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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	λ (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 (%).

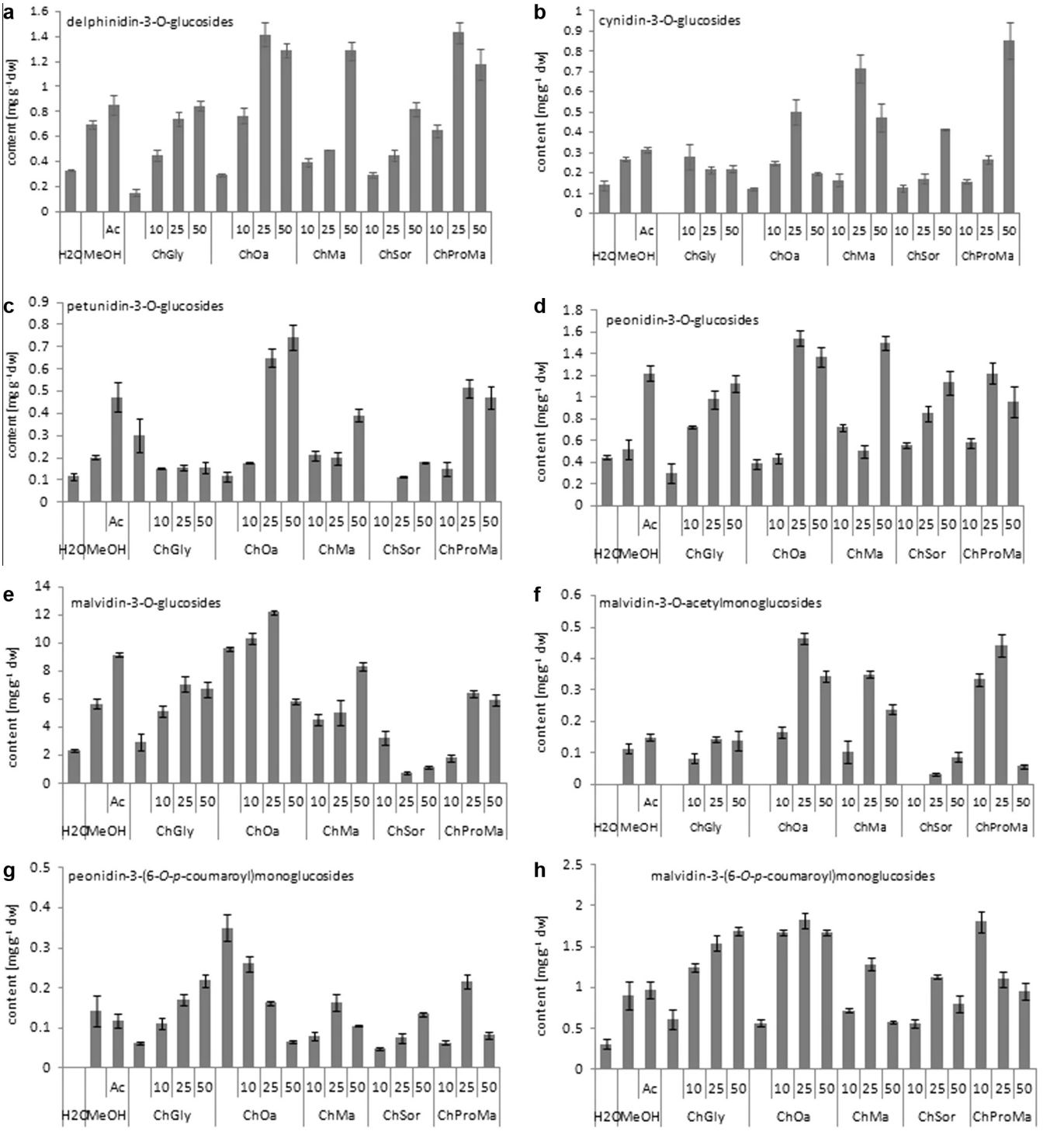

Fig. 1. Anthocyanin contents in grape skin extracted with different DES, water (H_2O), aqueous methanol (MeOH) and acidified aqueous solution of methanol (AcMeOH). The numbers in the graph (0–50) indicate water content in DES (%).

