemical extraction was performed using a planetary ball mill model S1(Retsch, Haan, Germany). The NADES components were mixed to produce 10 g of NADES in different molar ratios with 2.5 g of milled OL for 60 min at 25 °C in a ball mill speed of 100 rpm. Each mechanochemical extraction was performed in triplicate for each

NADES formulation tested.

After extracting high-added-value compounds from milled OL with the ball mill, the OL waste was separated from the extract by washing with distilled water, following the procedure described by Chen and Lahaye (2021) (Fig. 1). The mixture of OL waste and NADES was washed five times with 40 mL of distilled water. After extraction and washing, the mixture was filtered through filter paper (Resmas), and the filtrate was concentrated to 10 mL using vacuum rotary evaporator at 40 °C. The obtained extract was precipitated with 40 mL of 96% (v/v) ethanol to a final concentration of 77% (v/v). The precipitate was collected by centrifugation (3779×*g* for 20 min) and washed three times with EtOH:H_2O (70:30) and twice EtOH:Acetone (50:50). This precipitated material, referred to as the solid fraction, was subjected to dialysis (see section 2.5). The solid fraction was evaluated by gas chromatography to determinate individual neutral sugars. The solubilized extract obtained after the various washed was concentrated to 10 mL. The solubilized extract was evaluated for total sugars, uronic acid, total phenolic compounds (TPC), antioxidant activity, and individual phenolic compounds using HPLC.

2.4. Optimization of the extraction conditions by response surface methodology

A central composite design combined with response surface methodology was used to select the experimental conditions for the optimization of the effect of time of extraction (0.7–2.3 h), amount of milled OL (0.7–2.3 g/10 g of solvent), and types of solvent (traditional or green), on extraction efficiency of target compounds. Two types of solvents were tested. For classical solvents, hexane and methanol were selected as references for lignocellulosic material and phenolic compounds, respectively, while NADES obtained in the previous experiment were used as green solvents. The selection of the experimental conditions in this study was based on preliminary experiments (Table S1) and other studies (Jovanović et al., 2023; Santos-Martín et al., 2023; Wei et al., 2015). Two type of variables were selected as responses: those related to oligosaccharides from lignocellulosic material (solid fraction, total sugars, and uronic acids) and those related to phenolic compounds such as total phenolic compounds, antioxidant activity, reducing power and oleuropein and hydroxytyrosol content. The response variables were fitted to a second-order polynomial model to describe the relationship between the dependent and independent variables using the response surface methodology (RSM). Regression analyses were carried out according to Equation (1):

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i x_i+\sum_{i=1}^{3}\beta_{ii}x_i^2+\sum_{i,j=1}^{3}\beta_{ij}x_i x_j \qquad \text{Equation (1)}$$

where Y is the dependent variable (each of experimental response) β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients in the intercept, linear, quadratic, and the interaction terms of the model, respectively, and x_i and x_j ($i = 1, 3$; $j = 1, 3$; $i \neq j$) represent the non-coded independent variables. All experiments were performed in duplicate and randomized order to avoid systematic errors. Statistical analyses were conducted using the STATISTICA 7.0 software (StatSoft, Tulsa, OK).

The statistical validity of the models was tested by ANOVA, using the p-value to test for lack of fit and determining the coefficient of determination to check the quality of fit of the regression models. In addition, the desirability function method was used to predict single optimal extraction conditions valid for all dependent variables.

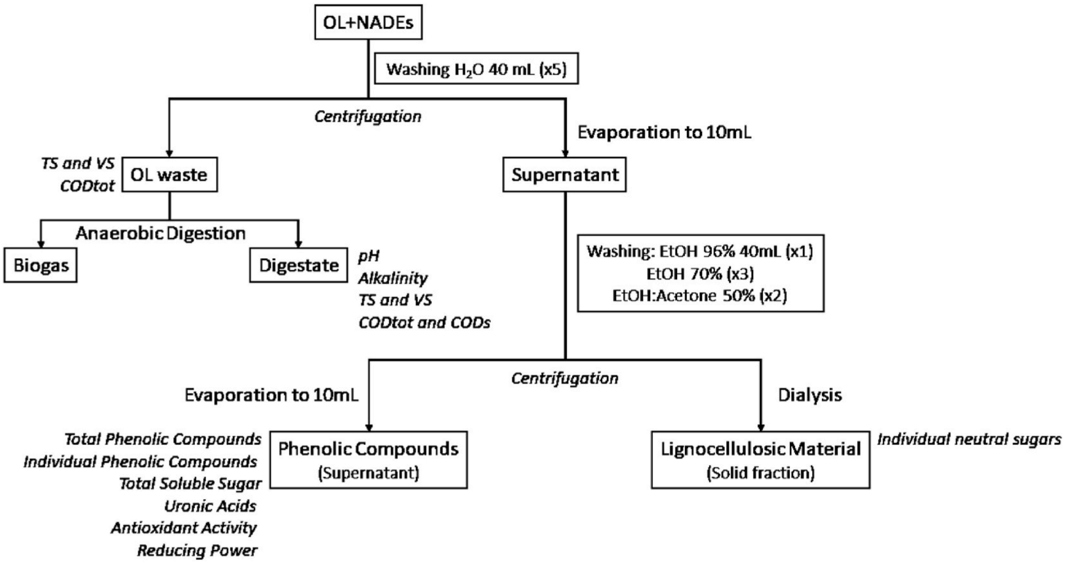

Fig. 1. Schematic representation of the High-added-value compounds obtained during mechanochemical extraction.

2.5. Biochemical methane potential test procedure

The AD process of the untreated milled OL, OL waste generated after extracting, NADES ChCl:Gl (3:1), and microcrystalline cellulose (Sigma Aldrich, US), used as a positive control, was studied following the biochemical methane potential (BMP) test

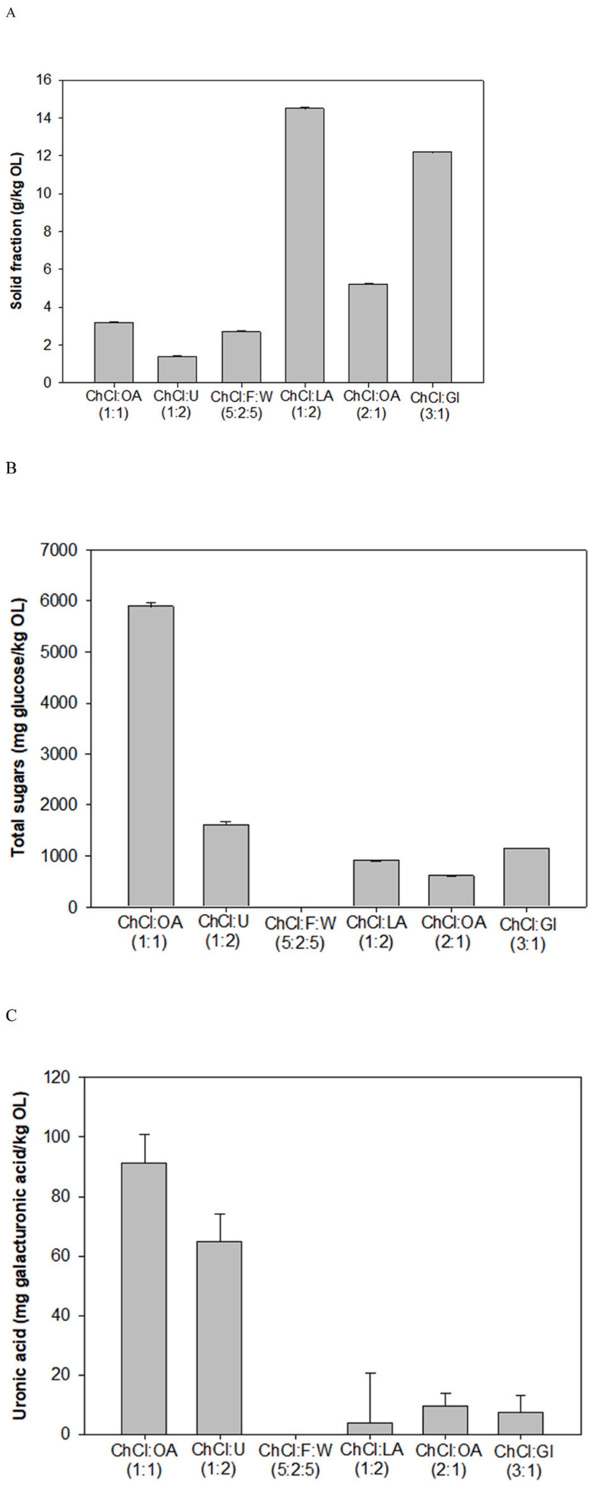

Fig. 2. Solid fraction (A), Total sugars (B) and uronic acid (C) of solubilized extract from milled OL using the ball mill with the different NADES studied (in brackets the ratio between each of the NADES components).

