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Analytical Methods

# Green extraction of grape skin phenolics by using deep eutectic solvents

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ABSTRACT

Conventional extraction techniques for plant phenolics are usually associated with high organic solvent consumption and long extraction times. In order to establish an environmentally friendly extraction method for grape skin phenolics, deep eutectic solvents (DES) as a green alternative to conventional solvents coupled with highly efficient microwave-assisted and ultrasound-assisted extraction methods (MAE and UAE, respectively) have been considered. Initially, screening of five different DES for proposed extraction was performed and choline chloride-based DES containing oxalic acid as a hydrogen bond donor with 25% of water was selected as the most promising one, resulting in more effective extraction of grape skin phenolic compounds compared to conventional solvents. Additionally, in our study, UAE proved to be the best extraction method with extraction efficiency superior to both MAE and conventional extraction method. The knowledge acquired in this study will contribute to further DES implementation in extraction of biologically active compounds from various plant sources.



## 1. Introduction

Plant phenolics, derived from a wide range of plant secondary metabolites, have attracted increasing attention for their antioxidant properties and marked effects in the prevention of various oxidative stress associated diseases such as cancer (Dai & Mumper, 2010). Therefore, in the last few years, the extraction and identification of phenolic compounds from different plants has become a major area of health and medical-related research. Due to their complex structure, there is no universal extraction method suitable for extraction of all plant phenolics whereby conventional extraction techniques are usually associated with high organic solvent consumption and long extraction times (Ignat, Volf, & Popa, 2011). Also, growing awareness of the human impact on the environment has pushed the "green extraction" in the spotlight of the scientific and industrial community. In general, green extraction is based on the discovery and design of extraction processes which would reduce energy consumption, allow use of alternative solvents and renewable natural products, and ensure safe and high quality extract/products (Chemat, Maryline Abert Vian, & Cravotto, 2012). Since Directive 2010/75/EU on industrial emissions requires plants to limit emissions of certain volatile organic compounds, a growing area of research in the development of green technologies including extraction is devoted to designing new, more environmentally friendly solvents (Cvjetko Bubalo, Vidović, Radojčić Redovniković, & Jokić, 2015).

Over the last few years, among neoteric solvents (neoteric = new, recent, modern) deep eutectic solvents (DES) have been dramatically expanding in popularity as promising alternatives to traditional organic solvents (Cvjetko Bubalo et al., 2015). DES present a new generation of liquid and are generally based on mixtures of cheap and readily available components: nontoxic quaternary ammonium salts (e.g., cholinium chloride) and a naturally-derived uncharged hydrogen-bond donor (e.g., vitamins, amines, sugars, alcohols and carboxylic acids). DES have unique physicochemical properties and thanks to the possibility of designing their properties for particular purpose, their low ecological footprint and attractive price, have become of growing interest for both research and industry (Paiva et al., 2014). Since their emergence, these solvents have attracted attention as solvents in organic synthesis and (bio)catalysis, polymer production, electrochemistry, nanomaterials, separation processes, analysis), biomedical applications and extraction of biologically active compounds from plant material (Cvjetko Bubalo et al., 2015; Paiva et al., 2014).

Since DES consist of simple, cheap, and naturally occurring compounds with a high safety profile, they may be used for very efficient extraction of natural products from plants, both polar and non-polar, such as pharmaceuticals, flavours, natural colourants, etc. (Young et al., 2011). Some authors have studied DES

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**Table 1**
List of the DES used in this study.

| Combination | Abbreviation | Molar ratio |
|---|---|---|
| Choline chloride:glycerol | ChGyl | 1:2 |
| Choline chloride:oxalic acid | ChOa | 1:1 |
| Choline chloride:malic acid | ChMa | 1.5:1 |
| Choline chloride:sorbose | ChSor | 1:1 |
| Choline chloride:proline:malic acid | ChProMa | 1:1:1 |

assisted extraction of phenolic compounds showing that many compounds are dissolved better than in water or lipids. Namely, DES have the ability of donating and accepting protons and electrons, which confers them the ability to form hydrogen bonds, thus increasing their dissolution capability (Bi, Tian, & Row, 2013; Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013; Dai, Witkamp, Verpoorte, & Choi, 2013; Woo Nam, Zhao, Sang Lee, Hoon Jeong, & Lee, 2015). Furthermore, excellent stability of phenolic compounds in sugar-based DES were noticed indicting possible novel application this solvent in food and pharmaceuticals industry (Dai, Verpoorte, & Choi, 2014).

Based on the aforementioned, the aim of the present study was to establish an environmentally friendly extraction method for grape skin phenolic compounds by using DES. We chose red grape skin as plant materials due to their high content of diverse flavonoids. Namely, grape flavonoids (anthocyanins, flavan-3-ols and flavonols) located in skin are extracted during maceration process into the red wine and hence are important contributors to wine quality. Initially, screening of five different DES as potential extraction solvents was performed. In order to optimize extraction methods, after the selection of optimal DES, alternative methods such ultrasound- or microwave-assisted extraction (UAE and MAE, respectively) was applied.