2.4. HPLC analysis

Samples were filtered through 0.45 μm cellulose acetate filters (Macherey–Nagel GmbH & Co., Düren, Germany) prior to the injection. Chromatographic analyses were performed on the Agilent 1100 Series HPLC system (Agilent, San Jose, CA, USA) equipped with a diode array detector. Flavan-3-ol monomer (+)-catechin, flavonol quercetin-3-*O*-glucoside and anthocyanins were separated on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 μm) using water/formic acid (95:5, v/v) (solvent A) and acetonitrile/-formic acid (95:5, v/v) (solvent B), as previously reported by Lorrain, Chira, and Teissedre (2011). Gradient conditions were as follows: 10–35% B linear 0–25 min, 35–100% B linear 25–26 min, 100% B isocratic 26–28 min, 100–10% B linear from 28 to 29 min with re-equilibration of the column for 5 min under initial gradient conditions; and flow rate 1 mL min^{-1}. UV–Vis spectra were measured in the wavelength range of 200–600 nm. Detection and identification of phenolic compounds were performed at the following

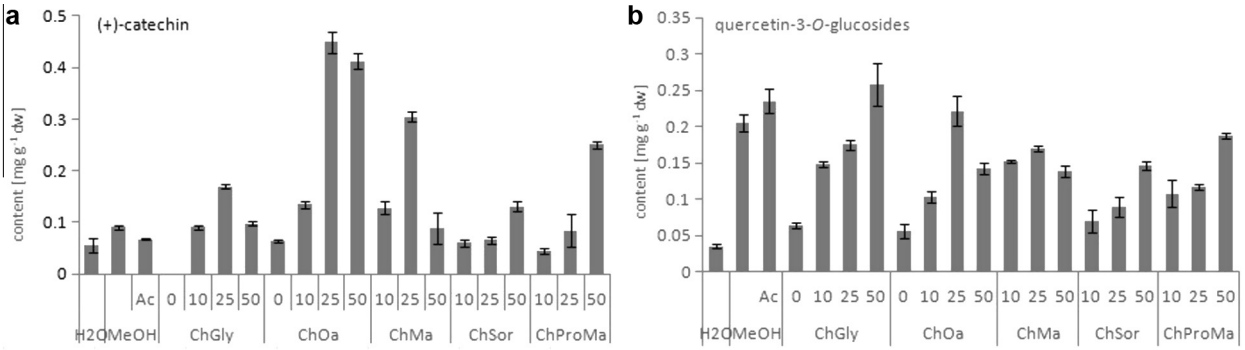

Fig. 2. (+)-Catechin and quercetin-3-*O*-glucoside content in grape skin extracted with different DES, water (H_2O), aqueous methanol (MeOH) and acidified aqueous solution of methanol (AcMeOH). The numbers in the graph (0–50) indicate water content in DES (%).

wavelengths: 280 nm (catechin), 360 nm (quercetin-3-glucoside) and 520 nm (anthocyanins). Identification and peak assignment of these compounds were based on the comparison of their retention times and spectral data with those of the authentic standards. Quantification was done by the external standard method at the wavelength of maximum absorbance for each compound as listed below. HPLC method was validated in the terms of linearity of calibration graphs, limits of detection (LOD), limits of quantification (LOQ) and precision, as shown Table 2. Linearity was evaluated on the basis of eight point calibration curves for each standard. Linear least-square regression analysis was employed to calculate slope, intercept and correlation coefficient of the calibration curve. Correlation coefficients of calibration curves for all standards were higher then 0.9997, indicating very good linearity. Limit of detection was determined form the amount of standard required to give signal-to-noise ratio of 3, and limit of quantification was determined as the lowest concentration giving signal-to-noise ratio of 10. The precision of method was determined in terms of intraday repeatability of peak area for all standards, with relative standard deviation (RSD%) being lower than 0.96% and indicating adequate degree of precision. HPLC analyses of skin phenolic compounds were conducted in triplicate.

