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Antioxidant activity of grape skin aqueous extracts from pressurized hot water extraction combined with electron paramagnetic resonance spectroscopy

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ABSTRACT

Pressurized hot water extraction (PHWE) was employed to prepare extracts from dried grape skin of two wine grape varieties (St. Laurent and Alibernet) at various temperatures (from 40 up to 120 °C) and amounts of sample (0.5, 1.0 and 1.5 g). To assess the antioxidant activity of the extracts, electron paramagnetic resonance (EPR) spectroscopy was applied involving •DPPH and ABTS•+ assays. Other extract characteristics including HPLC profile of anthocyanins and total phenolic compound content were obtained as well. PHWE has also been compared with earlier results of extractions of the same grape skin samples with compressed methanol and compressed ethanol under the conditions of pressurized fluid extraction (PFE). From this comparison, PHWE emerges as the more benign and efficient extraction method to recover valuable phenolic antioxidants from grape skins for the prospective use in functional food supplements.

1. Introduction

Wine production industries generate a large amount of waste including grape seeds, skins, stems, stalks, pomace, pulp, marc, wastewater and sludge (around 5–9 million tonnes per year, worldwide). Its efficient, inexpensive and environment-friendly utilization is of growing importance both from the higher profitability and environmental impact minimization points of view. Besides other applications, valuable compounds recovered from waste could be effectively used in pharmaceutical, cosmetics and food industries [1,2].

Growing evidence of the role of free radicals and antioxidants in health management has focused great interest on phenolic compounds isolated from grapes and wines. Phenolics are believed to have many human health beneficial effects [1–5]. Plant polyphenols, because of their diversity and extensive distribution, are the most important group of natural antioxidants. As one of their most important source, grape skins were recognized. It is well-documented that the antioxidant activities of plant polyphenols depend on their chemical structures and are also affected by the type of the extracting solvent. Diverse results were reported on the relationship between the phenolic content and antioxidant activity; some authors pointed on positive correlation whereas others found no relationship [6–8]. Anthocyanins, the major phenolics component in berries, revealed to be twice as effective as commercially available synthetic anti-oxidants including butylated hydroxyanisole (BHA) or α-tocopherol. However, only about 30–40% of polyphenols are extracted from grapes during the wine-making process [9].

Various extraction techniques and procedures for the isolation of valuable compounds from the above-mentioned wastes have been tested, involving at the same time the green chemistry philosophy [10], i.e., an effort to replace any hazardous solvents and practices and to maximize the efficiency of extraction process [3,4,11]. Moreover, there is a growing interest to exploit the residues generated by the food industry itself. For these reasons, a complex characterization of the extracts could represent an interesting advance in the maintenance of the environmental equilibrium.

In this context, the utilization of pressurized hot water as a green solvent to enable isolation and subsequent characterization of the compounds of interest is one of the possible "green philosophy" approaches, enabling also the development of analytical chemistry along the routes towards new ecological orientation and challenges [4,5,11,12]. Pressurized hot water extraction (PHWE) represents modern extraction technique employing the elevated temperature and pressure to extract the target analytes from the sample matrix [13]. Because of its lower cohesive energy density and relative permittivity in comparison to those of water at ambient conditions [14–16], pressurized hot water is closer to organic

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solvents as regards the solvent properties. The advantages of PHWE can be briefly summarized as follows: The use of higher temperature increases the solubility, diffusion rates and mass transfer of the compounds and decreases the viscosity and surface tension of the extraction solvent. These changes improve the contact of analytes with the solvent and enhance the extraction, which can thus be achieved more rapidly with less solvent consumption in comparison to the conventional/traditional extraction methods. Moreover, the elevated pressure improves the contact between the sample and extraction solvent, thereby facilitating solvent penetration into such matrices as grape skins [17]. PHWE has been successfully applied for extraction of bioactive and nutritional compounds from plant and food material, for removal of organic contaminants from foodstuff, for environmental samples and for pesticides and herbicides in soil and sediments [13].

Different methods have been used to monitor and compare the radical-scavenging or antioxidant activity of foods and biosystems, among which electron paramagnetic resonance (EPR) spectroscopy is considered to be one of the most efficient [18–20]. Most frequently, the ability of the studied system to scavenge various free radicals, e.g., 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) cation radical ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picrylhydrazyl ($^{\bullet}DPPH$), or hydroxyl radicals ($^{\bullet}OH$) added or generated directly in the experimental system is monitored [21–24]. As the redox reaction occurring in the experimental system is frequently accompanied with the development or disappearance of colour at the specific wavelengths, UV–vis spectroscopy is often involved for these purposes as well [2,4,5,25–29].

In this contribution, the off-line PHWE–EPR combination is presented for the first time as a tool for maintenance and characterization of the antioxidant status of some sensitive biological compounds. To the best of our knowledge, no such application of the PHWE–EPR combination has been previously described for any purpose. Besides that, to spread out the applicability of PHWE extraction system to anthocyanins extraction from grape skins, the results of PHWE experiments are compared with those obtained by the extractions of the same grape skin samples with pressurized hot methanol and ethanol under the conditions of Pressurised Fluid Extraction (PFE) previously reported by Polovka et al. [30].

