Food Chemistry 239 (2018) 671–678

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products

María de los Ángeles Fernández [1], Magdalena Espino [1], Federico J.V. Gomez, María Fernanda Silva *

Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina

ARTICLE INFO

Article history:
Received 1 March 2017
Received in revised form 1 June 2017
Accepted 28 June 2017
Available online 1 July 2017

Keywords:
Natural Deep Eutectic Solvents
Green chemistry
Phenolic compounds
Liquid chromatography
Industrial waste valorization

ABSTRACT

An environmentally friendly method for the phenolic compound extraction from agro-food industrial by-products was developed in order to contribute with their sustainable valorization. A Natural Deep Eutectic Solvent was chemometrically-designed for the first time and compared with traditional solvents in terms of analyte stabilization. The combination of lactic acid, glucose and 15% water (LGH-15) was selected as optimal. A high-efficiency ultrasound-assisted extraction mediated by LGH-15 prior to HPLC-DAD allows the determination of 14 phenols in onion, olive, tomato and pear industrial by-products. NADES synthesis as well as the extraction procedures were optimized by Response Surface Methodology. Thus, phenolic determination in these complex samples was achieved by a simple, non-expensive, eco-friendly and robust system. The application to different matrices demonstrated the versatility of the proposed method. NADES opens interesting perspectives for their potential use as vehicles of bioactive compounds as food additives or pharmaceuticals.

1. Introduction

Agro-food industry generates significant amounts of by-products that are discarded and can be a serious environmental problem. Nevertheless, food by-products are an extraordinary source of bioactive compounds, including phenolics, proteins, alkaloids, sugars and lipids (Abu-Reidah, Arráez-Román, Warad, Fernández-Gutiérrez, & Segura-Carretero, 2017; Petkowicz, Vriesmann, & Williams, 2017). Those products can be recovered in order to produce valuable metabolites via chemical and biotechnological processes (Ravindran & Jaiswal, 2016). Food-related phenolics are getting great interest due to their antimicrobial and antioxidant activity, strongly related to cancer prevention, inflammatory disorders and cardiovascular diseases (Alarcón Flores, Romero-González, Garrido Frenich, & Martínez Vidal, 2012; Działo et al., 2016; Lou et al., 2012). The concept of turning "waste to wealth" by means of industrial food residues can significantly contribute to sustainable development.

By-products of fruit and vegetable processing mainly contain leaves, peels, seeds, cull fruits and stones (García, Endermann, González-García, & Marina, 2015). The extraction, separation and determination of phenolic compounds is a challenging and important analytical task owing to the great number of phenolic compounds with similar structures along with the complexity of the samples involved. Usually, those analysis are performed by traditional extraction schemes; solid phase extraction (SPE) with organic solvents or water, in combination with high resolution separation methods such as high performance liquid chromatography (HPLC) (Aires, Carvalho, & Saavedra, 2016; Alarcón Flores et al., 2012).

One of the key subjects in Green Chemistry is to develop new green agents for the substitution of hazardous solvents (Nam, Zhao, Lee, Jeong, & Lee, 2015; Pena-Pereira, Kloskowski, & Namieśnik, 2015). In this sense, a new generation of green solvents has emerged in the last decade as promising green media (Cvjetko Bubalo, Vidović, Radojčić Redovniković, & Jokić, 2015). These solvents, called Natural Deep Eutectic Solvents (NADES) are constituted of metabolites that are naturally present in all types of cells and organisms (Choi et al., 2011; Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013). NADES are composed of a

Abbreviations: ACN, acetonitrile; Api, apigenin; Caf, caffeic acid; Cat, catechin hydrate; CGH, citric acidglucose-agua; Cin, cinnamic acid; Cou, p-coumaric acid; FA, formic acid; FCH, fructose-citric acidwater; Fer, *trans*-ferulic acid; Gal, gallic acid; H_20, water; Hty, 3-hydroxytyrosol; LGH, lactic acidglucose-water; Lut, luteolin; MeOH, methanol; Nar, naringenin; Ole, oleuropein; Quer, quercetin dihydrate; Rut, rutin hydrate; Tyr, tyrosol.

* Corresponding author at: Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almte. Brown 500, (CP 5505) Chacras de Coria, Mendoza, Argentina.

E-mail address: msilva@fca.uncu.edu.ar (M.F. Silva).

[1] Equal contribution of both authors.

http://dx.doi.org/10.1016/j.foodchem.2017.06.150
0308-8146/

mixture consisting of a hydrogen bond acceptor (HBA), with a hydrogen bond donor (HBD). The common components are sugars (glucose, sacarose, fructose, etc.); organic acids (lactic, malic, citric acids, etc.); urea and choline chloride (Espino, de los Ángeles Fernández, Gomez, & Silva, 2016). NADES have received growing attention in chemistry for the extraction and separation of analytes from natural sources (Dai, Rozema, Verpoorte, & Choi, 2016; Duan, Dou, Guo, Li, & Liu, 2016; Radošević et al., 2016; Rajan, Prabhavathy, & Ramesh, 2015; Wei, Qi et al., 2015). From an environmental and economic perspective, NADES offer many striking benefits including biodegradability, low toxicity, solute stabilization, sustainability and low cost. The most relevant advantages of NADES are their ease of preparation, the large number of combinations that could be made (around 10^6) and their "tunable" physicochemical properties. The adjustable properties of NADESs such as viscosity, surface tension and polarity offer endless opportunities for the design of extraction approaches (Dai, Witkamp, Verpoorte, & Choi, 2015; Paiva et al., 2014). Although there are several reports concerning the use of NADES as extraction media for bio-compounds from natural sources, there is scarce information about applications for the revalorization of agro-food industry waste and by-products (Bosiljkov et al., 2017; Huang et al., 2017; Jeong et al., 2015).

In the present study a chemometrically-designed NADES is presented for the first time. No systematic study for optimization of synthesis conditions of NADES through a Central Composite using Response Surface Design has been reported before. The tailor-made solvent was also evaluated in terms of analyte stabilization. An ultrasound assisted extraction method based on NADES (UAE-NADES) was developed prior to HPLC-DAD analysis. The optimal approach was successfully applied for the determination of phenolic compounds in by-products from olive oil industry, onion seed production, as well as tomato and pear industries. The phenolic compounds were selected considering their chemical nature (secoiridoids, simple phenol, flavonoids, hidroxycinnamic and hidroxybenzoic acids) as well as their occurrence in fruit and vegetable products. To our knowledge there are no reports on the use NADES for the extraction, separation and detection of phenolic compounds from these by-products. Taking into account that constituents of NADES are present at high concentrations in our diet, the extracts could be directly used in pharmaceutical, cosmetic, agricultural and food industries.