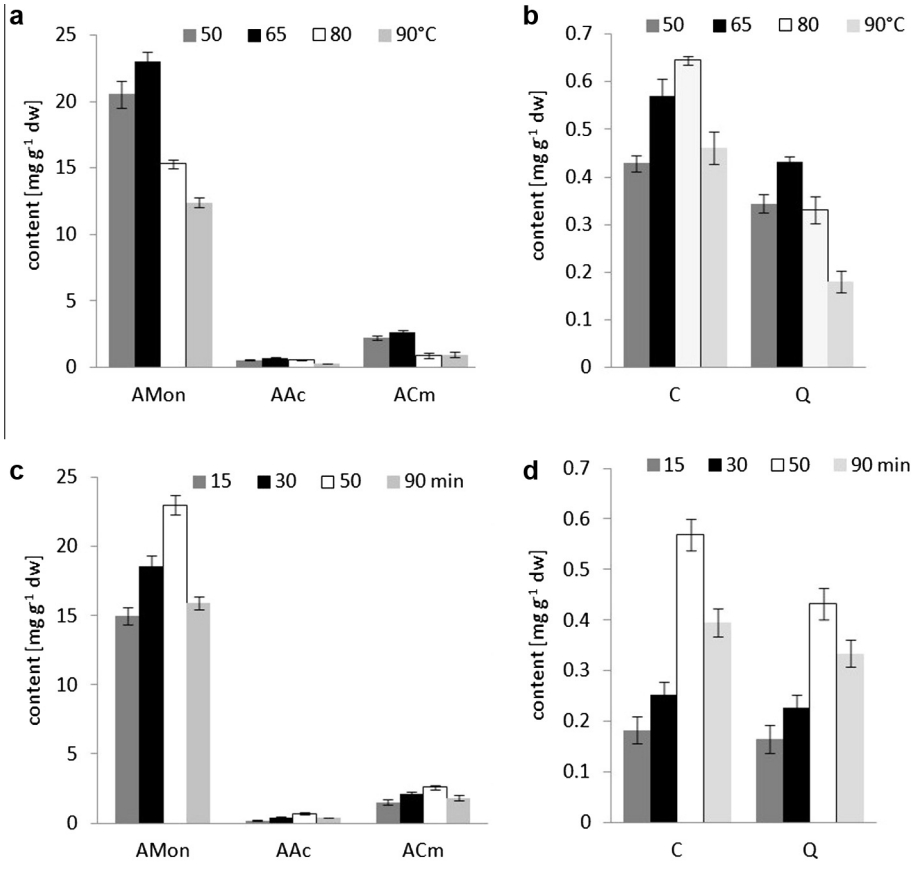

Fig. 3. Influence of temperature (a and b) and extraction time (c and d) during MAE on the extraction yield of sum of anthocyanin-3-*O*-monoglucosides (AMon), sum of anthocyanin-3-acetylmonoglucosides (AAc), sum of anthocyanin-3-(6-*O*-*p*-coumaroyl)monoglucosides (ACm), (+)-catechin (C) and quercetin-3-*O*-glucoside (Q). Mean values ($n = 3$) ± SD.

