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The present work aimed at the recovery and characterization of polyphenolic compounds extracted from red grape pomace (*Vitis vinifera* L.), a winemaking by-product. Polyphenolic compounds of wet (WP) and dried (DP) red pomace were recovered by enzymatic digestions and ethanol-based extractions. Fungamyl and Celluclast enzymes were found to be the most effective in enhancing polyphenol release from WP. WP samples showed the highest capacity of releasing polyphenols with 2 h control 24°C and 2 h 1% Celluclast resulting as the best treatments. A significantly lower amount of polyphenols was recovered from DP most probably as a consequence of the pomace drying. The best extracts contained high amounts of total polyphenols, flavonoids, tannins and anthocyanins and exerted antioxidant and cholesterol-lowering activities. The results support the possibility of exploiting the extracts coming from grape processing by-products as ingredients for functional and innovative products in the nutraceutical, pharmaceutical or cosmetic fields.

Introduction

The food processing industry annually produces large quantities of both liquid and solid waste and by products. These by products constitute a rich but yet underutilised source of valuable com pounds, which may find an application in the food, feed, cosmetic and pharmaceutical industries. Grape (*Vitis* sp.) is the world's largest fruit crop mostly used in wine making, a process during which approximately 20 30% of the weight of processed grapes ends up as pomace, its primary by product [1]. Pomace mainly consists of pressed skins, seeds and stems. These large amounts of by products constitute a serious environmental and disposal problem for wineries. However, they also represent a rich source of various high value molecules, such as phytochemicals with high antioxidant activity [1 4]. Grape pomace is characterized by a high content of polyphenol compounds that are only partially extracted during the winemaking process and whose range and extractability mainly depends on the technological parameters applied during vinification. Flavonoids (such as anthocyanins and catechins), phenolic acids and stilbenes are among the main constituents of grape pomace [2,4]. The beneficial influence on human health of grape and wine phenols has been increasingly investigated with evidences provided for their protective effects against chronic diseases such as cancer, neurodegeneration and cardiovascular pathologies [5]. Grape polyphenols have also been shown to have anti inflammatory and anti microbial properties [4,6,7]. Due to their biological and chemical properties, grape pomace extractable components may have many applications: as ingredients of functional foods and feeds, cosmetics and nutraceuticals; as natural colorants and preservatives of foods [2 4]. However, after ingestion dietary phenols are modified

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and degraded in the gastrointestinal tract and appear in the circulatory system at low concentration and in different chemical forms [5]. Comparison of several recovery methodologies showed that solvent (mainly water, ethanol and methanol) and supercritical fluid extractions are the most efficient for grape pomace extraction [3,8,9]. Enzymatic digestion using cell wall polysaccha ride degrading enzyme mixtures was also shown to enhance the release of grape phenolics still present in pomace [10,11].

In the present work, the recovery of polyphenolic compounds was studied on wet pomace (WP) and dried pomace (DP) from red grapes (*Vitis vinifera* L., mixture of Sangiovese and Montepulciano cultivars), by means of enzymatic digestions and ethanol based extractions. The extracts with the highest amount of compounds were tested for their biological activities in view of their possible future application in cosmetic, nutraceutical and pharmaceutical industries.

Materials and methods

Materials

Red wine pomace derived from a mix of *Vitis vinifera* cv. Sangiovese and Montepulciano, harvested in the year 2011, was supplied by the Cantine Moncaro wineries (Jesi, Ancona, Italy). The same day of wine production, pomace was either frozen (wet pomace, WP) or dried (dried pomace, DP) in an industrial vented oven (60°C for 24 h) and stored at 20°C until used for analyses. Both types of pomace contained berry skins, seeds, petioles and stalks.