2. Experimental

2.1. Equipment

The extraction procedure was performed in an in-lab-assembled PHWE system described before [31,32]. For individual extracts' analysis, a high performance liquid chromatography (HPLC) apparatus (Spectra SYSTEM HPLC, USA) coupled with diode array detector was used. The antioxidant activity measurements were carried out using a portable X-band EPR spectrometer e-scan (Bruker BioSpin GmbH, Karlsruhe, Germany) with accessory. A Specord M40 double-beam UV–vis spectrometer (Carl Zeiss, Jena, Germany) with appropriate accessories was employed to monitor the total phenolic compounds content (TPC). Water used as an extraction solvent was purified prior its utilization by reverse osmosis involving Ultra Clear UV system (SG Wasseraufbereitung und Regenerierstation GmbH, Barsbüttel, Germany).

2.2. Chemicals and material

Stable free radical 1,1-diphenyl-2-picrylhydrazyl ($^{\bullet}DPPH$, Fluka, Buchs, Switzerland) as well as 2,2′-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) salt (ABTS, Polysciences, Inc., Warrington, PA, USA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid monohydrate, Folin & Ciocalteu's phenol reagent (both purchased from Sigma Aldrich Ltd., Milwaukee, WI, USA), sodium carbonate (Na_2CO_3, Lachema, Brno, Czech Republic) and water purified as described above were used in the experiments. 3-O-monoglucosides of malvidin (Mv-3-glc), delphinidin (De-3-glc), peonidin (Pn-3-glc), cyanidin (Cy-3-glc), petunidin (Pt-3-glc) and pelargonidin (Pg-3-glc) of analytical grade purity (Polyphenols Laboratories AS, Sandnes, Norway) were used as anthocyanins' standards. It should be noted here that although the other anthocyanins and/or polyphenolic forms could be expected in the matrices under study, the selection of the anthocyanin standards applied followed from three basic aspects, i.e., expected concentration, price and availability of individual standards. Brillant blue FCF (Lachema, Brno, Czech Republic) was employed as an internal standard in HPLC measurements for the purposes of anthocyanins quantification.

Grape skins from two wine grape varieties, St. Laurent and Alibernet from Velké Pavlovice and Mikulov sub-regions (South Moravia region, Czech Republic), collected in 2007 vintage were used in all experiments. Harvested grapes were placed into polystyrene boxes filled with dry ice and transported to the laboratory. Grape skins were then manually separated from the pulps at inert atmosphere and lyophilized. The dried skins were ground to a fine powder under liquid nitrogen and stored at −20 °C in dark glass vials.

2.3. Extraction procedure

The portion of grape skin powder (0.5, 1.0, or 1.5 g) was placed into an 11 mL extraction cell containing inert material (glass beads, 570–700 μm) at the bottom. The extraction was performed under the following conditions: extraction temperature, 40–120 °C (20 °C step); pressure, 15 MPa; extraction solvent, purified water; extraction time, 3 × 5 min; rinsing time, 20 s; nitrogen purge time, 20 s after each cycle and 120 s after the extraction run. The collected extract was cooled to 5 °C and stored in a fridge prior the analysis. The first step in the development of the analytical protocol was to assess the extraction efficiency for PHWE-HPLC method. To prepare the in-house reference materials, a portion of 6 g grape skin powder was placed into a 30 mL extraction cell containing inert material at the bottom. The extraction was performed under the conditions mentioned above with optimized extraction temperature, 80 °C. To secure an analyte-free blank material, an additional extraction cycle was carried out so that the extraction time was 4 × 5 min. After extraction, the blank material was lyophilized. Then, the extraction efficiency was evaluated by triplicate analyses of two reference materials prepared by spiking the blank with different concentrations of the anthocyanins' standards representing different concentration regions of the calibration curve. The low-concentration material (C1) contained 300 μg/g of each target analyte whereas the high concentration material (C5) contained De-3-glc, Pt-3-glc, Pn-3-glc (50 mg/g of each), 3 mg/g of Cy-3-glc and 120 mg/g of Mv-3-glc. Then the spiked materials were processed in the same way as the samples. After the chromatographic analysis of the extracts, recoveries were calculated. When extracting the spiked materials, typical recoveries in PHWE ranged from 93 to 96% for C1 and C5 concentrations. The relative standard deviations were 7 and 5%, respectively. For the PHWE-EPR method, the same procedure and results were used.

2.4. UV/VIS experiments

The TPC values of individual extracts were determined following the modified Folin & Ciocalteu (F-C) procedure [33]. Briefly, the F-C reagent oxidised the reducing substances in samples and changed in colour to dark blue (λ = 765 nm), monitored by UV/VIS spectrophotometer [34]. First of all, the calibration plot of absorbance

versus gallic acid concentration was constructed. In this way, the total content of polyphenols as measured by the Gallic Acid Equivalent (GAE) values could be assigned to all samples. All UV/VIS measurements were carried out in the same quartz cells (1 cm path length) in duplicates. As regards the precision, the relative standard deviations of parallel experiments did not exceed 0.1%.