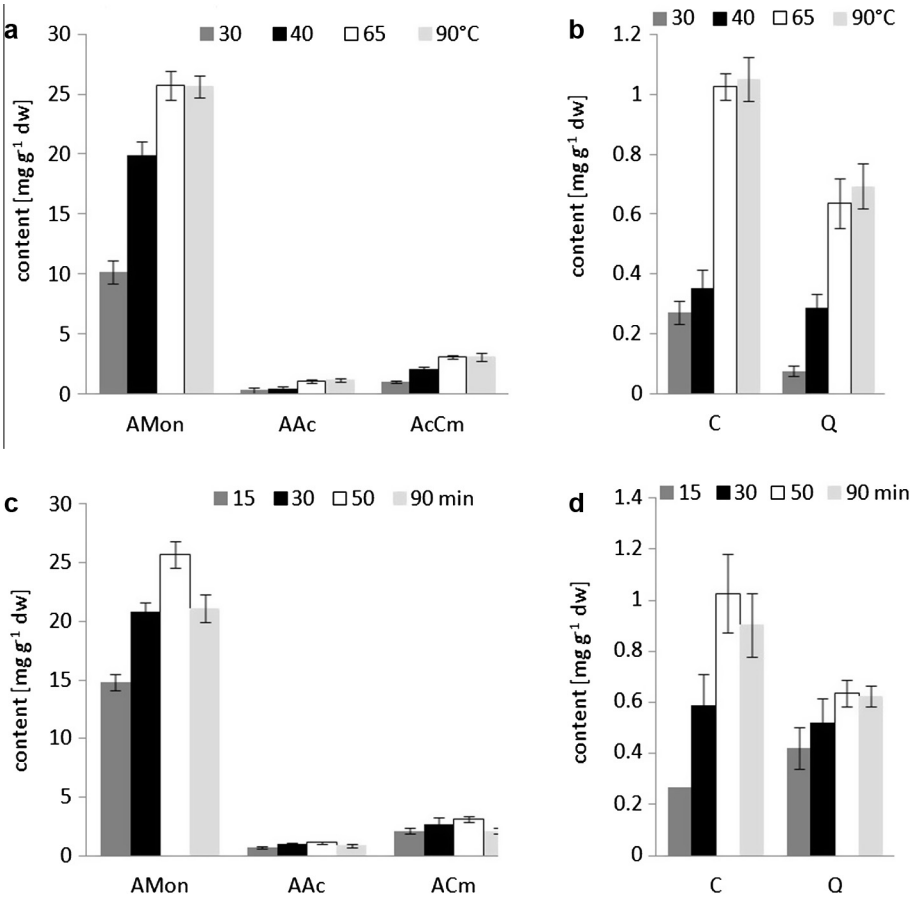

Fig. 4. Influence of temperature (a and b) and extraction time (c and d) during UAE on the extraction yield of sum of anthocyanin-3-*O*-monoglucosides (AMon), sum of anthocyanin-3-acetylmonoglucosides (AAc), sum of anthocyanin-3-(6-*O*-*p*-coumaroyl)monoglucosides (ACm), (+)-catechin (C) and quercetin-3-*O*-glucoside (Q). Mean values (n = 3) ± SD.

2.5. Data analysis

All experimental results were statistically analysed using the Statistica 9.1 software. Data in the text and tables were expressed as mean ± standard deviation (±SD), and error bars in the figures indicate standard deviation. Differences between means were analysed by the ANOVA test, followed by the post hoc Tukey's test. A significant difference was considered at the level of $P < 0.05$.

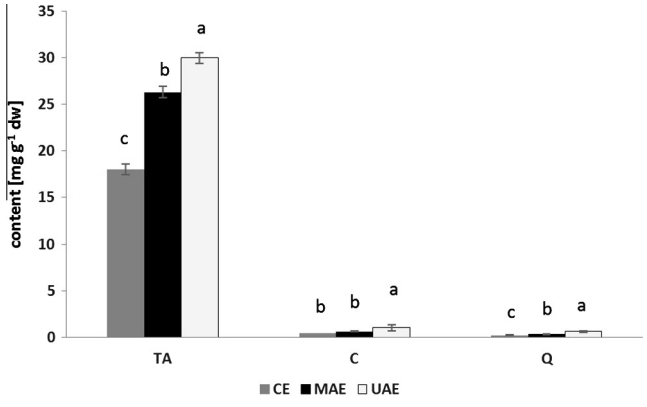

Fig. 5. Comparison between conventional extraction method (CE), MAE and UAE extraction yield for total free anthocyanins (TA), (+)-catechin (C) and quercetin-3-*O*-glucoside (Q). Mean values (n = 3) ± SD were obtained at optimal condition for each extraction method. Different letters on the top of column showed differences among different extraction methods for the same response measured by Tukey's HSD test ($P < 0.05$).