methodology described by Raposo et al. (2011). Batch mode tests were performed using reactors with a total volume of 250 mL and a working volume of 240 mL. The reactors were placed in thermostatic baths at 35 ± 2 °C, with constant stirring at 400 rpm. An inoculum-to-substrate ratio of 2 (as volatile solid per litter) was maintained in the reactors. The anaerobic flocculant inoculum was obtained from a mesophilic industrial reactor in the "Copero" wastewater treatment plant in Seville. The main characteristics of the inoculum were pH: 7.2 ± 0.1; chemical oxygen demand (COD): 10,500 ± 400 mg O_2/L; TS: 18.1 ± 0.3 g/kg; and VS: 11.0 ± 0.3 g/kg. Nitrogen gas was flushed into the reactors at the start of the experiments, which were then sealed with a rubber stopper to maintain anaerobic conditions. Triplicate reactors were performed for each substrate, to account for the inoculum's endogenous methane production. The biogas generated was passed through a 2 mol/L NaOH solution to retain the CO_2, and the displaced volume was assumed as methane. Methane production was expressed under standard pressure and temperature conditions, i.e., 25 °C and 1 atm. The biodegradability of the substrates was assessed by determining their total chemical oxygen demand (COD). It was considered that under standard conditions of 25 °C and 1 atm pressure, 382 mL of CH_4/g of O_2 is produced.

2.6. Physicochemical characterization

The solid fraction was subjected to dialysis against distilled water for 24 h at room temperature using cellulose membrane (Sigma Aldrich) of 12,000 Da. Subsequently, the dialyzed solid fraction was lyophilized using a Telstar model Lyoquest-55 lyophilizer (Terassa, Barcelona). The dry powder was weighed and expressed as grams of extracted material obtained per kilogram of olive leaves (OL).

Total sugars were analyzed using the colorimetric anthrone method described by Witham et al. (1971) using a spectrophotometer (BIO-RAD iMark Microplate Reader, USA). The results were expressed as grams of D-glucose per kilogram of OL. Uronic acids were quantified using the m-hydroxydiphenyl method, and the results were expressed as grams of galacturonic acid per kilogram of OL (Blumenkrantz and Asboe-Hansen, 1973).

Individual neutral sugars were analyzed in duplicate samples of solubilized fractions with and without initial TFA hydrolysis before reduction, acetylation, and analysis by gas chromatography (GC) (Hewlett–Packard 5890 series II), using the method described by Lama-Muñoz et al. (2012).

The total phenolic content was determined using the Folin-Ciocalteu spectrophotometric method and expressed as grams of gallic acid equivalents per kilogram of OL (Singleton and Rossi, 1965).

The antioxidant activity of the extracts was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method (Brand-Williams et al., 1995) and expressed as milligrams of Trolox per kilogram of OL. The reducing power (RP) was expressed as milligrams of Trolox per kilogram of OL from the equation described by Rodríguez-Gutiérrez et al. (2019).

The sample extracts were centrifuged at 3779×*g* for 3 min and filtered through 0.45 μm nylon filters. Individual phenolic compounds were determined using a previously optimized and validated methodology using a Hewlett-Packard 1100 HPLC system coupled to a photodiode array detector (DAD). Briefly, the separation of phenolic compounds was performed on a Teknokroma Tracer Extrasil OSD2 column (5 μm, 25 mm × 0.46 mm x 2.1). The injection volume was 20 μL, and the flow rate was set at 1 mL/min. Separation was achieved using solvent A (Milli-Q water, pH 2.5 adjusted with 20 mM TFA) and solvent B (acetonitrile). The elution gradient was programmed as follows: 0–3 min, 0% B; 3–16 min, 0–12% B; 16–16.5 min, 12–16% B; 16.5–21min, 16% B; 21–25 min, 16–20% B; 25–30 min, 20% B; 30–31 min, 20–100% B; 31–34 min, 100% B; 34–39 min, 0% B. The individual phenolic compounds were identified by comparing their retention times and UV/Vis spectra with those of commercial standards at a wavelength of 280 nm.

To monitor the AD process, the pH, alkalinity, total solids (TS), total volatile solids (VS), total chemical oxygen demand (CODtot), and soluble chemical oxygen demand (CODs) were assessed using the methods established by the American Public Health Association

Table 1
Glycoside composition (mg/g of solid fraction), total oligosaccharides, and glycoside composition percentage of total oligosaccharides.

mg/g	ChCl:OA (1:1)	ChCl:U (1:2)	ChCl:LA (1:2)	ChCl:OA (2:1)	ChCl:Gl (3:1)
Rhamnose	1.00 ± 0.01	n.d	0.55 ± 0.02	n.d	n.d
Fucose	n.d	n.d	n.d	n.d	n.d
Arabinose	2.10 ± 0.05	3.28 ± 0.08	n.d	0.95 ± 0.02	1.00 ± 0.01
Xylose	3.16 ± 0.08	5.06 ± 0.13	0.61 ± 0.03	1.00 ± 0.03	0.41 ± 0.02
Mannose	1.93 ± 0.05	2.66 ± 0.07	0.63 ± 0.04	2.00 ± 0.05	0.62 ± 0.04
Galactose	3.00 ± 0.08	n.d	n.d	n.d	n.d
Glucose	1.93 ± 0.05	2.31 ± 0.06	1.38 ± 0.2	0.80 ± 0.02	1.30 ± 0.03
Total oligosaccharides	174.9 ± 5.2	64.8 ± 2.6	134.1 ± 3.6	85.9 ± 2.15	90.8 ± 2.8
% Total oligosaccharides composition					
Rhamnose	4	7	4	0	7
Fucose	1	0	9	1	1
Arabinose	18	13	28	15	11
Xylose	36	3	24	8	6
Mannose	9	4	4	12	10
Galactose	10	15	4	33	20
Glucose	22	58	26	31	44

*n.d.: not detected.

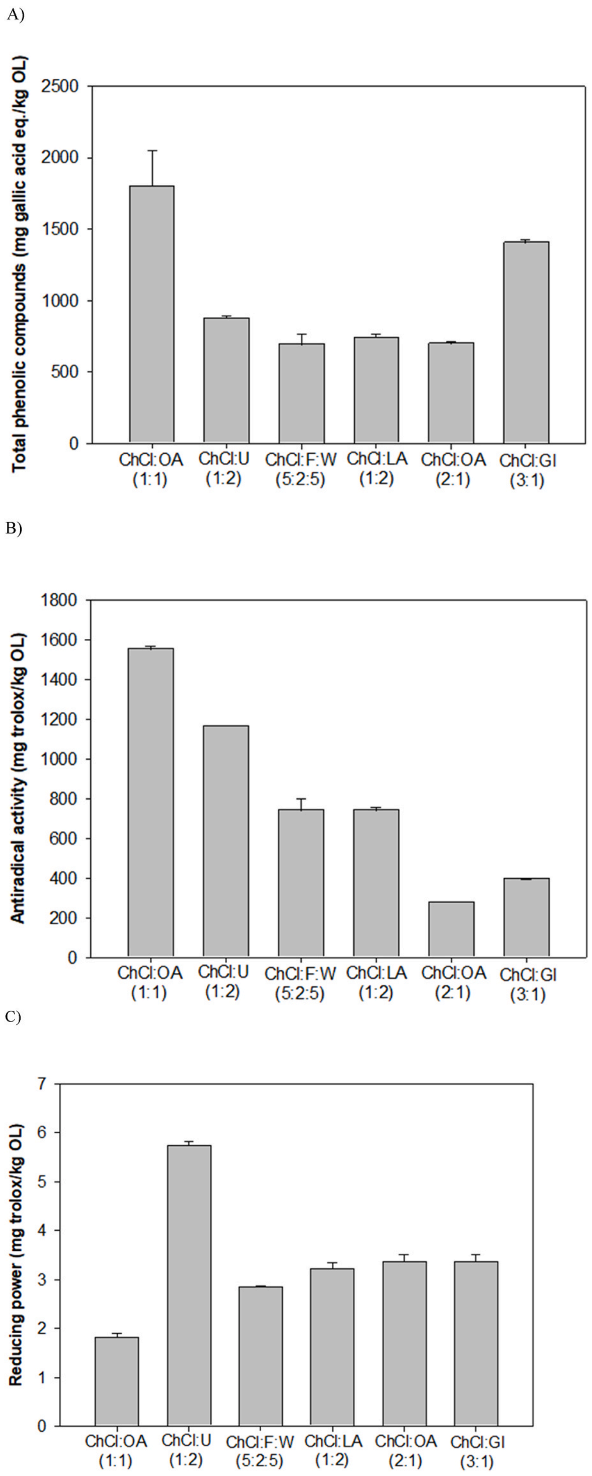

Fig. 3. Column plots with standard deviation bars representing the total phenolic compounds (A), antioxidant activity (B) and reducing power (C).