2. Material and methods

2.1. Chemicals, standards solutions and equipment

Analytical standards, 3-hydroxytyrosol ≥ 98% (Hty), p—coumaric acid 98% (Cou), apigenin 95% (Api), oleuropein 80% (Ole), cinnamic acid ≥ 99% (Cin), gallic acid ≥ 99% (Gal), (±)catechin hydrate (Cat), naringenin ≥ 95% (Nar) and caffeic acid ≥ 99% (Caf) were purchased from Sigma Aldrich (St. Louis, MO,USA). Quercetin dihydrate ≥ 97% (Quer) were obtained from Alfa Aesar (Haverhill, MA, USA), tyrosol > 99,5% (Tyr), luteolin ≥ 98% (Lut), rutin hydrate ≥ 94% (Rut) from Fluka Analytical (St. Louis, MO,USA), and *trans*-ferulic acid ≥ 99% (Fer) from SAFC (St. Louis, MO,USA). Compounds for NADES preparation including glucose anhydrous (≥99%), citric acid anhydrous (≥99%), D(-) fructose (≥99%), L(+)lactic acid (85–90%) were purchased from Biopack (Bs. As., Argentina). Formic acid (85%) (FA) was obtained from Sintorgan (Bs. As., Argentina). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). Methanol (MeOH) and acetonitrile (ACN) of chromatographic grade was purchased from J. T. Baker (USA).

Stock solutions were prepared by dissolving each phenolic standard at concentration of 1000 μg mL^{-1} in NADES, methanol (MeOH) or water (H_2O) at 0.1% with FA. Standard working solutions of each solvent at concentration of 25, 15, 10, 5 μg mL^{-1} were obtained from stock solutions. All these solutions were stored in dark-glass bottles at 4 °C.

Magnetic stirrer with temperature control Fisatom (model 752 A, Brazil) was used in the preparation of NADES. For extraction of phenolic compounds from by-products a centrifuge (Eppendorf 5804-R, Germany) and ultrasonic bath (Cleanson, Argentina) were used.

2.2. By-product samples

The by-products of olive oil industry (olive cake), onion seed production, tomato and pear canning industry were obtained from Experimental Industry of Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina. The olive cake (also called wet pomace or alperujo) is a semisolid waste from olive oil extraction by the two-phase centrifugation system. The by-product from onion seed production is constituted of dry scapes and umbels. Tomato and pear disposal (containing peels, seeds and cull fruits) were collected from canning industry. Then, samples were frozen in liquid nitrogen and lyophilized in darkness. Before the extraction, lyophilized material was grounded up to a fine powder with liquid nitrogen.

2.3. NADES synthesis

NADES evaluated were: LGH (lactic acid and glucose, 5:1); CGH (citric acid and glucose, 1:1) and FCH (fructose and citric acid, 1:1). They were prepared using a heating and stirring method previously described by Dai et al. (Dai et al., 2013). The component mixture was placed in a 20 mL amber glass vial and heated in a magnetic stirrer with temperature control for 60 min.

NADES synthesis was optimized by means of chemometric tools in order to determine the most appropriate components of NADES (LGH, FCH and CGH), temperature (40–80 °C), percentage of water (0–15%) and buffer concentration (0–0.1%). The effect of these parameters on the stability of NADES, pH and density were investigated at three levels (−1, 0 and +1). The pH of NADES synthesized was tested with an Altronix model TPX-I pH meter furnished with a combined glass electrode. The density (ρs) of the sample can be calculated from its mass and volume (ρs = ms/Vs), therefore a known volume of NADES (1 mL) was weighed. The stability was evaluated every 24 h; solvents were considered stable when the mixture remained in the liquid phase for a 5-day period.

2.4. HPLC-DAD analysis

Phenolic compounds were determined using a HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany). The HPLC instrument was a Dionex Ultimate 3000 consisting of vacuum degasser unit, autosampler, quaternary pump and chromatographic oven. The detector was a Dionex DAD-3000 (RS) model. Chromeleon 7.1 software was used to control all the acquisition parameters of the HPLC-DAD system and also to process the obtained data.

HPLC separations were carried out in a Zorbax SB-Aq column (4.6 mm × 150 mm, 5 μm) Agilent Technologies. Ultrapure water with 0.1% FA (A) and ACN (B) were used as mobile phase. Phenolic compounds were separated using the following gradient: 0–2.7 min, 5% B; 2.7–10.7 min, 30% B; 10.7–11 min, 32% B; 11–15 min, 40% B; 15–15.5 min, 50% B: 15.5–16 min 50% B; 16–16.5 min 30% B; 16.5–17 min 15% B; 17–17.5 min 5% B; 17.5–18 min 5% B. The mobile phase flow was 1 mL min^{-1}. The column temperature was held at 30 °C and the injection volume was 5 μL. The identification and quantification of phenolic compounds in the different solvents studied were based on the comparison of the

retention times (t_R) and absorbance values of detected peaks in solvents with those obtained by injection of pure standards of each analyte. Chromatograms were recorded at 254 (Ole), 280 (Gal, Hty, Tyr, Cat, Cin, Nar), 320 (Cou, Caf, Fer, Api) and 370 nm (Rut, Quer, Lut).

2.5. Analyte stability test

The effect of storage time and temperature on the stability of eight selected phenolic compounds in NADES, H_2O and MeOH was investigated. The compounds studied were: oleuropein (secoiridoids), 3-hydroxytyrosol (simple phenol), coumaric, cinnamic, caffeic and ferulic acids (hidroxycinnamic acids), quercetin (flavonolol), and apigenin (flavone). Solutions of standards were prepared at concentrations of 10 μg mL^{-1} in the different solvents. The stability was evaluated by monitoring the chromatographic area of standard solutions at three different temperatures (20, 4 and -18 °C) during a 60-day period. All tests were done in triplicate.