3. Results and discussion

3.1. DES selection for extraction of grape skin phenolic compounds

The structure of DES determines their physicochemical properties and consequently greatly influences extraction efficiency of biologically active compounds. Thus, we selected five different ChCl-based DES containing glycerol (ChGyl), oxalic acid (ChOa), sorbose (ChSor), malic acid (ChMa), and proline and malic acid (ChProMa) as hydrogen-bond donor (HBD) to test their extraction efficiency for the main grape skin flavonoid (delphinidin-3-*O*-monoglucoside, cyanidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, peonidin-3-*O*-monoglucoside, malvidin-3-*O*-monoglucoside, malvidin-3-O-acetylmonoglucosides, peonidin-3-(6-*O*-*p* coumaroyl)monoglucosides, and malvidin-3-(6-*O*-*p*-coumaroyl) monoglucosides as anthocyanins, (+)-catechin as a flavan-3-ol representative and quercetin-3-*O*-glucoside as a flavonol representative) (Table 1). Extraction of phenolic compounds was performed with DES containing different water contents in order to reduce viscosity, which is one of the major problems when using DES as extraction solvent (Dai, van Spronsen, et al., 2013). For example, in contrast to ChGyl and ChCa extraction with pure ChSor, ChMa and ChMaPro could not be performed. All DES were diluted up to 50% water in DES. Further dilutions of DES were not included since large excess of water in DES could break the halide-HBD

supramolecular complex and a simple aqueous solution of the individual components could be obtained (Gutierrez, Ferrer, Mateo, & del Monte, 2009). Also, in order to compare extraction efficiency with conventional solvents, extractions were performed by using water, aqueous methanol and acidified aqueous solution of methanol.

Generally, DES capacity to extract phenolic compounds varied considerably according to the target phenolic compounds and the DES itself (Figs. 1 and 2). In general, it was found that extraction efficiency strongly to depend on water content. In the case of total anthocyanin (sum of all quantified anthocyanins), the best extraction efficiency was obtained with ChOa, followed by ChMa > ChMaPro > ChGyl > ChSor. Anthocyanins are polar molecules and such difference in extraction efficiency among various DES could be explained by distinction in their polarity. For example, of the DES applied, the organic acid-based ones are most polar, thus probably showing best performance, whereas both sugar and polyalcohol based DES are least polar and show poorest results (Dai, Witkamp, et al., 2013). Another import feature for successful anthocyanin extraction is acidity of extraction solvent since anthocyanins can be found in different chemical forms depending on the solution pH. Namely, at pH 1 they are predominantly present in the form of red flavylium cation, and at pH between 2 and 4 as the blue quinoidal species. At pH between 5 and 6, colourless carbinol pseudobase and chalcone are observed, whereas at pH values higher than 7 anthocyanins are degraded (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Given that pH has great importance in the anthocyanin equilibrium forms and stability, it was no surprise that among the DES tested, acidic ChOa and ChMa enabled highest concentrations of total free anthocyanins. On the contrary, extraction efficiency decreased with acidity lowering (Dai et al., 2014).

Furthermore, the anthocyanin composition obtained in DES (Fig. 1) was in good agreement with previous studies carried out on grape skin, comprising of five anthocyanin-3-*O*-monoglucosides and their three major acylated derivatives (acetylmonoglucosides and coumaroylmonoglucosides), which differ in polarity (Lorrain et al., 2011). Anthocyanin polarity is a function of the number of hydroxyl groups and their degree of methylation of the B-ring (delphinidin < cyanidin < petunidin < peonidin < malvidin), along with their acylation pattern (acetylglucosides < coumaroyl-glucosides) (Novak, Janeiroa, Seruga, & Oliveira-Brett, 2008). However, the pattern observed among different DES was very similar for all individual anthocyanins, with a clear trend for more polar anthocyanins being extracted better with DES containing higher water content and *vice versa*, which is consistent with previously published data (Dai, Witkamp, et al., 2013). For instance, anthocyanin-3-*O*-monoglucosides are extracted better with DES containing 50% of water, whereas the less polar ones such as malvidin-3-*O*-monoglucosides and acylated derivative are extracted better with DES containing 25% of water.