(APHA) (APHA, 2017).

3. Results and discussion

3.1. Study of solid fraction obtained by mechanochemical extraction process

Fig. 2A shows the data obtained from the mechanochemical extraction process, expressed as grams of solid fraction extracted per kilogram of OL. Using NADES ChCl:LA (1:2) and ChCl:Gl (3:1) extracted more than 12 g of solid fraction/kg OL, while the other four NADES studied, i.e., ChCl:OA (1:1), ChCl:U (1:2), ChCl:F:W (5:2:5), and ChCl:OA (2:1), extracted less than 5 g of solid fraction/kg OL. In addition, ChCl:LA (1:2) yielded 18% more solid fraction than ChCl:Gl (3:1). In a study by Chen and Lahaye (2021), the NADES ChCl:OA, ChCl:U, and ChCl:LA were used with a 1:2 ratio to extract pectin material from apple pulp using thermal treatment. The authors concluded that the NADES with the highest pectin material extraction efficiency was ChCl:LA (1:2), which is consistent with the data obtained in this study. Fig. 2A also shows that NADES formed from oxalic acid had a low solid fraction yield. This could be due to the severe hydrolysis of oligosaccharides to monosaccharides during extraction. As shown in Fig. 2A, ChCl:OA (1:2) extracted almost twice as much solid fraction as the same NADES with a 1:1 ratio, demonstrating that the ratio of each NADES component influences the amount of solid fraction extracted. Other studies have shown that the viscosity of NADES is affected by the ratios of its components, which in turn affects the extraction of compounds (Kaoui et al., 2023; Santos-Martín et al., 2023).

Table 1 shows the results of the study on the glycosidic composition of the solid fraction of OL extracted using a ball mill and NADEs. The monosaccharides are shown first, expressed as milligrams of each monosaccharide per gram of solid fraction. The total oligosaccharides, expressed as milligrams per gram of solid fraction after hydrolysis, and the percentage of each individual glucoside are then indicated. The presence of 58% glucose in the sample extracted with NADES ChCl:U (1:2) is likely due to the hydrolysis cellulose fibers (Palamae et al., 2017). Cellulose extraction is a complex process, and generally requires more severe methods, such as high temperatures and more aggressive solvents. For example, in the study by Dinh Vu et al. (2017), cellulose and lignin were extracted using alkaline treatment with NaOH at 90 °C for 1.5 h in combination with ultrasound. Other samples, such as the one extracted with NADES ChCl:Gl (1:1), showed a reasonably large percentage of glucose and xylose, possibly from the hydrolysis of hemicellulose (Pauly et al., 2013). In this study, the oligosaccharides in the hemicellulose were mainly of the xyloglucan type (Table 1), with galactose and arabinose also present, likely due to substitution of xylose molecules (Pauly et al., 2013). Lama-Muñoz et al. (2020) quantified the composition of total oligosaccharides and glycoside composition in the Picual variety of olive oil, finding that it contained 4.1 ± 0.4 mg/g arabinose, 1.1 ± 0.1 mg/g xylose, 46.4 ± 2.3 mg/g mannose, 9.4 ± 0.3 mg/g galactose, and 73.3 ± 1.7 mg/g glucose.

3.2. Study of supernatant obtained by mechanochemical extraction process

Fig. 2B shows the data for total sugars in the supernatant, expressed in milligrams of glucose equivalents for each kilogram of OL. The NADES ChCl:OA (1:1) extracted the most monosaccharides, likely due to hydrolysis of oligosaccharides during the extraction process. Three NADES with higher solid fraction, ChCl:LA (1:2), ChCl:OA (2:1), and ChCl:Gl (3:1), had the lowest total sugar concentration. These three NADES did not significantly hydrolyze of oligosaccharides to monosaccharides. NADES ChCl:F:W (5:2:5) was discarded because the presence of fructose in the NADES inflated the extracted sugars values, causing overlap with the other results (54,406 ± 320 mg of glucose eq./kg OL). Fig. 2C shows the data for uronic acids in the sample, expressed in mg of galacturonic acid equivalents per kilogram of OL used in the extraction. NADES ChCl:OA (1:1) and ChCl:LA (1:2) had the highest uronic acid concentrations. Uronic acids are a component of acid carbohydrates or pectins, which are components of the lignocellulosic fraction. Pectins are of interest because they can be used as a gelling or thickening agent (Rodríguez-Gutiérrez et al., 2019).

Fig. 3A shows the results for total phenolic compounds in the supernatant, expressed in milligrams of gallic acid equivalents per kilogram of OL used in the extraction. Selectivity was observed in NADES ChCl:OA (1:1), which extracted the most total phenolic compounds, i.e., 1.7 mg g^{-1}. NADES ChCl:Gl (3:1) was the next most effective, extracting 28% less. It should also be noted that NADES ChCl:OA (2:1) extracted fewer than NADES ChCl:OA (1:1). In a previous study carried out by Alañón et al. (2020), NADES ChCl:OA (1:1) and ChCl:LA (1:2) were used to extract total phenolic compounds from olive leaves by microwave. The results were contradictory to those of this study, with NADES of the ChCl:LA (1:2) extracting more total phenolic compounds than NADES ChCl:OA (1:1), i.e., around 20 mg g^{-1}. Another study found high extraction yields using ultrasound-assisted extraction (UAE) with NADES ChCL:f:W (5:2:5) and ChCl:LA (1:2), ranging from 20 to 10 mg/g (Ünlü, 2021). Two solvents with the same molar ratio, such as ChCl:U (1:2) and ChCl:LA (1:2), demonstrated similar phenol extraction concentrations under identical conditions, despite the urea-based solvent having a higher viscosity (Sazali et al., 2023; Shekaari et al., 2017). This outcome can be attributed to the application of mechanochemistry, which effectively enhances solubilization by overcoming the limitations imposed by the high viscosity of NADES (Santana et al., 2019). A different profile of individual phenolic compounds was observed in the chromatograms obtained by HPLC-UV at 280 nm (Fig. S1). The phenolic profile obtained using the different NADES was not similar, due to the selectivity of the NADES for extracting specific types of phenolic compounds. Hydroxytyrosol and oleuropein were the two most abundant individual phenolic compounds, followed by lower concentrations of 3,4-dihydroxyphenylglycol, tyrosol, apigenin-7-O-glucoside, luteolin-7-O-glucoside, luteolin, and apigenin (Siamandoura and Tzia, 2023; Zurob et al., 2020).

Fig. 3B shows the antiradical activity values in the supernatant, expressed in milligrams of Trolox equivalents for each kilogram of OL used in the extraction. Bakirtzi et al. (2016) obtained a highly significant correlation between antiradical activity and the amount of total phenolic compounds extracted. They concluded that the antiradical activity of the phenolic extract depends to a certain extent on the amount of total phenolic compounds present in the sample (Ünlü, 2021). The findings of this study are consistent with those of Bakirtzi et al. (2016), as the NADES ChCl:OA (1:1) had the highest antiradical activity and also extracted the most total phenolic compounds. This suggests that it has a greater ability to scavenge free radicals. The NADES made up of urea was the next most effective at scavenging free radicals, with 33% less activity than NADES ChCl:OA (1:1). The NADES ChCl:LA (1:2), ChCl:OA (2:1), and ChCl:Gl (3:1) extracted fewer total phenolic compounds and had lower antiradical activity, suggesting that heir phenolic compounds have a lower capacity to scavenge free radicals. Interestingly, the NADES ChCl:OA (1:1) exhibited higher free radical scavenging capacity than its analogue with a (2:1) ratio, despite extracting fewer total phenolic compounds, This suggests that the specific types of phenolic

Table 2

Regression coefficients and analysis of variance of the models obtained by response surface methodology for lignocellulosic material and phenolic compound extraction. The p-values for each coefficient are indicated in parentheses.