2.6. UAE-NADES extraction

The extraction of phenolic compounds from by-products with the optimal NADES was performed mediated by Ultrasound Assisted Extraction (UAE). A chemometric approach was used for this purpose considering the following parameters: ultrasound time (15, 35, 60 min), sample material/solvent ratio (15, 45 and 75 mg mL^{-1}) and water dilution of the optimal NADES (0%, 40% and 75%). The temperature used for extraction was set at 40 °C. The chromatographic area of selected phenolic compounds was used to evaluate the extraction performance. As a result of the optimization, lyophilized material and NADES were placed in a 15 mL centrifuge tube (75 mg mL^{-1}) and homogenized by a vortex during 15 s. The suspensions were processed by ultrasound (200 W output power, 20 kHz frequency) during 60 min at 40 °C (±2 °C). Then, the system was centrifuged for 30 min and the supernatant were filtered (0.45 μm) before analyzed by HPLC-DAD. Each extraction was performed in triplicate.

2.7. Experimental design and statistical analysis

The synthesis of NADES and the UAE method were evaluated using Central Composite and Box-Behnken Design respectively. Response Surface Methodology was applied to evaluate and optimize the effects of the synthesis and extraction parameters according to various responses. The statistical analysis was performed using the RSM software Design-Expert, v.7.1 (Stat Ease, Minneapolis, USA). The results were tested statistically using analysis of variance (ANOVA) at the significance level of $p = 0.05$. The adequacy of the model was evaluated by the coefficient of determination (R^2) and the model p value. Mathematical models were established to describe the influences of the single process parameters and/or the interactions of multiple parameters on each response investigated. Response surface plots were generated with the same software, and were drawn using the function of two factors, and keeping the other factor constant.

Statistical analysis was done using software Statgraphics Centurion XV v15.2.06 and Graph Pad Prism v5.01. All data were reported as the mean ± SD for three replicates.

3. Results and discussion

3.1. Chemometric design of NADES

Statistics-based optimization of variables using Response Surface Methodology (RSM) can be advantageous over the classical one-variable-at-a-time (OVAT) approach because it allows the evaluation of interacting effects between variables and variable optimization in overall scope from fewer experiments (Nam et al., 2015). RSM is largely spread out and consolidated in the optimization of analytical procedures (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Central Composite experimental design (CCD) with Response Surface Methodology (RSM) was used to determine the optimal experimental conditions for NADES synthesis. The purpose was to reach the most appropriate NADES composition, temperature, percentage of water and buffer concentration for the extraction of phenolic compounds from solid matrices. Taking into account the chemical nature of the analytes under study as well as the matrices involved, density, stability and pH were considered.

The nature of the interactions that take place in the eutectic behavior depends on the type of the components where hydrogen bonds or Van der Waals forces are involved. For the selection of NADES, previous results obtained by several authors and our research group were taken into account (Dai et al., 2016; Dai, Witkamp, Verpoorte, & Choi, 2013; Gomez, Espino et al., 2016). Sugar-organic acids-water mixtures (LGH, CGH and FCH) were selected considering not only the desired extraction skills but also the potential applications of the bioextracts. The presence of several hydroxyl or carboxyl groups, allows more hydrogen bonds to be formed, thus increasing the stability of the liquids. Moreover, the selected NADES components are readily available, inexpensive and widely used as additives and / or food ingredients. Temperature range (40-80 °C), and water content (0–15%) were selected based on previous reports (Dai et al., 2013, 2015; Martins et al., 2014; Wei, Qi et al., 2015; Wei, Wang et al., 2015). Buffer addition (0–0.1% v/v FA) was also included in the design because stability of phenolic compounds is higher in acidic conditions (Bridgers, Chinn, & Truong, 2010; Friedman & Jürgens, 2000). The design consisted of sixty-six experiments divided in three blocks (three consecutive days). Eight replicates of center points, eight factorial points, and six axial points for tested NADES were included. The response variables were stability (hs), pH and density (g mL^{-1}) (Supplementary Table S1).

Analyzing the obtained results, CGH and FCH presented the highest density values. High density of some NADES causes severe errors during injection step in the HPLC, as well as decreases of extraction efficiency. Other important facts are that LGH is the most stable (solidcrystalline precipitate gradually appeared for CGH and FCH) and the only solvent that can be generated at lower temperatures (40 °C). Consequently, LGH was selected for further optimization.

The 2FI, quadratic and linear models better explained the behavior of responses stability, pH and density respectively (Supplementary Table S2). The resulting R^2 indicates that the experimental data were in satisfactory agreement with predicted responses for each model. F-values for the lack-of-fit of linear model were insignificant ($p \geq 0.1070$), indicating that this model accurately represents the experimental data. The models were expressed by the following equations (Eqs. (1)(3)):

$$\text{stability} = 84.98 + 2.94W + 0.47T - 0.04WT \quad (1)$$

$$(\text{pH} + 0.03)^{1.78} = 7.19 - 84.97A + 234.95A \quad (2)$$

$$\text{density} = 1.25 - 2.19e^{-3}W \quad (3)$$

where W: percentage of water; T: temperature and A: buffer concentration.

The content of water affected both stability and density (Eqs. (1) and (3)). The content of formic acid affects the pH of the NADES

obtained (Eq. (2)). Temperature influences the stability of the solvent (Eq. (1)).

For the simultaneous optimization of the three responses analyzed (pH, density and stability) the desirability function was used. Thus, target criteria for LGH were to minimize density and pH and maximize stability. As shown in Fig. 1 the desirability function increases when water and formic acid contents increase. The optimal experimental conditions (D = 0.854) for LGH synthesis (lactic acid and glucose, 5:1) were as follows: 40 °C, 15% of water and 0.1% (v/v) formic acid.

In order to validate the results, three additional experiments at the optimal conditions were conducted. The optimal synthesis conditions were thus confirmed. This NADES chemometrically designed was named LGH-15 and used for further studies.

3.2. HPLC-DAD analysis

A chromatographic procedure for the analysis of fourteen representative phenolic compounds was developed by means of the one-at a time optimization procedure. The following variables were evaluated: mobile phase composition and gradient, temperature and wavelength detection. Phenolic compounds were separated in 18 min and the optimal conditions are described in Section 2.4. Fig. 2 shows the chromatograms for phenolic compounds at 254 nm, 280 nm, 320 nm and 370 nm.