Additionally, DES extraction efficiency showed promising results when compared with conventional solvents. Extraction yields obtained with all DES with certain contents of water were higher than those obtained with water and 70% of methanol. ChOa with 25% water as the best extraction solvent of all DES tested showed 5- and 2-fold higher extraction yield recorded for water and aqueous methanol for total anthocyanin content, respectively. However, due to the stability of red flavylium cation in a highly acidic medium, extraction of anthocyanins is commonly performed with acidified organic solvents (based on previous studies, acidified aqueous solution of methanol was selected as a conventional procedure). ChOa and ChMa with 25% water showed higher content than acidified aqueous solution of methanol. In the case of ChOa with 25% water, higher content of all individual anthocyanins was noticed. Also, an interesting pattern was noticed for ChGyl, where selectivity for *p*-coumaroylmonoglucosides was observed. Namely, ChGyl extracts contained higher content of both *p*-coumaroylmonoglucosides than acidified aqueous solution of methanol, while for anthocyanin-3-*O*-monoglucosides a lower content was recorded. These observations indicate that by fine tuning of DES structure, it is possible to design an optimal solvent for structurally very similar compounds within the same class.

Moreover, DES also showed better extraction efficiency for (+)-catechin with the content higher for all studied DES with certain content of water in comparison to conventional solvents (Fig. 2). The best extraction efficiency was obtained with ChOa, followed by ChMa > ChMaPro > ChGyl > ChSor, which was similar to extraction efficiency for anthocyanin-3-*O*-monoglucosides, probably due to their comparable polarity. The best extraction efficiency for quercetin-3-*O*-glucoside was obtained with ChGyl, where higher content was obtained compared to conventional solvent. The rest of DES followed the order ChOa > ChMa > ChMaPro > ChSor and the quercetin-3-*O*-glucoside content was similar to methanol extracts (Fig. 2). Based on the data obtained in this study, ChOa with 25% of water was selected as the most promising solvent for extraction of grape skin phenolic compounds and could serve as promising replacement for volatile organic solvents.

3.2. Selection of the extraction methods

Besides the use of green solvents, one of the criteria for green extraction is to reduce energy consumption by using innovative technologies such as UAE and MAE (Chemat et al., 2012). Ultrasound and microwave have been recognized as outstanding energy sources to promote extraction, increase extraction yield with high product quality, as well as cutting down extraction time (Esclapez, García-Pérez, Mulet, & Cárcel, 2011; Mandal, Mohan, & Hemalatha, 2007). Herein, both extraction methods were applied. The influence of extraction temperature and extraction time on the extraction yield of sum of anthocyanin-3-*O*-monoglucosides (AMon), sum of anthocyanin-3-acetylmonoglucosides (AAc), sum of anthocyanin-3-(6-*O*-p-coumaroyl)monoglucosides (ACm), (+)-catechin and quercetin-3-*O*-glucoside was studied.

It is important to identify optimal extraction temperature, as it is one of the main factors contributing to extraction efficiency of MAE. In general, at higher temperatures the solvent power increases because of decrease in its viscosity and diffusivity, which is very important for viscous solvents such as DES. An increase in temperature also causes reduction of surface tension, as well as decrease in the interaction between target compounds and sample matrix, leading to enhanced desorption and dissolution of target component in the solvent (Bi et al., 2013; Mandal et al., 2007). On the other hand, high temperature may lead to thermal degradation of phenolic compounds (Xiao, Han, & Shi, 2008). Therefore, we decided to study moderate temperature range (50–90 °C). During the experiments, temperature was controlled by continuous adjustment of the microwave power output, which did not exceed 100 W. The upper limit of microwave power used was set in order to avoid possible overheating of extraction mixture and consequent phenolic degradation (Yan et al., 2010). As shown in Fig. 3a, the extraction efficiency of anthocyanins significantly increased when the temperature was raised from 50 to 65 °C, whereas further temperature increase caused decrease in anthocyanin contents. Similar values were obtained for quercetin-3-*O*-glucoside, while (+)-catechin content increased up to 80 °C, indicating its better thermal stability in extracts as compared with other compounds. However, 65 °C was selected as optimal temperature and monitoring of the irradiation time influence on extraction efficiency was done at this temperature. Extraction efficiency of all study