Regression coefficients	Responses of lignocellulosic material		
	Solid fraction	Total soluble sugars	Uronic acids
B_0	−32864,6 (0.000194)	−530585 (0.001367)	−658881 (0.077458)
B_1	644,4 (0.000168)	10277 (0.001370)	13005 (0.072325)
B_2	−66,9 (0,8252189	1058 (0.857695)	−8136 (0.568001)
B_3	−137,3 (0.611334)	6312 (0.234998)	2081 (0.869209)
B_{12}	0,8 (0.799239)	−11 (0.850630)	73 (0.603574)
B_{13}	1,3 (0.614163)	−67 (0.187420)	−31 (0.800199)
B_{23}	−1 (0.883245)	418 (0.002460)	349 (0.258168)
B_{11}	−3,2 (0.000151)	−50 (0.001430)	−64 (0.068734)
B_{22}	−2,3 (0.715016)	−199 (0.120363)	50 (0.867643)
B_{33}	−0,4 (0.940705)	−58 (0.622012)	130 (0.644805)
Validation of the model			
R^2	0.514	0.582	0.277
R^2_{adj}	0.358	0.448	0.045
p-value (model)	0.007	0.001	0.337
p-value (lack of fit)	0.1344	0.003	0.000

Regression coefficients	Responses of phenolic compound				
	Total phenolic compound	Antiradical activity	Reducing power	Oleuropein	Hydroxytyrosol
B_0	**−1367558** (0.018298)	**164966.9** **(0.039764)**	**−764282** **(0.000470)**	**−13223.4** **(0.012135)**	−1327.02 (0.767108)
B_1	**27329** **(0.0215240)**	**−2891.7** **(0.050526)**	**14906** **(0.000438)**	**260.8** **(0.010868)**	27.62 (0.750368)
B_2	−14032 (0.516925)	−6206.8 (0.047435)	−7109 (0.355281)	−232.4 (0.231696)	−114.79 (0.514378)
B_3	−21756 (0.262265)	−11378.0 (0.000289)	4700 (0.490557)	93.8 (0.582079)	67.44 (0.666049)
B_{12}	149 (0.482813)	63.7 (0.038809)	77 (0.305358)	2.3 (0.222671)	1.14 (0.510892)
B_{13}	191 (0.303746)	**108.1** **(0.000317)**	−43 (0.510424)	−1.2 (0.464136)	−0.73 (0.624499)
B_{23}	425 (0.363093)	37.4 (0.561359)	−87 (0.596057)	2.6 (0.527453)	−0.34 (0.928130)
B_{11}	**−136** **(0.012948)**	**12.5** **(0.050698)**	**−73** **(0.000416)**	**−1.3** **(0.010072)**	−0.14 (0.737459)
B_{22}	−685 (0.141943)	−128.6 (0.052239)	−243 (0.140507)	−4.2 (0.303873)	−0.40 (0.915215)
B_{33}	377 (0.381604)	38.9 (0.512409)	−73 (0.630342)	4.9 (0.204855)	2.18 (0.531900)
Validation of the model					
R^2	0.584	0.725	0.682	0.715	0.180
R^2_{adj}	0.450	0.637	0.580	0.642	0
p-value (model)	0.008	0.0000	0.035	0.0000	0.721
p-value (lack of fit)	0.00004	0.004	0.455	0.497	0.984

compounds extracted by NADES ChCl:OA (1:1) are more effective at scavenging free radicals.

Fig. 3C shows the reducing power data for the supernatant, expressed in milligrams of Trolox equivalents for each kilogram of OL used in the extraction. NADES ChCl:U (1:2) had the highest reducing power, despite having one of the highest values for total phenolic compounds and antiradical activity. This suggests that the phenolic compounds extracted by this solvent have a greater capacity to donate electrons or act as proton acceptors than those extracted with the other solvents, which had lower and very similar values among them. Ivanović et al. (2022) investigated the impact of NADES on the reducing power of phenolic compound extracts. They found that NADES containing lactic acid exhibited the highest reducing power values, while ChCl:U (2:1) had the highest values in this study.

3.3. *Optimization of the extraction conditions using an experimental design*

As shown previously, the extraction of lignocellulosic material and phenolic compounds depends on influencing different factors. Therefore, an experimental central composite design was used to optimize the process. A series of 19 assays were performed in

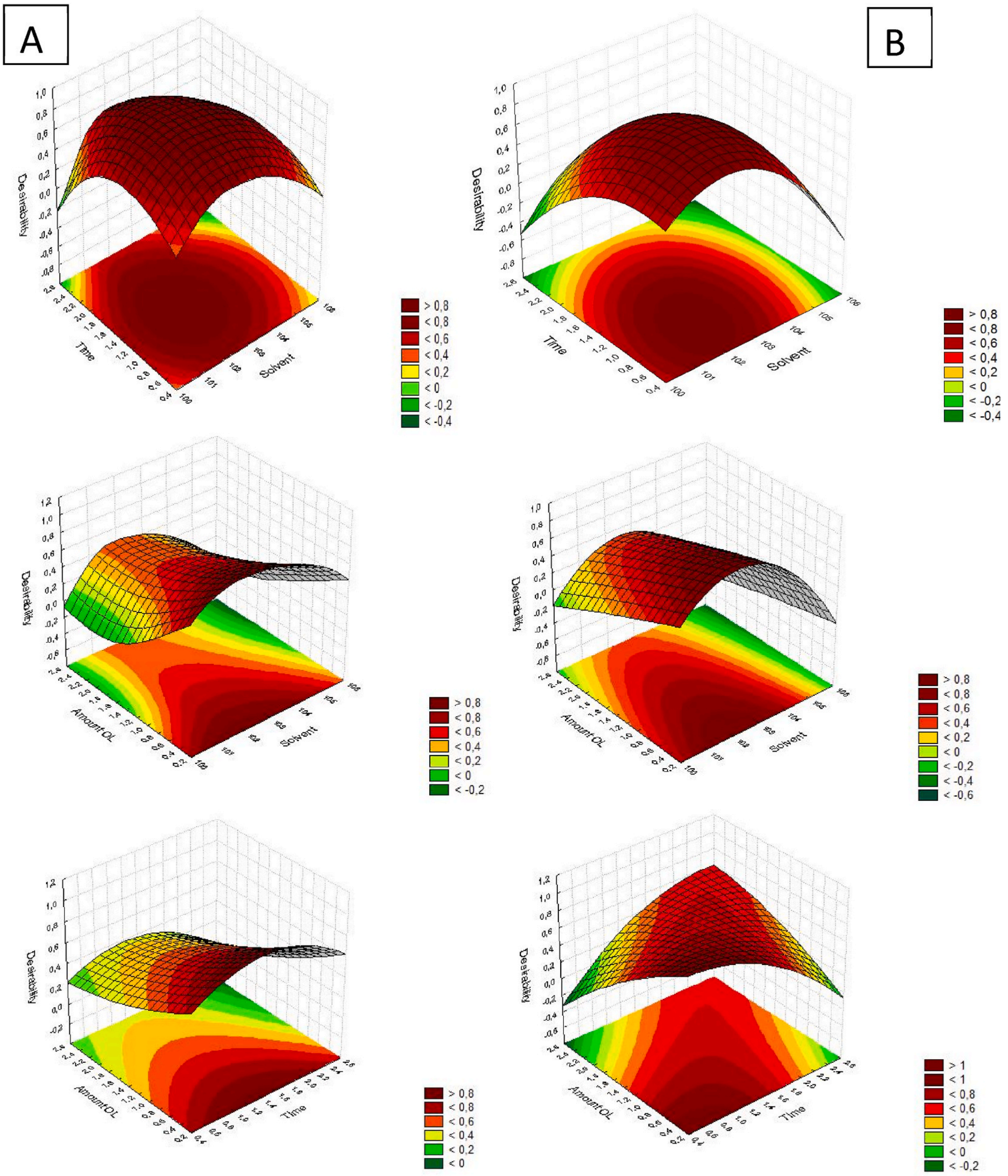

Fig. 4. Response surface plots for the phenolic compounds (A) and oligosaccharides from lignocellulosic material (B).

duplicate (Tables S2 and S3), considering these three factors: type of solvent (X_1) used (ChCl:AO (2:1), ChCl:LA (1:2), ChCl:Gl (3:1), hexane, and MeOH 80%), the extraction time (X_2), and the amount of OL (X_3). For this purpose, the response variables studied were the extracted amounts of lignocellulosic material, total soluble, sugars and uronic acids, on the one hand, and the contents of total phenolic compounds, hydroxytyrosol, oleuropein, antiradical activity and reducing power, on the other.