Pomace enzymatic digestion

WP was ground in a kitchen blender with the addition of distilled water (1:5 g/mL), while DP was ground directly and rehydrated with distilled water (1:5 g/mL for 1 h) just before enzymatic digestion. To determine the percentage of dry weight (DW), 10 g fresh weight (FW) of WP were placed at 80°C for 48 h and weighed (DW was about 54% of FW). Enzymatic digestions of ground WP and DP pomace suspension (20 mL aliquots) were carried out by adding different concentrations (0.5, 1 or 2% enzyme volume/pomace DW) of Pectinex 3XL (pectinase from *Aspergillus niger*, 3595 U/mL), Pectinex Ultra SPL (pectinase from *Aspergillus aculeatus*, 4218 U/mL), Termamyl (α amylase from *Bacillus licheniformis*, 605 U/mL), Fungamyl (α amylase from *Aspergillus oryzae*, 881 U/mL), Pentopan 500BG (xylanase from *Thermomyces lanuginosus*, 2.75 U/g), or Celluclast (cellulase from *Trichoderma reesei* ATCC 26921, 790 U/mL), all purchased from Sigma Aldrich (Milano, Italy). The different enzymatic treatments were incubated on an orbital shaker (150 rpm) at different incubation times and at the enzyme optimal working temperature according to the Sigma working certificate (Pento pan at 30°C, Celluclast at 37°C, all the other enzymes at 24°C). Controls performed without the addition of enzymes were incu bated under the same conditions as the treated samples. After incubation, the samples were centrifuged at 5000 rpm for 10 min at room temperature (Eppendorf centrifuge 5804 R, rotor A 4 44, Hamburg, Germany). The supernatant was removed and filtered under vacuum through Whatman GF/B filters using a Millipore funnel apparatus, and stored at 20°C until further analyses. After having selected the best treatment conditions for both WP and DP, the water enzymatic treatments were repeated and after removing the supernatant the residual pellet was incubated with 30 mL of 95% (v/v) ethanol at 24°C overnight on an orbital shaker (150 rpm). Subsequently, the ethanol supernatant was separated from the pellet by centrifugation for 10 min at room temperature at 5000 rpm and filtered through a Whatman GF/B filter. Both aqueous and ethanol supernatants were stored at 20°C until further analyses.

Spectrophotometric and HPLC-DAD analyses

Total polyphenol and total flavonoid content of aqueous and ethanol pomace extracts was quantified by using the Folin Cio - calteu assay and the Zhishen *et al.* [12] methods with minor modifications [13]. The results were, respectively, expressed as gallic acid (GA) and catechin (CAT) equivalents by means of calibration curves. The amount of tannins was analysed using the method of Porter *et al.* [14] with minor modifications. Anthocyanin content was determined as in Ferri *et al.* [15] while color density was determined as in Mazza *et al.* [16]. Phenolic com pounds were extracted both from water supernatants and ethanol pomace extracts [15] before being directly injected into the HPLC DAD system (column Gemini C18, 5 μm particles 250 mm × 4.6 mm, pre column SecurityGuard Ea, Phenomenex, Torrence CA, USA) equipped with an on line diode array detector (MD 2010, Plus, Jasco Instruments, Großumstad, Germany). The adopted HPLC DAD separation procedure allowed for the simul taneous analysis and identification of 28 different compounds among stilbenes, phenolic acids and flavonoids by direct compar ison both of retention time and absorbance spectra to the related compound standard [15].

Determination of biological activities

In vitro antioxidant activities were measured using the 2′ azino bis 3 ethylbenzothiazoline 6 sulfonic acid (ABTS) method with minor modifications [13]. The results were expressed as ascorbic acid (AA) equivalents by means of a dose response calibration curve. Cell based assays were performed to evaluate the effect of pomace extracts with the highest polyphenol amount on transcriptional regulation of cholesterol 7α hydroxylase (cyp7a1) and sterol 27 hydroxylase (cyp27a1). This dual color reporter assay allowed to preliminary investigation the cholesterol lowering activity of the samples. Human hepatocarcinoma HepG2 cells overexpressing the human farnesoid X receptor (FXR) (a generous gift from Prof. N. Carulli from the University of Modena, Italy) were used and cultured and assays were performed as previously reported [17].