2.5. HPLC measurements

All extracts were filtered through a 0.45 μm syringe filter prior the analysis. The separation was performed on a Synergi C12 Max-RP column (4.6 mm i.d., 250 mm length, 4 μm stationary phase particles) (Phenomenex, USA). A mixture of water/acetonitrile, 97:3 (A) and 40:60 (B) adjusted at pH 1.8 by formic acid was used as a mobile phase. The 0.5 mL/min flow rate was kept constant during the measurement (50 min run time) under the following linear gradient program: 0 min, 6% B; 20 min, 20% B; 35 min, 40% B; 40 min, 60% B; 45 min, 90% B; 47–55 min, 6% B. The peak assignment was accomplished by comparing their retention times with those of the respective standards. Quantification was performed by the internal standard method involving Brillant blue FCF as the internal standard. All analyses were performed in triplicates and the results obtained were expressed as the mean ± SD ($n = 3$).

2.6. EPR spectroscopy measurements

Determination of antioxidant activity by means of •DPPH or ABTS•+ was based on the scavenging of •DPPH/ABTS•+ radicals by antioxidants present in grape skins' extracts. The decrease of •DPPH/ABTS•+ concentration was monitored by electron paramagnetic resonance (EPR) spectroscopy. The respective reference samples were prepared by replacing the extracts with deionized water. Experimental systems used in these tests are summarized in Table 1. All components of the reaction mixture were mixed together in Eppendorf tubes and the reaction time was measured from the moment of •DPPH or ABTS•+addition. The so-prepared sample was transferred into a flat quartz EPR cell, closed with a stopper and inserted into the spectrometer cavity. All EPR measurements were performed at ambient temperature, starting precisely 3 min after the last reagent addition and 10 accumulated EPR spectra were recorded in time domain during 15 min. Each experimental spectrum represents an average of 30 individual scans. The actual concentration of •DPPH/ABTS•+ was calculated from double integrated EPR spectra recorded exactly at 10.5 min and finally converted to the Trolox equivalent antioxidant capacity (TEAC) in mmol/L of the extract in an identical manner as was previously described elsewhere [30,35]. The response and settings of the EPR spectrometer were checked by means of Strong pitch standards (Bruker) daily before the experiments and the processing and evaluation of experimental EPR spectra was carried out using WIN EPR program (Bruker) as described, e.g., in [30].

The EPR experiments were performed in triplicates, with the relative standard deviations among the individual measurements always less than 5%. Numerical values obtained were expressed as the mean ± SD ($n = 3$).

2.7. Calculations of water properties

Solubility parameter and cohesive energy density of pure water as the functions of temperature and pressure were calculated from the Wagner–Pruß equation of state [36] using the interactive software developed by Wagner and Overhoff [37]. In calculating the cohesive energy density, the ideal-gas limit was approximated by setting the pressure equal to 10^{-6} Pa. Relative permittivity and ionization constant of water were obtained from the correlations developed by Fernández et al. [38] and by Bandura and Lvov [39], respectively.

2.8. Statistical correlation

To gain a complex knowledge of the effects of extract preparation conditions on the antioxidant status and other important characteristics, all the data obtained from EPR and UV/VIS experiments were processed by multivariate statistics. Pearson's correlation coefficients were evaluated by means of Unistat® (Unistat, London, United Kingdom) statistical software.

3. Results and discussion

3.1. UV/VIS and HPLC analysis

It is generally accepted that the content of polyphenols has a significant impact on the various characteristics of foods including grapes and wines. Thus, as one of the extracts' basic characteristics, the content of total polyphenolic compounds was evaluated. Fig. 1 shows the TPC values determined in water extracts prepared by PHWE from different amounts of grape skin powders (0.5, 1.0 and 1.5 g) at different temperatures (40–120 °C). In accord with expectations, there is a clear dependence of TPC content on the amount of grape skins used for respective extracts preparation. Also, the content of polyphenols in the Alibernet variety was almost twice of that in the St. Laurent variety. As a result of the rising extraction temperature, a continuous increase of TPC was observed for both varieties throughout the whole interval of extraction temperatures used, in contradiction to the trends found previously in methanolic and ethanolic extracts prepared by PFE [30]. For St. Laurent extracts prepared from 0.5 g of skins, even a 400% increase of TPC was observed, taking into consideration the extracts prepared at 40 °C and 120 °C, respectively. In other cases, 90–120% increase of TPC content was noticed. It is supposed that this increase results directly from the extrability changes, similarly as was previously described for methanolic or ethanolic extracts prepared by pressurized fluid extraction [30].

Since anthocyanins including the 3-O-glucosides represent one of the most abundant fraction of polyphenols, their presence in the extracts prepared by PHWE has been monitored here in an identical way as previously performed by Polovka et al. [30] focusing on

Table 1
The composition of experimental systems used in EPR experiments.

System	Variety	Dilution[a]	Addition	Other components of experimental system
•DPPH	Alibernet	1:5	300 μl[b]	•DPPH in ethanol ($c_{0DPPH} = 5 \times 10^{-4}$ mol/L)[c]
	St. Laurent	1:5	300 μl[b]	
ABTS•+	Alibernet	1:40	150 μl	ABTS•+ in H_2O ($c_{0ABTS^{\bullet+}} = 8–10 \times 10^{-5}$ mol/L)[c]
	St. Laurent	1:30	150 μl	

[a] dilution prior the addition of extract into experimental system.
[b] for extracts from 1.5 g, 150 μl was used.
[c] final volume of experimental system after mixing = 1000 μL; c_{0DPPH} and $c_{0ABTS^{\bullet+}}$ are the initial concentrations of the radicals.