The results were then fitted to a second-order polynomial model (equation (1)). The corresponding regression coefficients describing the quantitative relationship between dependent variables and factors are shown in Table 2. ANOVA assessed the statistical validity of these models through the regression and lack of fit F tests. Statistical analysis showed that the proposed models were adequate and showed statistically valid fitness ($p < 0.05$ for the regression F test) except for hydroxytyrosol and uronic acids and non-significant lack of fit ($p > 0.05$) for reducing power, oleuropin and hydroxytyrosol content and extracted lignocellulosic material. Additionally, the coefficient of determination (R^2) and adjusted determination coefficient (R^2_{adj}) were also used to provide additional confirmation of the statistical validity of the model fit (López-Carbón et al., 2019). The values obtained showed that, in most cases, the multiple linear regression models could explain more than 50% of the variability of the data.

The significance of each coefficient of regression was also determined by using ANOVA. As can be observed in Tables 2 and **S2**, according to results from Fisher tests the value of all dependent variables, except hydroxytyrosol and uronic acids, were significantly affected by the type of solvent (linear and quadratic effect). Furthermore, an interaction effect between solvent and amount of OL (1x2) and between time and amount of OL (1x3) was also found for antioxidant activity and total soluble sugars, respectively.

In order to simultaneously maximize the extraction of both types of compounds, a desirability function approach was finally applied. This method transforms each response variable to give a desirability value proportional to the priority given to the response variable. The three-dimensional surface plots and contour plots were drawn to highlight the effects of the independent variables on the desirability function. The response surface analysis shows the optimal values of the different factors considered in the experimentation (Fig. 4). From these three-dimensional graphs, it is possible to deduce the direction in which to move to find the maximum value of each factor and, therefore, the optimal value. The plot of Fig. 4 shows the effect of all pairs of independent variables on overall response desirability obtained for the variables related to phenolic compounds (A) and lignocellulosic material (B). As can be observed, the type of solvent at the middle level produces the most desirable response on these dependent variables. Moreover, based on this graph. Keeping the solvent constant ChCl:Gl (3:1). a maximum point can be observed at the middle level of extraction time and the low amount of OL. The optimization process suggests that the use of NADES is more effective than organic solvents like methanol and hexane in extracting the desired compounds (Li et al., 2023). After applying the desirability function method, the optimal conditions for the extraction of lignocellulosic material and phenolic compounds from olive leaves using a mechanical procedure based on a ball mill were the following; extract 0.5 g of OL using 10 mL of a green solvent based on ChCl:Gl (3:1) and prolonging the extraction for 1.1 h in ball mill. Under these specified conditions, this technique has successfully extracted hydroxytyrosol, a relevant phenolic compound in current studies, widely studied for its antioxidant and beneficial properties for human health, having cardio and neuroprotective effects, among other properties positive (Zurob et al., 2020). The presence of hydroxytyrosol can also be observed, a catechol derived from oleuropein, which acts as an excellent natural antioxidant and can be found in olives or olive oil (Erkoç et al., 2003).

3.4. *Anaerobic digestibility study*

To assess the treatment effect using a BMP test, the anaerobic biodegradability of untreated OL and OL waste was evaluated. OL waste was generated by extraction with NADES and ball mills under the optimal conditions, i.e., 0.5 g of OL, 10 mL of ChCl:Gl (3:1), and prolonging the extraction for 1.1 h. The biodegradability of the NADES used in the extraction was also evaluated to determine the

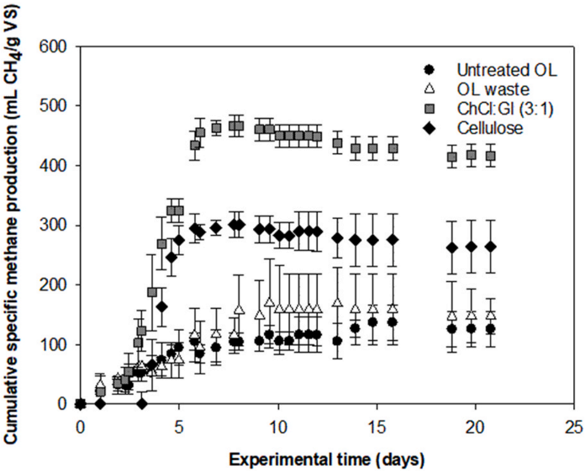

Fig. 5. Cumulative specific methane production (mL CH_4/g VS) to cellulose, untreated OL, ChCl:Gl (3:1), and OL waste generated after extracting the lignocellulosic material with NADES ChCl:Gl (3:1) and ball mill.

influence of its component biodegradability on the bonds formed in the NADES.

The cumulative specific methane production (mL CH_4/g VS) during the experimental time is shown in Fig. 5. Cellulose biodegradation showed a methane yield coefficient of 300 ± 22 mL CH_4/g VS (Fig. 5 and Table 3). Cellulose has often served as a positive control substrate in BMP test, consistent with findings from previous research (Raposo et al., 2020). These studies have reported methane yield coefficient values within a similar range, with a theoretical production of 414 mL CH_4/g VS (Garcia et al., 2019; Hafner et al., 2020). NADES ChCl:Gl (3:1) produced 417 ± 20 mL CH_4/g VS, which is 109 ± 5% of biodegradability due to the natural compound feasibility of the degradation. This methane production confirmed that the new bond formed in the NADES synthesis did not affect its biodegradability. Untreated OL showed a cumulative specific methane production of 126 ± 30 mL CH_4/g VS, which is 22 ± 5% of biodegradability (Fig. 5 and Table 3). This methane production is similar to that reported for other wastes generated in the olive oil industry, such as olive mill solid waste, whose methane production ranges between 154 and 415 mL CH_4/g VS (Serrano et al., 2021).

Extraction of lignocellulosic material from OL did not significantly affect methane production. The methane production obtained for OL waste generated after extraction was 142 ± 53 mL CH_4/g VS, which represents 24 ± 9% of biodegradability, an increase of 13% compared to untreated OL (Fig. 5 and Table 3). This increase may be due to the solubilization and hydrolysis of the OL caused by the combined effect of the ball mill and the ChCl:Gl (3:1) solvent, as seen in the optimization sections. Similar results have been reported for other biomass with high lignocellulosic material content. For example, ball milling of raspberry extrudate to solubilize the organic matter significantly increased methane production from 18 to 236 mL CH_4/g VS (Trujillo-Reyes et al., 2022). Similarly, sugarcane leaves were subjected to a similar pre-treatment process, using deep eutectic solvent derived from a mixture of ChCl and Monoethanolamine (MEA) to remove lignin and enhance the biodegradation of the leaves via fermentation, producing hydrogen and methane in two steps (Miftah et al., 2022).

Despite the pre-treatment effect, the AD processes were stable, whit pH values within the optimal range for methanogenic activity, i.e., 6.5–8.5, and alkalinity concentrations less than 1000 mg $CaCO_3$/L for all tested substrates, which is lower than recommended value in the literature (Table 2) (Trujillo-Reyes et al., 2023a). Non-biodegradable organic matter in OL waste is also shown in 32 % VS and 9% COD, with a concentration higher than cellulose. Volatile fatty acids were not detected in the final effluent of any of the substrates studied, using the method described by Trujillo-Reyes et al. (2023b). These results suggest that the remaining organic matter is difficult to degrade into simpler compounds and that a stronger pre-treatment is needed to solubilize it. Additionally, continuous research on mechanochemistry has shown a notable rise in scientific and patent output, pointing to efforts to industrialize this method for various applications (Reynes et al., 2023). A prominent example is its use in the mining industry, where the ball mill technique holds significant economic and operational importance in managing mineral processing plants (Tohry et al., 2020). Future studies should focus on lowering the production costs of the NADES components, since these components are present in several agro-industrial by-products, these by-products should be used as raw material for their production, and the use of these components not in pure form but in cheaper extracts should be studied. The regeneration of NADES should also be studied so that they can be used many times, substantially reducing their costs (Yiin et al., 2024).