components enhanced with increasing irradiation time up to 50 min. Further increase of treatment time caused decrease in the extraction yield indicating that prolonged MAE led to thermal degradation and oxidation of target compounds (Mandal et al., 2007). There are many literature reports on the successful usage of MAE in extraction of plant phenolic compounds at various operating conditions, however, frequently with opposite conclusions concerning optimal conditions (Jokić et al., 2012; Mandal et al., 2007; Song, Li, Liu, & Zhang, 2011). For example, some studies report that the usage of high microwave power for a short period of time led to high extraction efficiency (Song et al., 2011), whereas other studies (including ours, $T = 65$ °C, $t = 50$ min) demonstrated a combination of moderate temperature and longer time of exposure to lead to optimal operating approach (Jokić et al., 2012).

Ultrasound-assisted extraction enhancement of extraction yield is mainly due to cavitation bubbles in the solvent produced by the ultrasonic wave passage causing microjet impacts. Also, shockwave-induced damage of plant cell wall occur causing releasing cell content into the solvent (Esclapez et al., 2011). On the other hand, ultrasonic waves could cause some changes in chemical composition as a consequence of target compound degradation and production of free radicals within the gas bubbles (Paniwnyk, Beaufoy, Lorimer, & Mason, 2001). During UAE extraction temperature (40–90 °C) and extraction time (15–90 min) were considered. Fig. 4a and b shows that the extraction yield of all study phenolic compounds increased by more than 30% with temperature increase from 30 to 60 °C. Further temperature increase from 65 to 90 °C led to only slight increase in the extraction yield, therefore the influence of irradiation time on extraction yield was monitored at 65 °C. Fig. 4c and d illustrates a similar effect of extraction time as for MAE. It can be observed that the contents of all study components significantly increased with increasing irradiation time up to 50 min, whereas degradation clearly occurred with further increase of irritation time, which is in agreement with previous studies (Carrera, Ruiz-Rodríguez, Palma, & Barroso, 2012; Tao, Wu, Zhang, & Sun, 2014).

Additionally, comparison among MAE, UAE and conventional extraction (CE) by shaker indicated that extraction efficiency increased in the following order CE < MAE < UAE. This is not surprising, since many studies demonstrated MAE and UAE to be the techniques of choice in comparison to conventional methods (Esclapez et al., 2011; Mandal et al., 2007). Additionally, UAE showed better performance than MAE. There are a few literature reports dealing with comparison between UAE and MAE and there are ambiguous conclusions about method efficiency (Ghassempour et al., 2008; Rajaei, Barzegar, Hamidi, & Sahari, 2010). For example, Ghassempour et al. (2008) compared UAE and MAE in the recovery of anthocyanins from red grape skin and UAE showed slightly lower recoveries than MAE, while Rajaei et al. (2010) indicate that UAE is the method of choice for extraction of pistachio green hull phenolic compounds. UAE method was used in the extraction of phenolic compounds of *Carthamus tinctorius* L. and *Chamaecyparis obtuse* by using different DES and extraction efficiency was not improved in comparison to conventional extraction method including heating or heating and string. The possible reason for such a low productivity of UAE could be due to extraction temperature because in previous studies UAE was conducted at room temperature. In our study, 65 °C was selected as optimal temperature, indicting that temperature is one of the most important factors in extraction of phenolic compounds with DES (Bi et al., 2013; Dai, Witkamp, et al., 2013). However, in our study, UAE proved to be the best extraction method in the following conditions: extraction temperature of 65 °C and extraction time of 50 min (see Fig. 5).

4. Conclusion

Among the DES tested, ChOa with 25% of water was selected as the most promising solvent and proved to be effective in the extraction of grape skin phenolic compounds compared to conventional organic solvents. Additionally, in our study, the best extraction method was UAE with extraction efficiency better than MAE and CE. The use of DES from natural sources in the extraction processes could lead to highly efficient and truly eco-friendly extraction methods. However, further study such as biological activity of obtained extracts and/or stability of phenolic compounds in DES should be perform before truly implementation of DES in the extraction of phenolic compounds.