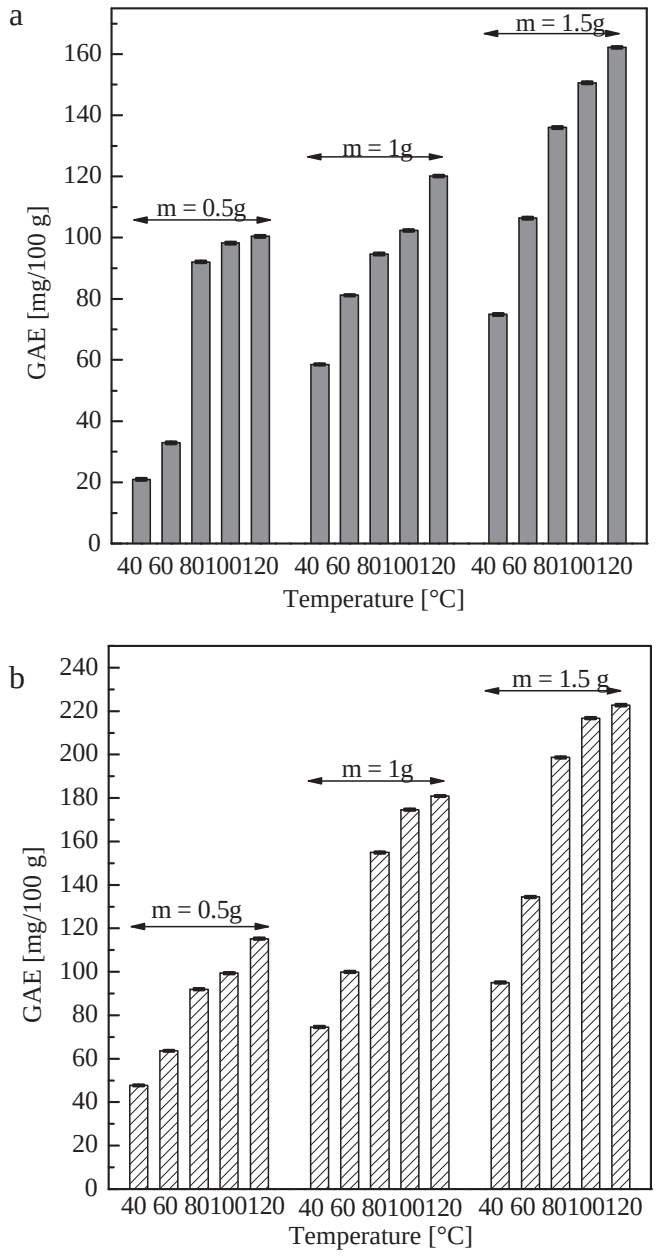

Fig. 1. Total phenolic content (TPC) in extracts of St. Laurent (a) and Alibernet (b) varieties prepared from different amount of grape skin powders (0.5, 1 and 1.5 g, respectively) at different temperatures (40–120 °C), expressed as Gallic Acid Equivalent (GAE). Measurements were performed at 25 °C. Results are expressed as mean ± SD (n = 3).