Statistical analyses

All the treatments were performed two times independently and the two extracts were analysed in two technical replicate each. The results are expressed as the mean of four data ± SD. Cell based assays were performed three times and each data point had at least three technical replicates. The results are expressed as the mean of nine data ± SD. Statistically significant differences between data sets were analysed using the Student's *t* test ($p < 0.05$) (Statistica 6 programme, Statasoft Inc., USA).

Results and discussion

Red pomace enzymatic digestions

Enzymatic digestions of both WP and DP were carried out by adding different concentrations (0.5, 1 or 2% enzyme volume/pomace DW)

of the enzymes Pectinex 3XL, Pectinex Ultra SPL, Termamyl, Fun gamyl, Pentopan, Celluclast. The samples were incubated for 2 h at the optimal temperatures of the enzymes. The total content of polyphenolic compounds was subsequently quantified by means of the Folin Ciocalteu assay on the aqueous supernatants (data not shown). Wet pomace (WP) contained in general 2 fold more poly phenols than dried pomace extracts (DP). In particular, total poly phenols of WP samples incubated at 37°C (with or without the enzyme) showed on average 2.4 fold higher polyphenol levels (346.1 mg GA eq/L) than DP samples (142.6 mg GA eq/L). Overall, the best treatments for WP were 1% Celluclast, 37°C control, 2% Fungamyl and 24°C control. For DP the best results were obtained with controls at 30°C and 37°C (144.1 and 157.8 mg GA eq/L respectively). Previous published data indicated that the sample preparation method had a significant impact on the recovery of polyphenolic compounds from berry pomace [8,9]. In particular, moisture content was found to be very important and pomace drying at high temperatures before extraction could cause a significant reduction in the amount of extractable polyphenols [8,9]. This result was attributed to a quicker solvent diffusion through crude wet pomace and a faster rate of reaching a kinetic equilibrium [9]. Similarly, the present results show a significantly higher polyphenol yield from WP than from DP, suggesting it is preferable to treat the pomace directly after its production during wine making. In a second set of digestions, combinations of two or three enzymes each at 1% concentration were simultaneously added to WP and DP samples and incubated at the average temperature of 30°C for 2 h. For comparison, control samples and single enzyme treatments were also performed at 30°C for 2 h. None of the combined treat ments significantly increased polyphenol content of the aqueous supernatant. Moreover as the enzymes Pectinex 3XL, Pectinex Ultra SPL, Termamyl and Pentopan showed a lower performance in extracting polyphenols from both WP and DP samples in the previous tests, they were not further utilized for following experi ments or combined enzyme digestions. To increase yields, the four best treatments for WP and the two best for DP were performed at successively increasing times (2, 6 and 24 h) and the total polyphe nol levels in the water supernatant were measured (Fig. 1). For WP the highest content was detected in samples incubated for 2 h (on average 318 mg GA eq/L) (Fig. 1a), while a 2.3 fold decrease was detected in both 6 h and 24 h samples. On the other hand, in DP there was no striking difference among samples treated at different incubation times, with only a small not significant increase after 24 h (on average 165 mg GA eq/L) with respect to the 2 h samples (150 mg GA eq/L) (Fig. 1b). The WP results (Fig. 1) are in agreement with those reported for Merlot pomace [10] in which a loss of up to 27% of extracted phenols was detected after treatment with or without Celluclast at increasing incubation times (8 48 h). Long time enzyme treatments seem to have a significantly negative effect on phenol recovery, probably due to metabolite degradation during extended incubation [10].