3.3. Stability test

Previous reports involving the mechanism of the stabilizing ability of NADES suggest that the formation of hydrogen bonds or chelation can stabilize phenolic compounds. The H-bond interactions between solute and NADES provide an explanation for the high stabilizing ability of the sugar-based NADES (Dai, Verpoorte, & Choi, 2014).

In the present study, the stability of eight phenolic compounds (Ole, Hty, Cou, Cin, Caf, Fer, Quer and Api) belonging to different families was evaluated following the procedure described in Section 2.5 (Supplementary Fig. S1). At −18 and 4 °C, all phenolic compounds remained stable in LGH-15 and methanol over a two-month period while degradations up to 90% were observed in water (Api and Quer). At 25 °C, degradations within 30–50% for Ole, Hty, Api and Quer were observed in LGH-15 after a two-month storage time. Interestingly, all other phenolic compounds were stable over 60 days in the eutectic solvent.

Fig. 3 shows the degradation curve for Cin and Api at −18, 4 and 25 °C over a 60-day period.

Our results for hydrophobic phenols are in agreement with previous studies that reported an important NADES-enhanced stability

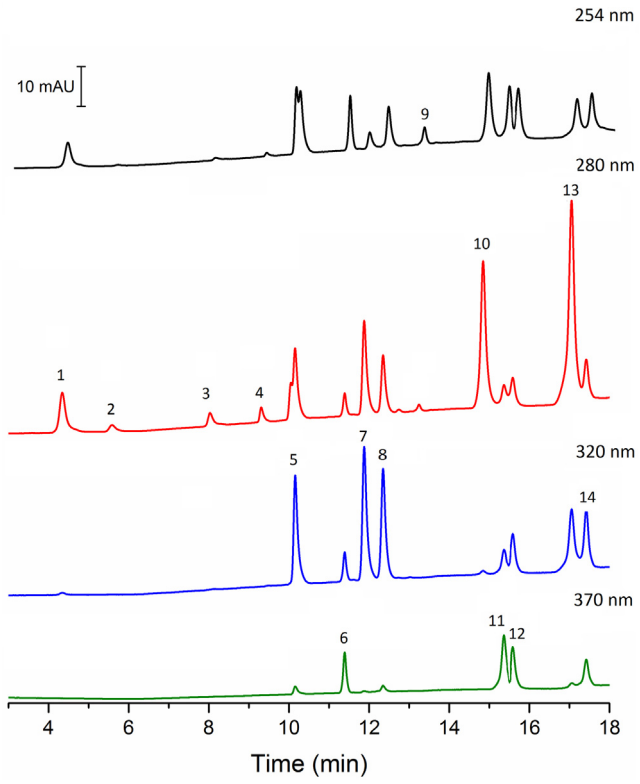

Fig. 2. HPLC-DAD chromatograms of phenolic standards in LGH-15 at 254, 280, 320, 370 nm. Gallic acid (1), hydroxytyrosol (2), tyrosol (3), catechin (4), caffeic acid (5), rutin (6), coumaric acid (7), trans-ferulic acid (8), oleuropein (9), cinnamic acid (10), quercetin (11), luteolin (12), naringenin (13), apigenin (14).

of compounds unstable in aqueous solutions. Dai and co-workers reported that natural colorant (carthamin) was preserved in a deep eutectic solvent (sucrose-choline chloride) at 4 °C for at least 1 month and at −20 °C for at least 3 months (Dai et al., 2014). It has to be pointed out that the stability in NADES of phenolic compounds which are water-soluble has not been studied before.

3.4. UAE-NADES extraction

Taking into account that a considerable number of variables affect the extraction efficiency in the NADES-based ultrasound-assisted procedure, optimization was carried out through a multivariate approach for each sample under study as described in Section 2.6. The extraction temperature is a critical parameter; so it was set at 40 °C to avoid degradation. Treatment of multiple

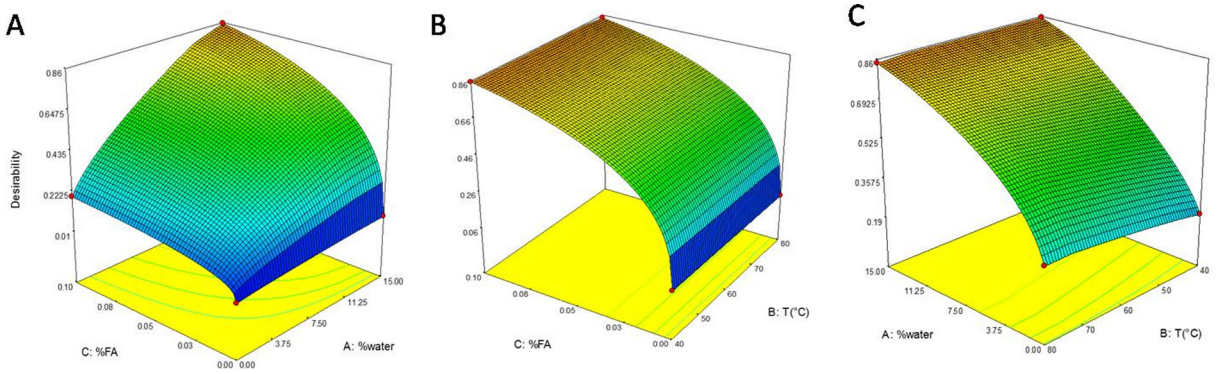

Fig. 1. Response surface plots (desirability function) for LGH-15 synthesis when optimizing acidification vs. percentage of water (A), acidification vs. temperature (B) and temperature vs. percentage of water (C).The third variable was kept constant (optimal value).