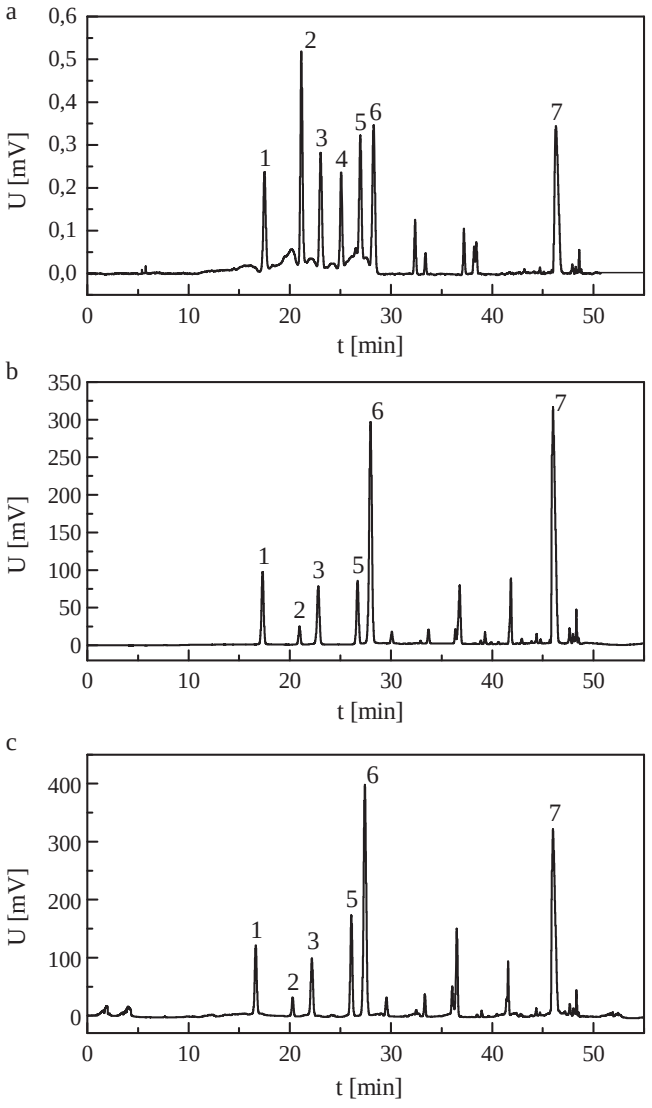

Fig. 2. Representative HPLC chromatograms recorded for 3-monoglucosides anthocyanins standard (a) and extracts from St. Laurent (b) and Alibernet (c) grape skins. Extracts were prepared from 0.5 g of dry matter by PHWE at 80 °C. Peaks assignment: (1) De-3-glc = delphinidin-3-glucoside, (2) Cy-3-glc = cyanidin-3-glucoside, (3) Pt-3-glc = petunidin-3-glucoside, (4) Pg-3-glc = pelargonidin-3-glucoside, (5) Pn-3-glc = peonidin-3-glucoside, (6) Mv-3-glc = malvidin-3-glucoside, (7) Brillant blue FCF – internal standard.

3-O-monoglucosides of delphinidin (De), cyanidin (Cy), petunidin (Pt), peonidin (Pn) and malvidin (Mv). As indicated above, Brillant Blue FCF was used as an internal standard for the quantification purposes. This approach allows at the same time the comparison of PHWE and PFE from the extraction efficiency point of view. In Fig. 2, a typical HPLC chromatographic record is depicted of the standard solution of six 3-monoglucosides and of the extracts of St. Laurent and Alibernet grape skins prepared by PHWE at 80 °C. Chromatographic record of standard solution of monoglucosides under the particular conditions of analysis showed a group of well resolved chromatographic peaks corresponding to the individual analyzed compounds and the internal standard employed, respectively. The limit of detection (corresponding to signal-to-noise ratio (S/N) = 3) was 48.8 ng/mL for De-3-glc and Mv-3-glc, and 24.4 ng/mL for Cy-3-glc, Pt-3-glc and Pn-3-glc. The respective limits of quantification (S/N = 10) were 147 ng/mL and 74.5 ng/mL. Calibration curves showed very good linear regression (R^2 > 0.9995), with the 5 calibration points (C1–C5) ranging from 1 mg/L (C1) to 100 mg/L (C5 for De-3-glc, Pt-3-glc, Pn-3-glc), 50 mg/L (C5 for Cy-3-glc) and 2000 mg/L (C5 for Mv-3-glc). Three injections were performed for each calibration level. The relative standard deviation was always less than 3%. Analysis of real extracts confirmed the presence of five 3-monoglucosides anthocyanins, namely, De-3-glc, Cy-3-glc, Pt-3-glc, Pn-3-glc and Mv-3-glc in the extracts of both varieties. The comparison of concentrations of individual compounds on an absolute basis is not fully appropriate, however, it can be concluded that the highest concentration was determined for Mv-3-glc with the concentration ranging from 31.7 to 69.2 mg/g (St. Laurent) and from 39.9 to 102 mg/g (Alibernet) followed by De-3-glc and Pn-3-glc for St. Laurent and Alibernet variety extracts, respectively. Vice-versa, the lowest concentration was found for Cy-3-glc, ranging from 0.39 to 1.08 mg/g (St. Laurent) and from 0.54