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References

Bi, W., Tian, M., & Row, K. H. (2013). Evaluation of alcohol-based deep eutectic solvent in extraction and determination of flavonoids with response surface methodology optimization. *Journal of Chromatography A, 1285*, 22–30.

Carrera, C., Ruiz-Rodríguez, A., Palma, M., & Barroso, C. G. (2012). Ultrasound assisted extraction of phenolic compounds from grapes. *Analytica Chimica Acta, 732*, 100–104.

Castañeda-Ovando, A., Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113*, 859–871.

Chemat, F., Maryline Abert Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences, 13*, 8615–8627.

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

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*, 7313–7352.

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 new extraction media for phenolic metabolites in *Carthamus tinctorius* L. *Analytical Chemistry, 85*, 6272–6278.

Esclapez, M. D., García-Pérez, J. V., Mulet, A., & Cárcel, J. A. (2011). Ultrasound-assisted extraction of natural products. *Food Engineering Reviews, 3*, 108–120.

Ghassempour, A., Heydari, R., Talebpour, Z., Fakhari, A. R., Rassouli, A., Davies, N., & Aboul-Enein, H. (2008). Study of new extraction methods for separation of anthocyanines from red grape skin and analysis by HPLC and LC–MS/MS. *Journal of Liquid Chromatography & Related Technologies, 31*, 2686–2703.

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

Ignat, I., Volf, I., & Popa, V. I. (2011). Critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. *Food Chemistry, 126*, 1821–1835.

Jokić, S., Cvjetko, M., Božić, Đ., Fabek, S., Toth, N., Vorkapić-Furač, J., & Radojčić Redovniković, I. (2012). Optimization of microwave-assisted extraction of phenolic compounds from broccoli and its antioxidant activity. *International Journal of Food Science and Technology, 47*, 2613–2619.

Lorrain, B., Chira, K., & Teissedre, P.-L. (2011). Phenolic composition of merlot and cabernet-sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages. *Food Chemistry, 126*, 1991–1999.

Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Review article: Microwave assisted extraction – An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews, 1*, 7–16.

Novak, I., Janeiroa, P., Seruga, M., & Oliveira-Brett, A. M. (2008). Ultrasound extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse-phase high-performance liquid chromatography with electrochemical detection. *Analytica Chimica Acta, 630*, 107–115.

Paiva, P., 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*, 1063–1071.

Paniwnyk, L., Beaufoy, E., Lorimer, J. P., & Mason, T. J. (2001). The extraction of rutin from flower buds of *Sophora japonica*. *Ultrasonics Sonochemistry, 8*, 299–301.

Rajaei, A., Barzegar, M., Hamidi, Z., & Sahari, M. A. (2010). Optimization of extraction conditions of phenolic compounds from pistachio (*Pistachia vera*) green hull through response surface method. *Journal of Agricultural Science and Technology, 12*, 605–615.

Song, J., Li, D., Liu, C., & Zhang, Y. (2011). Optimized microwave-assisted extraction of total phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. *Innovative Food Science and Emerging Technologies, 12*, 282–287.

Tao, Y., Wu, D., Zhang, Q.-A., & Sun, D.-W. (2014). Ultrasound-assisted extraction of phenolics from wine lees: Modeling, optimization and stability of extracts during storage. *Ultrasonics Sonochemistry, 21*, 706–715.

Woo Nam, M., Zhao, J., Sang Lee, M., Hoon Jeong, J., & 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*, 1718–1727.

Xiao, W., Han, L., & Shi, B. (2008). Microwave-assisted extraction of flavonoids from *Radix astragali*. *Separation and Purification Technology, 62*, 614–618.

Yan, M. M., Liu, W., Fu, Y. J., Zu, Y. G., Chen, C. Y., & Luo, M. (2010). Optimisation of the microwave assisted extraction process for four main astragalosides in *Radix astragali*. *Food Chemistry, 119*, 1663–1670.

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