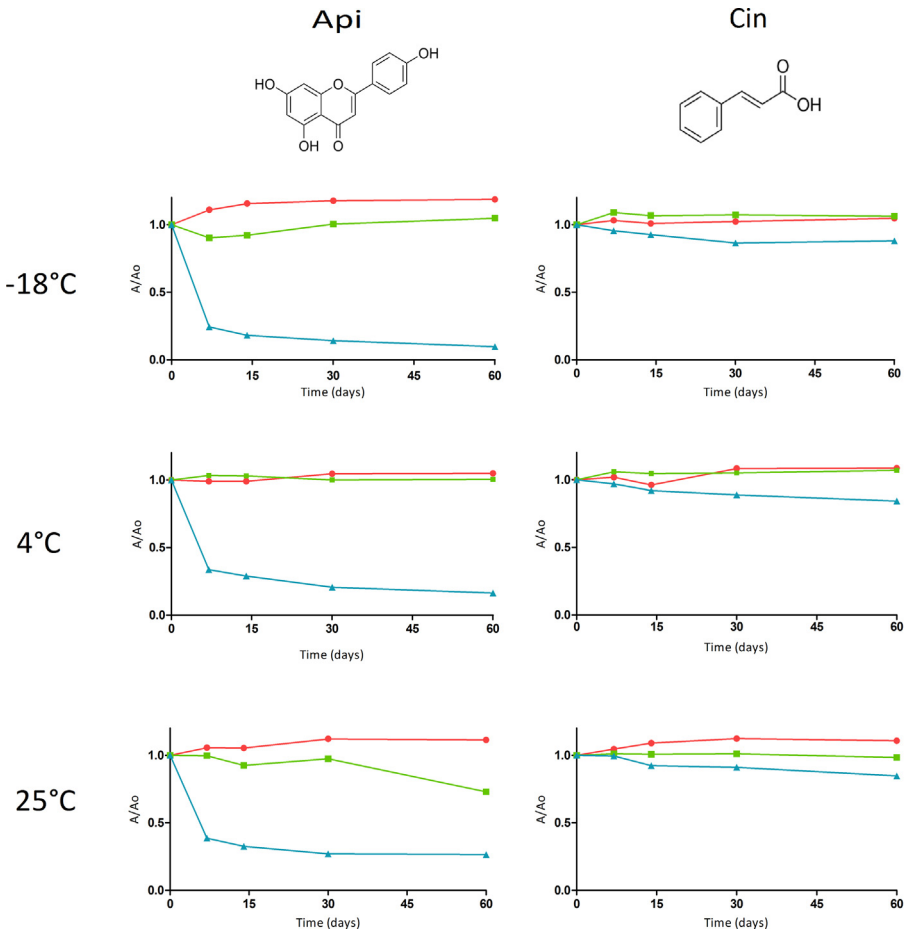

Fig. 3. Stability of cinnamic acid (Cin) and apigenin (Api) in LGH-15 (green), MeOH (red) and H_2O (blue) at −18, 4 and 25 °C over 60-day period. A_0 is the initial chromatographic area and A is the chromatographic area after storage time at a given temperature. Results are based on triplicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

responses and selection of optimal conditions were based on desirability function. In this study, the desirability was set to achieve the best conditions that maximize extraction efficiency.

The optimal ultrasound time was between 30 and 60 min according to evaluated matrix. The longer time was observed in olive cake; the sample with biggest particle size. It is also worth mentioning that conventional extractions of phenolic compounds generally demand much more time and energy. On the other hand, in onion seed by-product, 30 min resulted optimal for the extraction; this residue with the smallest particle size. The influence of particle size in extraction efficiency could be due to diffusion path length and effective area.

Also, the sample material/solvent ratio considerably affects the extraction of phenolics. In agreement with previous results (Pinelo, Rubilar, Jerez, Sineiro, & Núñez, 2005), increments in the sample material/solvent ratio allowed improvements of the extraction yield. A ratio of 75 mg mL^{-1} was chosen as optimal for all samples.

The importance of the water content in NADES for extraction efficiency has already been discussed in previous studies. Low level of added water decreases NADES viscosity, which is essential to improve the extraction yield. On the other hand, an excess of water in NADES appears to interfere with the halide hydrogen bond donor supramolecular complex (Dai et al., 2015; Gutiérrez, Ferrer, Mateo, & Monte, 2009). Consequently, it is crucial to consider water content optimization when applying DES as extraction solvents (Dai et al., 2013; Nam et al., 2015).

Our results indicated that, LGH-15 without dilution performed better for the extraction of phenolics from the by-products evaluated. It is worth mentioning that water is a component of LGH-15. The latter demonstrates the versatility of LGH-15 for direct application in different matrices. This fact represents clear advantages for extraction process; conditions could be extrapolated for other waste/ by-products materials.

A growing number of studies concerning the use of NADES as extractants of phenolic compounds from plant matrices indicate the great potential of these tailor-made green solvents (Bosiljkov et al., 2017; Peng et al., 2016; Ruesgas-Ramón, Figueroa-Espinoza, & Durand, 2017). Nevertheless, the long way to apply their pharmaceutical and food applications is beginning to be walk. Toxicity and stability studies as well as basic research to reveal NADES supramolecular structure and their interaction with analytes are needed.

Finally, to illustrate the potential of NADES in extraction of phenolic compounds, comparisons of the extraction efficiencies with traditional solvents such as methanol and water were performed (Fig. 4). Our results demonstrated that LGH-15 shows outstanding extractability for both polar and weak polar compounds compared to conventional solvents.

3.5. Analytical performance

After selecting the optimal extraction and chromatographic conditions, the effect of the matrix was assessed by comparing the signal of the analytes in standard solutions to the signal in the sample matrices. Calibration curves were obtained in pure LGH-15 and in the by-products extracts (onion seed, olive cake, tomato and pear)