## 2. Materials and methods

### 2.1. Chemicals and materials

Methanol, hydrochloric acid and acetic acid were obtained from Merck (Darmstadt, Germany). Choline chloride (ChCl), glucose, sorbose, glycerol, malic acid, oxalic acid were purchased from Sigma (St. Louis, MO, USA). Analytical standards of quercetin-3-glucoside (⩾98%) and (+)-catechin (⩾99%) were purchased from Sigma (St. Louis, MO, USA), while analytical standards of delphinidin-3-*O*-monoglucoside (⩾97%), cyanidin-3-*O*-monoglucoside (⩾97%), petunidin-3-*O*-monoglucoside (⩾97%), peonidin-3-*O*-monoglucoside (⩾97%), and malvidin-3-*O*-monoglucoside (⩾97%), were purchased from Polyphenols AS (Sandnes, Norway).

Grapes of the Croatian native red grape cultivar, *Vitis vinifera* cv. Plavac mali, originating from Dalmatia (Croatia southern vine-growing region) were harvested in their technological maturity in October 2012. The amount of 2 kg of randomly selected grapes was used in the study, where skins were immediately manually separated from the pulp, freeze-dried (Alpha 1-2 LD plus Christ, Germany) for three days at −40 °C and stored at −20 °C until analysis.

### 2.2. Preparation of DES

All chemicals for preparation were dried in a vacuum concentrator (Savant SPD131DDA SpeedVac Concentrator) at 60 °C for 24 h before use. DES were synthesized at certain ratios of ChCl to hydrogen donor (glucose, sorbose, glycerol, proline, malic acid and oxalic acid) to obtain liquids at room temperature, as shown in Table 1. The mixture of ChCl and hydrogen donor was stirred in a flask at 80 °C for 2–6 h until a homogeneous transparent colourless liquid was formed. DES samples were vacuum dried prior to further use. Additionally, different DES solutions in water were prepared by dilution of a certain volume of DES in deionised water (water solution of DES containing 10%, 25% and 50% of water (w/w) were prepared).

### 2.3. Preparation of extracts

Solid–liquid ratios of 0.1 g of freeze dried and ground Plavac mali grape skin per millilitre of the respective solvent (DES or conventional solvents) were extracted using three different extraction techniques (shaker, MAE and UAE). Then, extracts were centrifuged for 15 min at 5000 rpm and the supernatant was decanted and adjusted to a final volume of 5 mL (0.04 mg $mL^{-1}$).

Selection of optimal DES was performed in a shaker for 12 h at room temperature (conventional extraction, CE). In order to compare extraction efficiency with extraction performed by conventional solvents, extraction using water, aqueous methanol (MEOH; 70:30, v/v) and acidified aqueous solution of methanol (AcMeOH; methanol/water/12 M HCl, 70:29:1, v/v/v; with pH = 1.25) was performed under the same conditions as described above.

In order to optimize extraction methods after the selection of optimal DES, MAE and UAE were applied. A microwave extraction apparatus (Micro SYNTH platform, Milestone, Italy) was used for the MAE. The apparatus was equipped with a digital control system for temperature, time and power. The parameters observed during MAE were extraction temperature (50–90 °C) and extraction time (15–90 min). Temperature measurements were performed at the reactor wall by IR sensor and a fully automated system carried out temperature control by continuous adjustment of the microwave power output (maximal power was set at 100 W). For UAE, an ultrasonic bath with temperature regulation (Sonorex DL102H, Bandelin, Germany) was used. The parameters observed during UAE were extraction temperature (30–90 °C) and extraction time (15–90 min), while radiation was at a fixed frequency of 35 kHz. All extraction procedures using DES or conventional organic solvents were conducted in triplicate.

**Table 2**
Parameters of linear regression, LOD, LOD and RSD (%) for phenolic compounds by HPLC analysis.

| Compound | $\lambda$ (nm) | Concentration range (mg $L^{-1}$) | Regression equation | $R^2$ | LOD (mg $L^{-1}$) | LOQ (mg $L^{-1}$) | RSD (%) |
|---|---|---|---|---|---|---|---|
| (+)-Catechin | 280 | 1.25–200 | 81.853 $x$ + 72.301 | 0.9997 | 0.37 | 1.24 | 0.40 |
| Delphinidin-3-*O*-glucoside | 520 | 1–100 | 163.873 $x$ − 59.780 | 0.9998 | 0.18 | 0.60 | 0.96 |
| Cyanidin-3-*O*-glucoside | 520 | 1–100 | 172.127 $x$ − 10.533 | 0.9999 | 0.21 | 0.71 | 0.57 |
| Petunidin-3-*O*-glucoside | 520 | 1–100 | 191.604 $x$ − 6863 | 0.9999 | 0.24 | 0.80 | 0.83 |
| Peonidin-3-*O*-glucoside | 520 | 1–150 | 160.710 $x$ + 5131 | 0.9999 | 0.19 | 0.65 | 0.71 |
| Malvidin-3-*O*-glucoside | 520 | 1–500 | 123.595 $x$ + 101.097 | 0.9998 | 0.30 | 0.90 | 0.30 |
| Quercetin-3-*O*-glucoside | 360 | 0.5–50 | 111.572 $x$ − 70.855 | 0.9997 | 0.05 | 0.46 | 0.77 |

LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation (%).