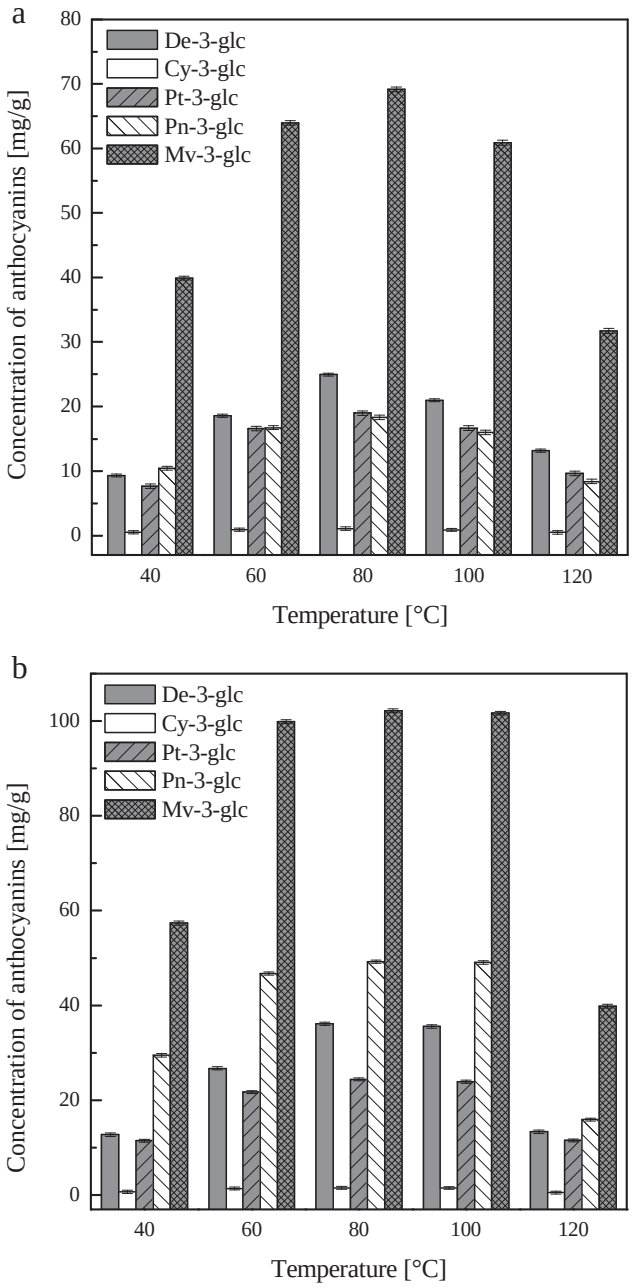

Fig. 3. Averaged concentration of 3-monoglucosides anthocyanins determined in St. Laurent (a) and Alibernet (b) grape skin extracts prepared from 0.5 g of dry matter by PHWE at 40–120 °C. Each value is expressed as mean ± SD ($n = 3$). Abbreviations of individual anthocyanins are explained in text.

to 1.52 mg/g (Alibernet). As regards the relationship between the anthocyanins recovery and extraction temperature, it is obvious from Fig. 3 that the highest concentrations of individual polyphenols were found in the extracts prepared at 80 °C for both varieties. With a further increase in temperature above 80 °C, a decrease of the anthocyanins content was observed, apparently because of degradation of anthocyanins at higher temperatures as noted by others before [40,41]. Anyway, the problem of thermal stability of polyphenols in individual foods is currently only poorly discussed and available data are frequently contradictious [42–44]. It should be noted here that the results obtained for water extracts prepared by PHWE are in good agreement and practically of the same trend with those for ethanolic extracts of the same varieties prepared by PFE [30], although the absolute concentrations (extraction yields) of individual anthocyanins determined in the extracts under the current study are somewhat lower. The combination of PHWE and HPLC methods provided good reproducibility.

3.2. Antioxidant activity assessed by EPR spectroscopy

Radical-scavenging ability assessments traditionally employ the •DPPH and $ABTS^{\bullet+}$ radicals assays adopted either for UV–vis or for EPR spectroscopy. The ways of evaluation of the results may differ; however, the most effective seems to be the relative comparison of the antioxidant (radical-scavenging) action of any investigated sample to that of a known antioxidant, e.g., Trolox – water soluble form of Vitamin E. Following the approach of Pellegrini et al. [45], the Trolox-equivalent (TEAC) values of each extract were calculated for the reactions with both •DPPH and $ABTS^{\bullet+}$ as depicted in Fig. 4. It is obvious that the extracts of both varieties demonstrated a significant ability to terminate both •DPPH and $ABTS^{\bullet+}$. In a similar way like that described above for the TPC content, for both radicals, the extracts' termination ability is significantly dependent on the amount of grape skins extracted. Also other findings correlate well with TPC – generally the higher TEAC values of Alibernet extracts in comparison to those of St. Laurent, and, last but not least, the effect of extraction temperature on radical-scavenging ability. As also clearly follows from Fig. 4, the increase of extraction temperature resulted in significantly increased TEAC values of Alibernet extracts. For St. Laurent extracts, the same dependence on extraction temperature was observed except for the extracts prepared from 0.5 g of grape skins. For the last mentioned, the increase of TEAC values in the extracts prepared up to 80 °C was noticed, whereas a further rise of extraction temperature caused a slight worsening of radical-scavenging properties, indicating a complete extraction of antioxidants from the sample at 80 °C and a possible decomposition of the matter at higher extraction temperatures. However, this phenomenon was not observed for the extracts prepared from higher amounts of grape skins.

As regards the numerical values of TEAC, those obtained for •DPPH ranged from 0.6 to 4.6 mmol/L for St. Laurent and from 1.3 to 5.8 mmol/L for Alibernet variety, whereas those for $ABTS^{\bullet+}$ from 1.0 to 7.4 mmol/L and from 2.2 to 10.3 mmol/L, respectively. Previously, Pellegrini et al. [45] published TEAC values of plant foods, beverages and oils. The TEAC values for wines ranged from 1.6 to 12 mmol/L and the present results on grape skin extracts fall within this interval. The radical-scavenging ability of St. Laurent variety is significantly weaker than that of the highly pigmented Alibernet variety. Moreover, as was proved, the radical-scavenging ability of the extracts was in both varieties strongly dependent on the extraction temperature and on the amount of sample. In fact, identical observations followed from our previous experiments with methanolic and ethanolic extracts prepared by PFE of the same grape skins varieties.

The correlation was evaluated between the TPCs and TEACs values obtained from individual antioxidant testing assays. Pearson's correlation coefficients for the extracts of both varieties are listed in Table 2. The data presented clearly indicated a significant corre-

Table 2
Correlation matrices between the antioxidant activities of extracts and total polyphenols content without respect to the amount of dry matter used for extract preparation.