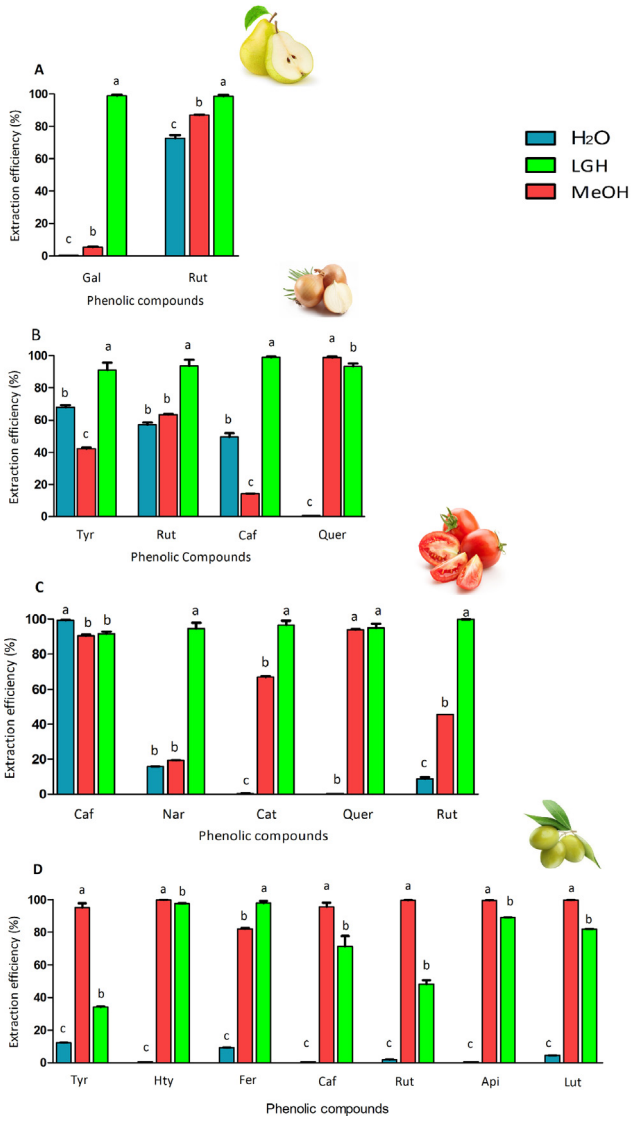

Fig. 4. Calibration plots from spiked by-products and spiked pure solvent: A (Olive cake); B (onion seed production); C (pear) and D (tomato).

in the concentration range: 5–25 mg L^{-1}. The percentage of the quotient of the slopes (b) in the spiked and solvent samples was used as an indicator of the extent of the suppression or enhancement signal, which was calculated as shown in Eq. (4).

$$Matrix effect\ (\%) = 100 - \left[\left(\frac{b_{spiked}}{b_{solvent}}\right)100\right] \quad (4)$$

It was found that the different matrices caused changes in the baseline as well as peak areas. Nonetheless, no differences at retention times of phenolic compounds were detected for all samples. Differences on intersect as well as slopes of all analytical curves (solvent × matrix) were also observed. This behavior was attributed to a proportional systematic error, caused by matrix components. No signal enhancement, but response reductions within the range 21–74% due to matrix interference was observed except for Tyr in onion, Quer in tomato and Api in olive. Linear regressions of analytes showing the highest matrix effects are presented in Supplementary Fig. S2. Consequently, quantification was carried out following the standard addition method.

Table 1 shows the figures of merit for the phenolic compounds in each matrix under study. Correlation coefficients of area ratio equations were >0.99 for all target compounds. All of them showed a linear range from the LOQ to 100 mg L^{-1} least. The limits of detection (LODs) and quantification (LOQs) were evaluated on the basis of signal-to-noise ratios (S/N) of 3 and 10, respectively.

Taking into account that a certified reference material of the studied matrices with an informed value for phenolic compounds does not exist, the trueness of the measurements was evaluated through recovery of additions of known amounts of the analytes to samples (Table 1). For this purpose, a pool of samples for each sample was used.

With the aim to estimate the trueness, intra-day repeatability, and inter-day reproducibility, spiked samples were analyzed: 5 blank samples, 3 replicate measurements at 5, 10 and 25 mg L^{-1} phenolic concentration levels; respectively. The same experiment was repeated on four other independent occasions with at least one week interval.

In summary and taking into account the matrix complexity, the reported values for the method assessment parameters could be considered highly satisfactory.

Table 1
Analytical figures of merit for phenolic compounds in different by-products.

Sample	Phenolic compound	Retention time (min)	Calibration curve	R^2	LOD[a]	LOQ[a]	Repeatability (RDS %)		Reproducibility %	Recovery %
							tr	area		
Olive cake	Hty	5.54	y = 0.0305x − 0.0213	1.000	0.023	0.0769	0.0634	2.3979	3.5702	97.9890
	Tyr	8.0133	y = 0.0329x + 0.1521	0.9941	0.0891	0.2970	0.0721	0.9986	2.6794	109.3659
	Caf	10.1257	y = 0.2135x + 0.0854	0.9977	0.0130	0.0434	0.0798	7.0277	9.6845	101.0523
	Rut	11.3767	y = 0.309x + 0.011	0.9999	0.0030	0.0099	0.0051	8.6113	10.3678	100.0367
	Fer	12.3497	y = 0.2118x + 0.5041	0.9896	0.0101	0.0336	0.0247	1.5468	3.1547	109.6325
	Lut	15.5653	y = 0.106x - 0.2405	0.9911	0.0128	0.0428	0.0134	0.3508	1.5908	89.7874
	Api	17.4077	y = 0.1858x - 0.3306	0.9993	0.0088	0.0293	0.0066	1.4467	2.6793	92.5654
Pear waste	Gal	4.1100	y = 0.1207x + 0.3607	0.9915	0.0020	0.0068	0.3406	0.9956	1.4698	95.9381
	Van	10.0100	y = 0.2314x + 0.4845	0.9999	0.0044	0.0146	0.0999	0.3728	1.8754	106.1360
	Rut	11.3790	y = 0.3266x + 0.4008	0.9991	0.0018	0.0059	0.0465	1.3340	3.2145	99.7961
Onion	Tyr	8.0460	y = 0.0443x − 0.1544	0.9959	0.0293	0.0976	0.0008	8.3412	10.6538	85.2219
	Caf	10.0930	y = 0.2679x + 0.2808	0.9999	0.0052	0.0174	0.0001	0.8264	1.2368	104.3254
	Rut	11.4153	y = 0.1346x − 0.0049	0.9990	0.0155	0.0516	0.0011	7.0170	9.6754	99.4601
	Quer	15.3433	y = 0.1682x + 0.0481	0.9999	0.0077	0.0257	0.0008	3.9296	5.8954	101.0432
Tomate waste	Cat	9.2957	y = 0.0247x − 0.1138	0.9956	0.0807	0.2691	0.1345	4.5608	7.8698	82.3643
	Caf	10.1400	y = 0.3432x − 0.4616	0.9984	0.0064	0.0212	0.1734	2.2464	4.6324	95.0749
	Rut	11.3847	y = 0.4555x − 0.7767	0.9982	0.0018	0.0059	0.0947	0.1515	2.2134	93.6647
	Quer	15.3567	y = 0.2276x − 0.4383	0.9991	0.0044	0.0146	0.0995	5.4574	7.9856	92.6473
	Nar	17.0390	y = 0.8377x − 2.8156	0.9980	0.0006	0.0021	0.1115	7.1372	9.8137	86.0335

[a] LOD and LOQ expressed in μg g^{-1}.