4. Conclusions

The efficient utilization of olive leaves (OL) through innovative and sustainable biorefinery approaches could convert these wastes into high-value products, reduce their accumulation and disposal, and mitigate adverse environmental problems. Mechanochemical extraction, which combines the extraction process and NADES formation in a single step, is a promising and cost-effective novel technique. Among the various NADES tested, ChCl:Gl (3:1) proved to be the optimal solvent for extracting phenolic compounds and lignocellulosic material. The following conditions achieved the maximum yield of high-value compounds: 0.5 g of milled OL, 10 mL of ChCl:Gl (3:1) and 1.1 h of extraction in a ball mill. This resulted in the production of 14.5 ± 2.9 g of solid fraction and 1.3 ± 0.3 g of gallic acid equivalent per kilogram of OL. The OL waste generated after the extraction was tested for methane production using BMP test. It produced a similar amount of methane to untreated OL, i.e., 142 ± 53 mL CH_4/g VS. Thus, the proposed biorefinery scheme has the potential to enhance the production of phenolic compounds, lignocellulosic material, and biomethane. NADES represent promising solvents for reducing dependence on organic solvents. However, further research is required to fully understand and address the current limitations of NADES, particularly concerning their industrial applicability. Therefore, reusing and saturating these NADES, studying new formulations, or seeking synergies with other treatments could help reduce the solvent-to-feedstock ratio.

Table 3
Analytical characterization of effluents from the AD process at the end of the BMP tests with their standard deviations.

		Untreated OL	OL waste	ChCl:Gl (3:1)	Cellulose
pH		6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.1
Alkalinity	mg $CaCO_3$/L	943 ± 36	928 ± 26	867 ± 19	802 ± 15
TS	mg/kg	4400 ± 1319	3804 ± 73	3038 ± 39	3010 ± 147
VS	mg/kg	2317 ± 147	2685 ± 195	1964 ± 89	1823 ± 192
CODs	mg O_2/L	203 ± 118	139 ± 59	116 ± 32	126 ± 35
Phenolic compounds	mg gallic acid/L	36.0 ± 0.1	32.8 ± 1.3	29.9 ± 0.5	30.6 ± 1.8
Methane production	mL CH_4/g VS	126 ± 30	142 ± 53	417 ± 20	300 ± 22
Biodegradability (COD)	%	22 ± 5	24 ± 9	109 ± 5	79 ± 6

CRediT authorship contribution statement

Juan Cubero-Cardoso: Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manuel Hernández-Escaño:** Writing – original draft, Methodology, Formal analysis, Data curation. **Ángeles Trujillo-Reyes:** Writing – original draft, Supervision, Methodology, Formal analysis. **Fernando G. Fermoso:** Resources, Funding acquisition. **Ma Ángeles Fernández-Recamales:** Writing – original draft, Formal analysis, Data curation. **Juan Fernández-Bolaños:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Guillermo Rodríguez-Gutiérrez:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Juan Urbano:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Funding

This work has received funding from Junta de Andalucía, within the framework of the “Programa Operativo FEDER 2014–2020” (UHU-202063).

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.

Acknowledgments

The authors also thank ALGRY Química S.L. for kindly supplying the choline chloride reagent**.**

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2024.101879.

Data availability

Data will be made available on request.

References

Alañón, M.E., Ivanović, M., Gómez-Caravaca, A.M., Arráez-Román, D., Segura-Carretero, A., 2020. Choline chloride derivative-based deep eutectic liquids as novel green alternative solvents for extraction of phenolic compounds from olive leaf. Arab. J. Chem. 13, 1685–1701. https://doi.org/10.1016/j.arabjc.2018.01.003.

APHA, 2017. Standard Methods for Examination of Water and Wastewater. Am. Public Heal. Assoc., Washington, DC, USA doi.org/ISBN9780875532356.

Bakirtzi, C., Triantafyllidou, K., Makris, D.P., 2016. Novel lactic acid-based natural deep eutectic solvents: efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants. J. Appl. Res. Med. Aromat. Plants 3, 120–127. https://doi.org/10.1016/j.jarmap.2016.03.003.

Benvenutti, L., Sanchez-Camargo, A., del, P., Zielinski, A.A.F., Ferreira, S.R.S., 2020. NADES as potential solvents for anthocyanin and pectin extraction from Myrciaria cauliflora fruit by-product: in silico and experimental approaches for solvent selection. J. Mol. Liq. 315, 113761. https://doi.org/10.1016/j.molliq.2020.113761.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489. https://doi.org/10.1016/0003-2697(73)90377-1.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Brandt, A., Gräsvik, J., Hallett, J.P., Welton, T., 2013. Deconstruction of lignocellulosic biomass with ionic liquids. Green Chem. 15, 550–583. https://doi.org/10.1039/c2gc36364j.

Carbonell-Rozas, L., Canales, R., Lara, F.J., García-Campaña, A.M., Silva, M.F., 2021. A natural deep eutectic solvent as a novel dispersive solvent in dispersive liquid-liquid microextraction based on solidification of floating organic droplet for the determination of pesticide residues. Anal. Bioanal. Chem. 413, 6413–6424. https://doi.org/10.1007/s00216-021-03605-z.

Chen, M., Lahaye, M., 2021. Natural deep eutectic solvents pretreatment as an aid for pectin extraction from apple pomace. Food Hydrocolloids 115, 106601. https://doi.org/10.1016/j.foodhyd.2021.106601.

Chen, Z., Bai, X., Lusi, A., Wan, C., 2018. High-solid lignocellulose processing enabled by natural deep eutectic solvent for lignin extraction and industrially relevant production of renewable chemicals. ACS Sustain. Chem. Eng. 6, 12205–12216. https://doi.org/10.1021/acssuschemeng.8b02541.

Clodoveo, M.L., Crupi, P., Annunziato, A., Corbo, F., 2022. Innovative extraction technologies for development of functional ingredients based on polyphenols from olive leaves. Foods 11. https://doi.org/10.3390/foods11010103.

Cortés-Triviño, E., Cubero-Cardoso, J., Tenorio-Alfonso, A., Fernández-Recamales, M.A., Valencia, C., Urbano, J., Franco, J.M., 2022. Structuring natural deep eutectic solvents with epoxidised lignin-enriched residues: a green alternative to petroleum-based thickened formulations. J. Mol. Liq. 360, 119433. https://doi.org/10.1016/J.MOLLIQ.2022.119433.

Dinh Vu, N., Thi Tran, H., Bui, N.D., Duc Vu, C., Viet Nguyen, H., 2017. Lignin and cellulose extraction from vietnam's rice straw using ultrasound-assisted alkaline treatment method. Int. J. Polym. Sci. 2017. https://doi.org/10.1155/2017/1063695.

Douard, L., Belgacem, M.N., Bras, J., 2022. Extraction of carboxylated nanocellulose by combining mechanochemistry and NADES. ACS Sustain. Chem. Eng. 10, 13017–13025. https://doi.org/10.1021/ACSSUSCHEMENG.2C02783/ASSET/IMAGES/LARGE/SC2C02783_0011.JPEG.

Elgharbawy, A.A.M., Hayyan, A., Hayyan, M., Mirghani, M.E.S., Salleh, H.M., Rashid, S.N., Ngoh, G.C., Liew, S.Q., Nor, M.R.M., Zulkifli, M.Z., Alias, Y., 2019. Natural deep eutectic solvent-assisted pectin extraction from pomelo peel using sonoreactor: experimental optimization approach. Processes 7, 416. https://doi.org/10.3390/pr7070416.

Erkoç, F., Keskin, N., Erkoç, Ş., 2003. Theoretical investigation of hydroxytyrosol and its radicals. J. Mol. Struct. THEOCHEM 625, 87–94. https://doi.org/10.1016/S0166-1280(03)00006-X.

Fermoso, F.G., Serrano, A., Alonso-Fariñas, B., Fernández-Bolaños, J., Borja, R., Rodríguez-Gutiérrez, G., 2018. Valuable compound extraction, anaerobic digestion, and composting: a leading biorefinery approach for agricultural wastes. J. Agric. Food Chem. 66, 8451–8468. https://doi.org/10.1021/acs.jafc.8b02667.

Fernández-Prior, M.Á., Fatuarte, J.C.P., Oria, A.B., Viera-Alcaide, I., Fernández-Bolaños, J., Rodríguez-Gutiérrez, G., 2020. New liquid source of antioxidant phenolic compounds in the olive oil industry: alperujo water. Foods 9, 962. https://doi.org/10.3390/foods9070962.

Garcia, N.H., Mattioli, A., Gil, A., Frison, N., Battista, F., Bolzonella, D., 2019. Evaluation of the methane potential of different agricultural and food processing substrates for improved biogas production in rural areas. Renew. Sustain. Energy Rev. 112, 1–10. https://doi.org/10.1016/J.RSER.2019.05.040.

Hafner, S.D., de Laclos, H.F., Koch, K., Holliger, C., 2020. Improving inter-laboratory reproducibility in measurement of biochemical methane potential (BMP). Water 12, 1752. https://doi.org/10.3390/W12061752.