Ethanol extracts

After selecting the best conditions for both WP and DP (2 h and 24 h, respectively), the water enzymatic treatments were repeated, the aqueous supernatant was separated from the residual pellet (still deeply colored) which was then further incubated overnight with 95% (v/v) ethanol at 24°C. Both ethanolic and aqueous

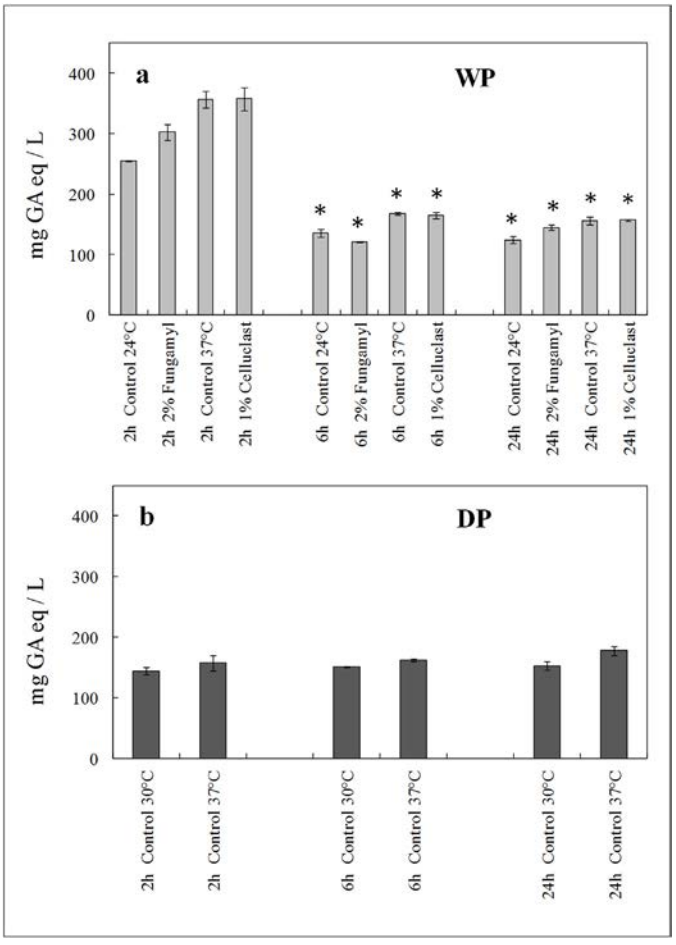

FIGURE 1

Total levels of phenolic compounds in water supernatants of the four best wet pomace (**a,** WP) and the two best dried pomace (**b,** DP) samples treated at increasing incubation times (2, 6 and 24 h). The data are expressed as mg of gallic acid (GA) equivalent per L of extract (mg GA eq/L). The star symbol indicates a statistically significant difference (Student's *t* test, $p < 0.05$) between the treatments incubated for 6 h or 24 h compared to the 2 h treatment. The results are expressed as the mean of four data ± SD.

extracts were used for spectrophotometric analyses (Fig. 2). As expected, ethanol 95% was able to improve the extraction effi ciency of phenolic compounds from the residual pellet with respect to pure water based (with or without the enzyme) treatments. A beneficial effect of using a water and ethanol mixture instead of pure solvents has been observed previously for extracts of different grape varieties [8]. In WP samples, the total polyphenol content was on average 2.7 fold higher in ethanol with respect to water extracts, while only a 1.3 fold average increase was obtained in ethanol extracts of DP samples (Fig. 2a). Similar results were obtained for total flavonoids (Fig. 2b) and tannins (Fig. 2c). In WP, the best polyphenol yields were obtained with the 1% Celluclast sample for water extraction (340.6 mg GA eq/L corresponding to 920 mg GA eq/kgDW) and with the 24°C control for ethanol extraction (843.0 mg GA eq/L corresponding to 2276 mg GA eq/kgDW) (Fig. 2a). The lower polyphenol recovery from water extractions may be ascribed to the very high amount of antho cyanins present, compared with non anthocyanin flavonoids and phenolic acids, which may result in a saturation of the aqueous solution or, less probably, to anthocyanin degradation following