Table 2
Concentration of phenolic compounds detected in each sample.

Phenolic compound	By-products[a]			
	Onion seed	Olive cake	Pear	Tomato
Rut	67.169 ± 4.679	12.620 ± 1.128	7.946 ± 0.112	325.132 ± 0.458
Tyr	139.012 ± 7.719	1.356 ± 0.048	nd	nd
Caf	136.314 ± 1.242	3.225 ± 0.601	nd	98.087 ± 1.801
Quer	2.056 ± 0.231	nd	nd	62.605 ± 2.015
Lut	nd	453.690 ± 1.485	nd	nd
Api	nd	79.328 ± 0.804	nd	nd
Fer	nd	8.161 ± 0.617	nd	nd
Hty	nd	111.053 ± 2.440	nd	nd
Gal	nd	nd	24.862 ± 0.149	nd
Nar	nd	nd	nd	116.531 ± 5.118
Cat	nd	nd	nd	491.120 ± 19.120
Cin	nd	nd	nd	nd
Cou	nd	nd	nd	nd
Ole	nd	nd	nd	nd

nd: non detected.
[a] concentration expressed as μg of phenolic compound in g of dry by-products ± Standard error (n = 3).

3.6. Sample analysis

In this work, the optimized UAE-LGH15 -HPLC-DAD procedure was applied for the determination of phenolic compounds in the following agro-food industrial by-products from olive, onion, tomato and pear. The results are shown in Table 2. Supplementary Fig. S3 shows the chromatograms of by-products.

Previous studies demonstrated that the amounts of phenolic compounds in peels and seeds fruit are much higher than in edible pulp; therefore removal of them results in a significant loss of antioxidants and their potential health benefits (Raja, Hernández-Revelles, Hernández-Cassou, & Saurina, 2014; Stajčić et al., 2015). In this work, the results reveal that tomato and pear by-products should be regarded as a potential source of bioactive compounds. In tomato residue, Rut and Cat were found at the highest concentrations; while pear waste presented Gal as the most abundant compound. The results obtained are in agreement with previous studies (Gharbi et al., 2017; Raja et al., 2014). It is worth mentioning that although fruits waste has been studied, there is scarce information about pear residues.

The onion seed production by-product is generally collected and incinerated after the seed harvest season consequently there are no reports on its chemical composition. In order to contribute to the valorization of this residue, the phenolic composition were determinate being Tyr and Caf the most representative compounds.

Olive oil contains 2% of the total phenolic content of whole olives, while the remaining 98% is present in olive waste (Alu'datt, Alli, Ereifej, Alhamad, Al-Tawaha, & Rababah, 2010). The results obtained show that olive cake is rich in Lut and Hty. Hydroxytyrosol possesses the most potent antioxidant activity similar to those of BHT (2,6-ditert-butyl-phydroxytoluene) as well as of other synthetic antioxidants. At the present time, it is obtained by total synthesis and commercialized at very high market price. The presence of significant concentrations of hydroxytyrosol in Olive cake makes this by-product of great importance for its direct recovery (Federici, Fava, Kalogerakis, & Mantzavinos, 2009).

4. Conclusions

In this work, the chemometric design of a Natural Deep Eutectic Solvent, LGH15, is presented and evaluated for the first time. The stabilization "power" of NADES opens interesting possibilities for their use as vehicles of bioactive compounds as food additives or pharmaceuticals.

The proposed optimized UAE-LGH15 approach coupled to HPLC-DAD opens up an attractive alternative in the area of sustainable analytical characterization of agro-food by-products particulary in view of the excellent extraction efficiencies. The phenolic determination in complex matrices was achieved by a simple, non-expensive, eco-friendly and robust system. The application to different matrices such as onion, olive, tomato and pear by products demonstrated the versatility of the proposed method. Our results support the revalorization of bioextracts from agro-food residues to be directly used for health and nutrition applications. Further researches are needed for the applicability of this by-products extracts in commercial processes

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo (Mendoza,Argentina).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.06.150.

References

Abu-Reidah, I. M., Arráez-Román, D., Warad, I., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2017). UHPLC/MS2-based approach for the comprehensive metabolite profiling of bean (Vicia faba L.) by-products: A promising source of bioactive constituents. *Food Research International, 93*, 87–96.

Aires, A., Carvalho, R., & Saavedra, M. J. (2016). Valorization of solid wastes from chestnut industry processing: Extraction and optimization of polyphenols, tannins and ellagitannins and its potential for adhesives, cosmetic and pharmaceutical industry. *Waste Management, 48*, 457–464.

Alarcón Flores, M. I., Romero-González, R., Garrido Frenich, A., & Martínez Vidal, J. L. (2012). Analysis of phenolic compounds in olive oil by solid-phase extraction and ultra high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry, 134*(4), 2465–2472.

Alu'datt, M. H., Alli, I., Ereifej, K., Alhamad, M., Al-Tawaha, A. R., & Rababah, T. (2010). Optimisation, characterisation and quantification of phenolic compounds in olive cake. *Food Chemistry, 123*(1), 117–122.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*(5), 965–977.

Bosiljkov, T., Dujmić, F., Cvjetko Bubalo, M., Hribar, J., Vidrih, R., Brnčić, M., ... Jokić, S. (2017). Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. *Food and Bioproducts Processing, 102*, 195–203.

Bridgers, E. N., Chinn, M. S., & Truong, V. D. (2010). Extraction of anthocyanins from industrial purple-fleshed sweetpotatoes and enzymatic hydrolysis of residues for fermentable sugars. *Industrial Crops and Products, 32*(3), 613–620.

Cvjetko Bubalo, M., Vidović, S., Radojčić Redovniković, I., & Jokić, S. (2015). Green solvents for green technologies. *Journal of Chemical Technology and Biotechnology, 90*(9), 1631–1639.

Choi, Y. H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W. C. E., ... Verpoorte, R. (2011). Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology, 156*(4), 1701–1705.

Dai, Y., Rozema, E., Verpoorte, R., & Choi, Y. H. (2016). Application of natural deep eutectic solvents to the extraction of anthocyanins from Catharanthus roseus with high extractability and stability replacing conventional organic solvents. *Journal of Chromatography A, 1434*, 50–56.

Dai, Y., van Spronsen, J., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2013). Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta, 766*, 61–68.