Hajiali, F., Vidal, J., Jin, T., De La Garza, L.C., Santos, M., Yang, G., Moores, A., 2022. Extraction of chitin from green crab shells by mechanochemistry and aging. ACS Sustain. Chem. Eng. 10, 11348–11357. https://doi.org/10.1021/ACSSUSCHEMENG.2C02966/ASSET/IMAGES/LARGE/SC2C02966_0005.JPEG.

Ivanović, M., Grujić, D., Cerar, J., Razboršek, M.I., Topalić-Trivunović, L., Savić, A., Kočar, D., Kolar, M., 2022. Extraction of bioactive metabolites from Achillea millefolium L. with choline chloride based natural deep eutectic solvents: a study of the antioxidant and antimicrobial activity. Antioxidants 11, 724. https://doi.org/10.3390/antiox11040724.

Jovanović, M.S., Krgović, N., Radan, M., Ćujić-Nikolić, N., Mudrić, J., Lazarević, Z., Šavikin, K., 2023. Natural deep eutectic solvents combined with cyclodextrins: a novel strategy for chokeberry anthocyanins extraction. Food Chem. 405, 134816. https://doi.org/10.1016/J.FOODCHEM.2022.134816.

Kaoui, S., Chebli, B., Zaidouni, S., Basaid, K., Mir, Y., 2023. Deep eutectic solvents as sustainable extraction media for plants and food samples: a review. Sustain. Chem. Pharm. 31, 100937. https://doi.org/10.1016/J.SCP.2022.100937.

Lama-Muñoz, A., del Mar Contreras, M., Espínola, F., Moya, M., Romero, I., Castro, E., 2020. Characterization of the lignocellulosic and sugars composition of different olive leaves cultivars. Food Chem. 329, 127153. https://doi.org/10.1016/j.foodchem.2020.127153.

Lama-Muñoz, A., Rodríguez-Gutiérrez, G., Rubio-Senent, F., Fernández-Bolaños, J., 2012. Production, characterization and isolation of neutral and pectic oligosaccharides with low molecular weights from olive by-products thermally treated. Food Hydrocolloids 28, 92–104. https://doi.org/10.1016/j.foodhyd.2011.11.008.

Li, K., Li, Z., Men, L., Li, J., Gong, X., 2023. Deep eutectic solvent-based ultrasound-assisted strategy for simultaneous extraction of five macamides from lepidium meyenii walp and in vitro bioactivities. Foods 12. https://doi.org/10.3390/FOODS12020248/S1.

López-Carbón, V., Sayago, A., González-Domínguez, R., Fernández-Recamales, Á., 2019. Simple and efficient green extraction of steviol glycosides from Stevia rebaudiana leaves. Foods 8, 402. https://doi.org/10.3390/FOODS8090402.

Ma, Y., Li, P., Li, Y., Willot, S.J.P., Zhang, W., Ribitsch, D., Choi, Y.H., Verpoorte, R., Zhang, T., Hollmann, F., Wang, Y., 2019. Natural deep eutectic solvents as multifunctional media for the valorization of agricultural wastes. ChemSusChem 12, 1310–1315. https://doi.org/10.1002/CSSC.201900043.

Miftah, A.K., Sittijunda, S., Imai, T., Salakkam, A., Reungsang, A., 2022. Biohydrogen and methane production from sugarcane leaves pretreated by deep eutectic solvents and enzymatic hydrolysis by cellulolytic consortia. Fermentation 8, 396. https://doi.org/10.3390/FERMENTATION8080396.

Millati, R., Wikandari, R., Ariyanto, T., Hasniah, N., Taherzadeh, M.J., 2023. Anaerobic digestion biorefinery for circular bioeconomy development. Bioresour. Technol. Reports 21, 101315. https://doi.org/10.1016/j.biteb.2022.101315.

Mir-Cerdà, A., Nuñez, O., Granados, M., Sentellas, S., Saurina, J., 2023. An overview of the extraction and characterization of bioactive phenolic compounds from agri-food waste within the framework of circular bioeconomy. TrAC Trends Anal. Chem. 161, 116994. https://doi.org/10.1016/J.TRAC.2023.116994.

Mišan, A., Nađpal, J., Stupar, A., Pojić, M., Mandić, A., Verpoorte, R., Choi, Y.H., 2020. The perspectives of natural deep eutectic solvents in agri-food sector. Crit. Rev. Food Sci. Nutr. 60, 2564–2592. https://doi.org/10.1080/10408398.2019.1650717.

Muñoz-Batista, M.J., Rodriguez-Padron, D., Puente-Santiago, A.R., Luque, R., 2018. Mechanochemistry: toward sustainable design of advanced nanomaterials for electrochemical energy storage and catalytic applications. ACS Sustain. Chem. Eng. 6, 9530–9544. https://doi.org/10.1021/ACSSUSCHEMENG.8B01716/ASSET/IMAGES/LARGE/SC-2018-01716V_0013.JPEG.

New, E.K., Tnah, S.K., Voon, K.S., Yong, K.J., Procentese, A., Yee Shak, K.P., Subramonian, W., Cheng, C.K., Wu, T.Y., 2022. The application of green solvent in a biorefinery using lignocellulosic biomass as a feedstock. J. Environ. Manage. 307, 114385. https://doi.org/10.1016/j.jenvman.2021.114385.

Palamae, S., Dechatiwongse, P., Choorit, W., Chisti, Y., Prasertsan, P., 2017. Cellulose and hemicellulose recovery from oil palm empty fruit bunch (EFB) fibers and production of sugars from the fibers. Carbohydr. Polym. 155, 491–497. https://doi.org/10.1016/j.carbpol.2016.09.004.

Palos-Hernández, A., Gutiérrez Fernández, M.Y., Escuadra Burrieza, J., Pérez-Iglesias, J.L., González-Paramás, A.M., 2022. Obtaining green extracts rich in phenolic compounds from underexploited food by-products using natural deep eutectic solvents. Opportunities and challenges. Sustain. Chem. Pharm. 29, 100773. https://doi.org/10.1016/J.SCP.2022.100773.

Pauly, M., Gille, S., Liu, L., Mansoori, N., de Souza, A., Schultink, A., Xiong, G., 2013. Hemicellulose biosynthesis. Planta 238, 627–642. https://doi.org/10.1007/s00425-013-1921-1.

Raposo, F., Borja, R., Ibelli-Bianco, C., 2020. Predictive regression models for biochemical methane potential tests of biomass samples: pitfalls and challenges of laboratory measurements. Renew. Sustain. Energy Rev. 127, 109890. https://doi.org/10.1016/j.rser.2020.109890.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., de Wilde, V., 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. J. Chem. Technol. Biotechnol. 86, 1088–1098. https://doi.org/10.1002/jctb.2622.

Reimer, M., Van Opdenbosch, D., Zollfrank, C., 2021. Fabrication of cellulose-based biopolymer optical fibers and their theoretical attenuation limit. Biomacromolecules 22, 3297–3312. https://doi.org/10.1021/acs.biomac.1c00398.

Reynes, J.F., Isoni, V., García, F., 2023. Tinkering with mechanochemical tools for scale up. Angew. Chemie Int. Ed. 62, e202300819. https://doi.org/10.1002/ANIE.202300819.

Rodríguez-Gutiérrez, G., Cubero Cardoso, J., Rubio-Senent, F., Serrano, A., Borja, R., Fernández-Bolaños, J., Fermoso, F.G., 2019. Thermally-treated strawberry extrudate: a rich source of antioxidant phenols and sugars. Innov. Food Sci. Emerg. Technol. 51, 186–193. https://doi.org/10.1016/j.ifset.2018.05.017.

Ruesgas-Ramón, M., Figueroa-Espinoza, M.C., Durand, E., 2017. Application of deep eutectic solvents (DES) for phenolic compounds extraction: overview, challenges, and opportunities. J. Agric. Food Chem. 65, 3591–3601. https://doi.org/10.1021/acs.jafc.7b01054.

Santana, A.P.R., Mora-Vargas, J.A., Guimarães, T.G.S., Amaral, C.D.B., Oliveira, A., Gonzalez, M.H., 2019. Sustainable synthesis of natural deep eutectic solvents (NADES) by different methods. J. Mol. Liq. 293, 111452. https://doi.org/10.1016/j.molliq.2019.111452.