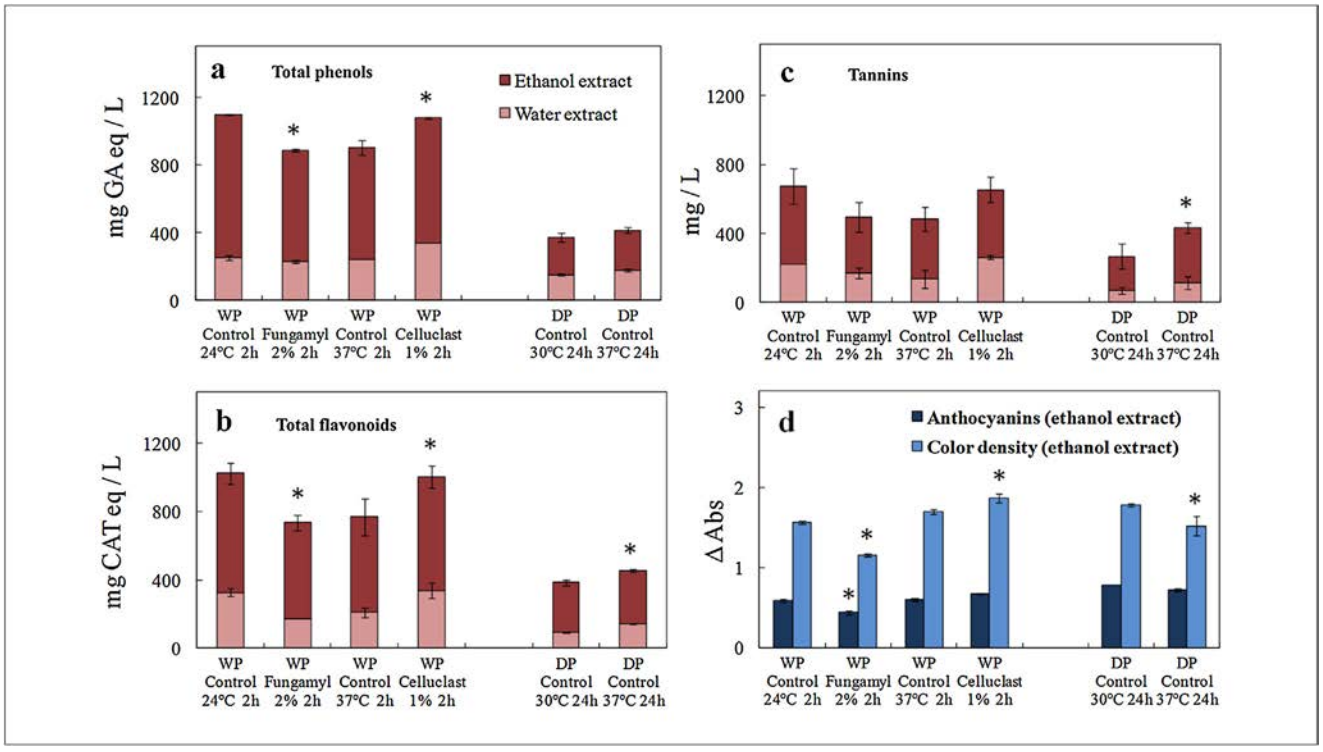

FIGURE 2

Total levels of phenols **(a)**, total flavonoid **(b)**, tannin **(c)**, anthocyanin and color density levels **(d)** in water and ethanol extracts of the four best wet pomace (WP) and the two best dried pomace (DP) samples incubated, respectively, for 2 h and 24 h. Total phenol data are expressed as mg of gallic acid (GA) equivalent per L of extract (mg GA eq/L); total flavonoid data are expressed as mg of catechin (CAT) equivalent per L of extract (mg CAT eq/L); total tannins are expressed as mg/L; anthocyanins and color density are expressed as the variation of absorbance units per L of extract (Δ_{Abs}/L). The star symbol indicates a statistically significant difference (Student's *t* test, $p < 0.05$) between the total level (water plus ethanol extracts) of an enzymatic treated sample compared to the respective control (WP) or between the two shown samples (DP). The results are expressed as the mean of four data ± SD.