Dai, Y., Verpoorte, R., & Choi, Y. H. (2014). Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (Carthamus tinctorius). *Food Chemistry, 159*, 116–121.

Dai, Y., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2013). Natural deep eutectic solvents as a new extraction media for phenolic metabolites in Carthamus tinctorius L.. *Analytical Chemistry, 85*(13), 6272–6278.

Dai, Y., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2015). Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chemistry, 187*, 14–19.

Duan, L., Dou, L. L., Guo, L., Li, P., & Liu, E. H. (2016). Comprehensive evaluation of deep eutectic solvents in extraction of bioactive natural products. *ACS Sustainable Chemistry and Engineering, 4*(4), 2405–2411.

Działo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J., & Kulma, A. (2016). The potential of plant phenolics in prevention and therapy of skin disorders. *International Journal of Molecular Sciences, 17*(2).

Espino, M., de los Ángeles Fernández, M., Gomez, F. J. V., & Silva, M. F. (2016). Natural designer solvents for greening analytical chemistry. *TrAC - Trends in Analytical Chemistry, 76*, 126-136.

Federici, F., Fava, F., Kalogerakis, N., & Mantzavinos, D. (2009). Valorisation of agro-industrial by-products, effluents and waste: Concept, opportunities and the case of olive mill waste waters. *Journal of Chemical Technology and Biotechnology, 84*(6), 895–900.

Friedman, M., & Jürgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry, 48*(6), 2101–2110.

García, M. C., Endermann, J., González-García, E., & Marina, M. L. (2015). HPLC-Q-TOF-MS identification of antioxidant and antihypertensive peptides recovered from cherry (Prunus cerasus L.) subproducts. *Journal of Agricultural and Food Chemistry, 63*(5), 1514–1520.

Gharbi, S., Renda, G., La Barbera, L., Amri, M., Messina, C. M., & Santulli, A. (2017). Tunisian tomato by-products, as a potential source of natural bioactive compounds. *Natural Product Research, 31*(6), 626–631.

Gutiérrez, M. C., Ferrer, M. L., Mateo, C. R., & Monte, F. D. (2009). Freeze-drying of aqueous solutions of deep eutectic solvents: A suitable approach to deep eutectic suspensions of self-assembled structures. *Langmuir, 25*(10), 5509–5515.

Huang, Y., Feng, F., Jiang, J., Qiao, Y., Wu, T., Voglmeir, J., & Chen, Z. G. (2017). Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. *Food Chemistry, 221*, 1400–1405.

Jeong, K. M., Zhao, J., Jin, Y., Heo, S. R., Han, S. Y., Yoo, D. E., & Lee, J. (2015). Highly efficient extraction of anthocyanins from grape skin using deep eutectic solvents as green and tunable media. *Archives of Pharmacal Research, 38*(12), 2143–2152.

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., & Li, J. (2012). P-Coumaric acid kills bacteria through dual damage mechanisms. *Food Control, 25*(2), 550–554.

Martins, M., Aroso, I. M., Reis, R. L., Duarte, A. R. C., Craveiro, R., & Paiva, A. (2014). Enhanced performance of supercritical fluid foaming of natural-based polymers by deep eutectic solvents. *AIChE Journal, 60*(11), 3701–3706.

Nam, M. W., Zhao, J., Lee, M. S., Jeong, J. H., & Lee, J. (2015). Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: Application to flavonoid extraction from Flos sophorae. *Green Chemistry, 17*(3), 1718–1727.

Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R. L., & Duarte, A. R. C. (2014). Natural deep eutectic solvents – Solvents for the 21st century. *ACS Sustainable Chemistry and Engineering, 2*(5), 1063–1071.

Pena-Pereira, F., Kloskowski, A., & Namieśnik, J. (2015). Perspectives on the replacement of harmful organic solvents in analytical methodologies: A framework toward the implementation of a generation of eco-friendly alternatives. *Green Chemistry, 17*(7), 3687–3705.

Peng, X., Duan, M. H., Yao, X. H., Zhang, Y. H., Zhao, C. J., Zu, Y. G., & Fu, Y. J. (2016). Green extraction of five target phenolic acids from Lonicerae japonicae Flos with deep eutectic solvent. *Separation and Purification Technology, 157*, 249–257.

Petkowicz, C. L. O., Vriesmann, L. C., & Williams, P. A. (2017). Pectins from food waste: Extraction, characterization and properties of watermelon rind pectin. *Food Hydrocolloids, 65*, 57–67.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry, 53*(6), 2111–2117.

Radošević, K., Ćurko, N., Gaurina Srček, V., Cvjetko Bubalo, M., Tomašević, M., Kovačević Ganić, K., & Radojčić Redovniković, I. (2016). Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. *LWT - Food Science and Technology, 73*, 45–51.

Raja, M., Hernández-Revelles, J., Hernández-Cassou, S., & Saurina, J. (2014). Determination of polyphenols in the pear pulp matrix by solvent extraction and liquid chromatography with UV-Vis detection. *Analytical Methods, 6*(24), 9769–9776.

Rajan, M., Prabhavathy, A., & Ramesh, U. (2015). Natural deep eutectic solvent extraction media for Zingiber officinale Roscoe: The study of chemical compositions, antioxidants and antimicrobial activities. *Natural Products Journal, 5*(1), 3–13.

Ravindran, R., & Jaiswal, A. K. (2016). Exploitation of Food Industry Waste for High-Value Products. *Trends in Biotechnology, 34*(1), 58–69.

Ruesgas-Ramón, M., Figueroa-Espinoza, M. C., & Durand, E. (2017). Application of deep eutectic solvents (DES) for phenolic compounds extraction: Overview, challenges, and opportunities. *Journal of Agricultural and Food Chemistry, 65*(18), 3591–3601.

Stajčić, S., Četković, G., Čanadanović-Brunet, J., Djilas, S., Mandić, A., & Četojević-Simin, D. (2015). Tomato waste: Carotenoids content, antioxidant and cell growth activities. *Food Chemistry, 172*, 225–232.

Wei, Z., Qi, X., Li, T., Luo, M., Wang, W., Zu, Y., & Fu, Y. (2015). Application of natural deep eutectic solvents for extraction and determination of phenolics in Cajanus cajan leaves by ultra performance liquid chromatography. *Separation and Purification Technology, 149*, 237–244.

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. *Industrial Crops and Products, 63*, 175–181.