Santos-Martín, M., Cubero-Cardoso, J., González-Domínguez, R., Cortés-Triviño, E., Sayago, A., Urbano, J., Fernández-Recamales, Á., 2023. Ultrasound-assisted extraction of phenolic compounds from blueberry leaves using natural deep eutectic solvents (NADES) for the valorization of agrifood wastes. Biomass Bioenergy 175, 106882. https://doi.org/10.1016/J.BIOMBIOE.2023.106882.

Satija, P., Chambyal, A., Singh, Gurleen, Ritvik, Singh, Gurjaspreet, Devi, S., Singh, J., 2024. Natural deep eutectic solvents (NADES): manufacture, characteristics, and their significance as designer solvents. ChemistrySelect 9, e202401212. https://doi.org/10.1002/slct.202401212.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K., 2015. Anaerobic digestion of lignocellulosic biomass: challenges and opportunities. Bioresour. Technol. 178, 178–186. https://doi.org/10.1016/j.biortech.2014.09.103.

Sazali, A.L., AlMasoud, N., Amran, S.K., Alomar, T.S., Pa'ee, K.F., El-Bahy, Z.M., Yong, T.L.K., Dailin, D.J., Chuah, L.F., 2023. Physicochemical and thermal characteristics of choline chloride-based deep eutectic solvents. Chemosphere 338, 139485. https://doi.org/10.1016/J.CHEMOSPHERE.2023.139485.

Serrano, A., Villa-Gomez, D., Fermoso, F.G., Alonso-Fariñas, B., 2021. Is anaerobic digestion a feasible alternative to the combustion of olive mill solid waste in terms of energy production? A critical review. Biofuels, Bioprod. Biorefining 15, 150–162. https://doi.org/10.1002/BBB.2159.

Shekaari, H., Zafarani-Moattar, M.T., Mohammadi, B., 2017. Thermophysical characterization of aqueous deep eutectic solvent (choline chloride/urea) solutions in full ranges of concentration at T = (293.15–323.15) K. J. Mol. Liq. 243, 451–461. https://doi.org/10.1016/J.MOLLIQ.2017.08.051.

Siamandoura, P., Tzia, C., 2023. Comparative study of novel methods for olive leaf phenolic compound extraction using NADES as solvents. Molecules 28, 353. https://doi.org/10.3390/molecules28010353.

Singleton, V.L.V.L., Rossi, J.A.J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Talhaoui, N., Taamalli, A., Gómez-Caravaca, A.M., Fernández-Gutiérrez, A., Segura-Carretero, A., 2015. Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits. Food Res. Int. 77, 92–108. https://doi.org/10.1016/J.FOODRES.2015.09.011.

Tohry, A., Chehreh Chelgani, S., Matin, S.S., Noormohammadi, M., 2020. Power-draw prediction by random forest based on operating parameters for an industrial ball mill. Adv. Powder Technol. 31, 967–972. https://doi.org/10.1016/J.APT.2019.12.012.

Trujillo-Reyes, Á.G., Cuéllar, S., Jeison, D., Serrano, A., Zahedi, S., G Fermoso, F., 2023a. Anaerobic digestion of organic solid waste: challenges derived from changes in the feedstock. In: Solid Waste and Landfills Management - Recent Advances. IntechOpen. https://doi.org/10.5772/intechopen.107121.

Trujillo-Reyes, Á., Serrano, A., Pérez, A.G., Peces, M., Fermoso, F.G., 2023b. Impact of monoterpenes in the stability of the anaerobic digestion of mediterranean wholesale market waste. J. Environ. Chem. Eng. 11. https://doi.org/10.1016/j.jece.2023.109653.

Trujillo-Reyes, Á., Sinisgalli, É., Cubero-Cardoso, J., Pérez, A.G., Serrano, A., Borja, R., Fermoso, F.G., 2022. Assessment of different mechanical treatments for improving the anaerobic biodegradability of residual raspberry extrudate. Waste Manag. 139, 190–198. https://doi.org/10.1016/J.WASMAN.2021.12.034.

Umai, D., Kayalvizhi, R., Kumar, V., Jacob, S., 2022. Xylitol: bioproduction and applications-A review. Front. Sustain. 3, 2. https://doi.org/10.3389/frsus.2022.826190.

Ünlü, A.E., 2021. Green and non-conventional extraction of bioactive compounds from olive leaves: screening of novel natural deep eutectic solvents and investigation of process parameters. Waste and Biomass Valorization 12, 5329–5346. https://doi.org/10.1007/s12649-021-01411-3.

Wang, T., He, F., Chen, G., 2014. Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: a concise review. J. Funct.Foods 7, 101–111. https://doi.org/10.1016/j.jff.2014.01.033.

Wei, Z.F., Wang, X.Q., Peng, X., Wang, W., Zhao, C.J., Zu, Y.G., Fu, Y.J., 2015. Fast and green extraction and separation of main bioactive flavonoids from Radix Scutellariae. Ind. Crops Prod. 63, 175–181. https://doi.org/10.1016/J.INDCROP.2014.10.013.

Witham, F.H., Blaydes, D.F., Devlin, R.M., 1971. Experiments in Plant Physiology, Co245. Van Nostrand Reinhold, New York.

Yiin, C.L., Lai, Z.Y., Chin, B.L.F., Lock, S.S.M., Cheah, K.W., Taylor, M.J., Al-Gailani, A., Kolosz, B.W., Chan, Y.H., 2024. Green pathways for biomass transformation: a holistic evaluation of deep eutectic solvents (DESs) through life cycle and techno-economic assessment. J. Clean. Prod. 470, 143248. https://doi.org/10.1016/J.JCLEPRO.2024.143248.

Zhang, Y., Lin, J., Song, T., Su, H., 2022. Anaerobic Digestion of Waste for Biogas Production. Waste-to-Energy, pp. 177–206. https://doi.org/10.1007/978-3-030-91570-4_6.

Zurob, E., Cabezas, R., Villarroel, E., Rosas, N., Merlet, G., Quijada-Maldonado, E., Romero, J., Plaza, A., 2020. Design of natural deep eutectic solvents for the ultrasound-assisted extraction of hydroxytyrosol from olive leaves supported by COSMO-RS. Sep. Purif. Technol. 248, 117054. https://doi.org/10.1016/j.seppur.2020.117054.

Update

Sustainable Chemistry and Pharmacy
Volume 43, Issue , February 2025, Page

DOI: https://doi.org/10.1016/j.scp.2024.101884

Sustainable Chemistry and Pharmacy 43 (2025) 101884

Contents lists available at ScienceDirect

Sustainable Chemistry and Pharmacy

journal homepage: www.elsevier.com/locate/scp

Corrigendum to “Mechanochemical-assisted natural deep eutectic solvent as a platform for an olive leaves biorefinery: Extraction of bioactive compounds and methane production” [Sustain. Chem. Pharm. 43 (2025) 101879]

Juan Cubero-Cardoso [a,b], Manuel Hernández-Escaño [a,b], Ángeles Trujillo-Reyes [a], Fernando G. Fermoso [a], Ma Ángeles Fernández-Recamales [c], Juan Fernández-Bolaños [a], Guillermo Rodríguez-Gutiérrez [a,**], Juan Urbano [b,*]

[a] *Instituto de la Grasa, Spanish National Research Council (CSIC), Campus Universitario Pablo de Olavide, building 46, Ctra. de Utrera, km. 1, Seville, Spain*
[b] *Laboratory of Sustainable and Circular Technology, CIDERTA and Chemistry Department, Faculty of Experimental Sciences, Campus de “El Carmen”, University of Huelva, 21071, Huelva, Spain*
[c] *AgriFood Laboratory, Faculty of Experimental Sciences, University of Huelva, 21007, Huelva, Spain*

The authors regret not including the following phrase in the acknowledgments section:

"Funding for open access charge: University of Huelva/CBUA"

The authors would like to apologise for any inconvenience caused.

DOI of original article: https://doi.org/10.1016/j.scp.2024.101879.

* Corresponding author. University of Huelva, Huelva, Spain.

** Corresponding author. Instituto de la Grasa, Spanish National Research Council (CSIC), Seville, Spain

E-mail addresses: j.cubero@dqcm.uhu.es (J. Cubero-Cardoso), manuelhdezesca@gmail.com (M. Hernández-Escaño), angeles.trujillo@ugr.es (Á. Trujillo-Reyes), fgfermoso@ig.csci.es (F.G. Fermoso), recamale@uhu.es (M.Á. Fernández-Recamales), j.fb.g@csic.es (J. Fernández-Bolaños), guirogu@ig.csic.es (G. Rodríguez-Gutiérrez), juan.urbano@dqcm.uhu.es (J. Urbano).

https://doi.org/10.1016/j.scp.2024.101884