	St.Laurent			Alibernet		
	TPC	$TEAC_{ABTS^{\bullet+}}$	$TEAC^{\bullet}_{DPPH}$	TPC	$TEAC_{ABTS^{\bullet+}}$	$TEAC^{\bullet}_{DPPH}$
TPC	1	0.9431	0.9296	1	0.9758	0.9384
$TEAC_{ABTS^{\bullet+}}$	0.9431	1	0.9078	0.9758	1	0.9727
$TEAC^{\bullet}_{DPPH}$	0.9296	0.9078	1	0.9384	0.9727	1

TPC = Total phenolic content, TEAC = Trolox-equivalent antioxidant capacity.

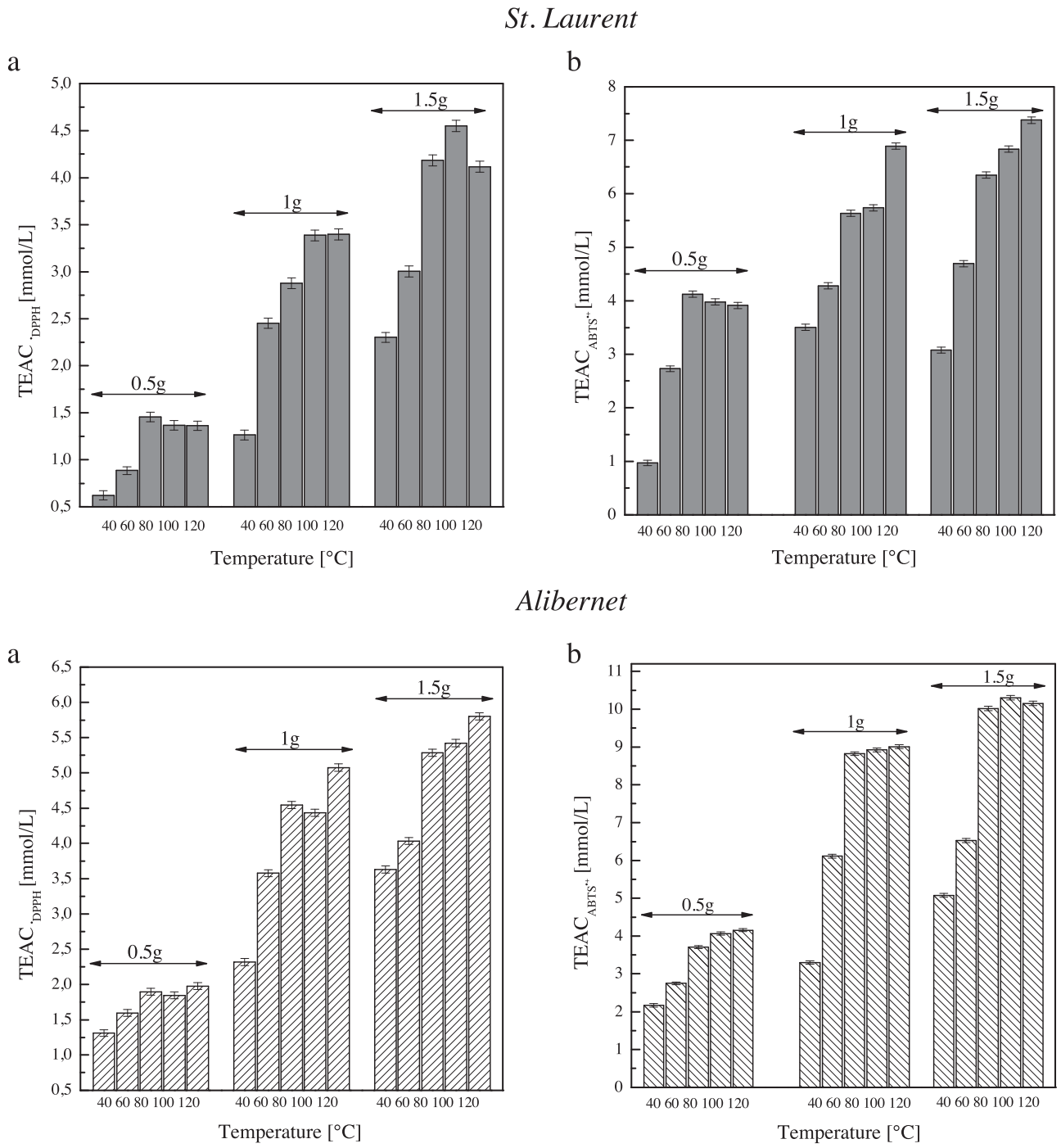

Fig. 4. Trolox-equivalent antioxidant capacity (TEAC) of St. Laurent and Alibernet grape skins water extracts prepared at various temperatures from 0.5 g, 1.0 g and 1.5 g of crude grape skins evaluated from EPR spectra recorded 10.5 min after the addition of •DPPH (a) and $ABTS^{\bullet+}$ (b) into the experimental system. Each value is expressed as mean ± SD ($n = 3$).

lation between TPCs and TEACs for both varieties, reaching $P \approx 0.95$ (Alibernet) and $P \approx 0.93$ (St. Laurent).

3.3. Antioxidant activity versus water properties

Physico-chemical properties and behavior of the extraction solvent have been recognized as the most important parameters governing both qualitative and quantitative aspects of any extraction process. Fig. 5 shows how several pertinent properties of pure water vary with temperature along the 15 MPa isobar in the temperature range of the extraction experiments. The properties of extraction media were expressed relative to their respective values at the temperature of 40 °C and pressure of 15 MPa, for which the value of each characteristics was set to 1. All the properties showed the decreasing trend with rising temperature, except for the ion product of water as the drop in relative pK_w from 1 to 0.884 corresponds to an increase in K_w from 3.28×10^{-14} mol^2/kg^2 to 1.22×10^{-12} mol^2/kg^2. The second largest property change is that in the relative permittivity (a drop by about 31%). Fig. 5 indicates important shifts in the solvent characteristics of water across the temperature interval from 40 °C to 120 °C. From the evaluation of water properties relationship to the experimental characteristics of

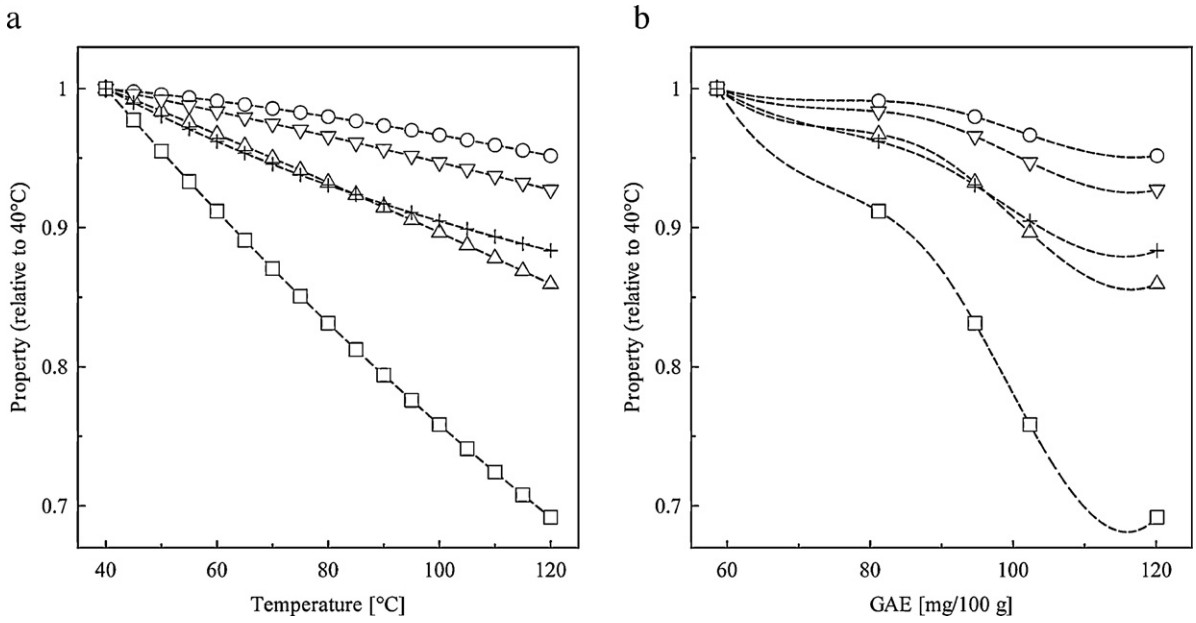

Fig. 5. (a) Temperature dependence of selected properties of pure water at 15 MPa: (○) density, (△) cohesive energy density, (▽) solubility parameter, (□) relative permittivity, (+) pK_w. The property values at 40 °C and 15 MPa are: density 998.65 g cm^{-3}, cohesive energy density 2262.7 J cm^{-3}, solubility parameter 47.57 (J cm^{-3})$^{1/2}$, relative permittivity 73.77, pK_w 13.48. The lines only serve to guide the eye. b) Properties of water versus GAE values of the extracts prepared from 1 g of Alibernet grape skins: (○) density, (△) cohesive energy density, (▽) solubility parameter, (□) relative permittivity, (+) pK_w. The lines only serve to guide the eye.

the extracts under study it is obvious that there exist some empirical correlations between the TPCs of the extracts and the water properties (Fig. 5b). As was discussed above, the TPC contents of all grape skin extracts increase with rising temperature (refer to Fig. 1). Obviously, the temperature-driven shifts in water properties may also be partly responsible for the observed TPC increase – taking into account the increasing fugacities of the antioxidants and diminishing antioxidant – matrix interactions as other important factors.

4. Conclusion

Characterization of water extracts prepared from grape skins of St. Laurent and Alibernet wine grape varieties by PHWE was performed, proving that PHWE provides a gentle, fast, effective and environment-friendly method for the extraction of monoglucoside anthocyanins and other compounds of interest from grape skins. The extracts reveal significant radical-scavenging abilities that are affected by the extraction temperature. The elevated temperature influences significantly also the recovery of anthocyanins.

The off-line PHWE–EPR spectroscopy combination provides enhanced sensitivity and improved information content in antioxidant activity assessment as compared to conventional determination by UV/VIS spectrophotometry. This coupling represents efficient and powerful tool with potential industrial application for the extraction and antioxidant assessment of natural samples. Because of the unique combination of benign solvent and extraction efficiency, PHWE presents a promising extraction route towards the production of high-concentration aqueous solutions of natural antioxidants for functional food supplements utilizing, e.g., highly pigmented wine grape varieties.

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