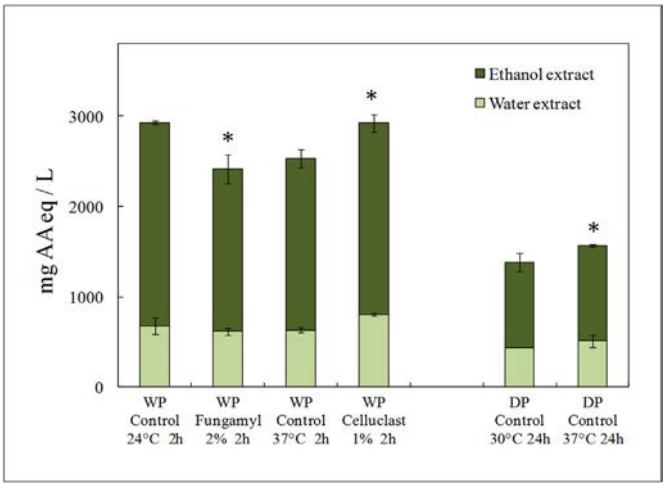

FIGURE 3

Antioxidant activity measured by means of the ABTS method in water and ethanol extracts of the four best wet pomace (WP) and the two best dried pomace (DP) samples incubated, respectively, for 2 h and 24 h. The data are expressed as mg of ascorbic acid (AA) equivalent per L of extract (mg AA eq/L). The star symbol indicates a statistically significant difference (Student's *t* test, $p < 0.05$) between the total level (water plus ethanol extracts) of an enzymatic treated sample compared to the respective control (WP) or between the two shown samples (DP). The results are expressed as the mean of four data ± SD.

extraction [11]. With regard to flavonoid yields (Fig. 2b), previous data on pomace from the Greek red cultivar Agiorgitiko [18] reported 5 25 fold higher amounts than those obtained here from the mixed Sangiovese and Montepulciano pomace. This difference may be ascribed to different used cultivar, grape culture conditions and vinification techniques. The levels of solubilised tannins (water plus ethanol extracts) reached a maximum of 650 mg/L in WP treated with 1% Celluclast (1735 mg/kgDW) and of 430 mg/L in DP 37°C control (2150 mg/kgDW) (Fig. 2c), values slightly lower than those obtained for pomace skins and seeds of different grape cultivars [19]. The anthocyanin and color density levels were almost comparable between WP and DP samples (Fig. 2d). Antho cyanin levels of both WP and DP (Fig. 2d) were between 0.44 and 0.79 ΔAbs, corresponding to 7.7 and 13.8 mg malvidin 3 glucoside (MDV) per litre (MDV/L). The same data expressed per kgDW (on average 26.4 and 66.3 mg MVD eq/kgDW, respectively, in WP and DP) seem to be slightly lower than those obtained from pomace of several different cultivars [9]. Color density (up to 1.8 ΔAbs, Fig. 3d) was of the same order of magnitude than that found in wine (3.0 9.6 Abs units) [16]. In general, anthocyanin yields (per kgDW) were higher in WP than in DP, in agreement with previous data [9]. The use of some pectinases (e.g., Pectinex) in a more efficient water based extraction of red grape pigments has been reported to lead to a 2 4 times higher color density with respect to pure water extraction [20]. It is well known that cellulose present in plant cell walls may form highly ordered crystalline structures,

TABLE 1

Polyphenol levels (mg/L) of water and ethanol extracts of wet (WP) and dried (DP) red grape pomace determined by HPLC-DAD analysis. The star symbol indicates a statistically significant difference (Student's *t*-test, $p < 0.05$) between the water or ethanol extract data of an enzymatic-treated sample and the respective 24°C or 37°C control (WP) or between water and ethanol extract data of the two samples (DP). Data are the mean ± SD of four replicates.

Compound	Type of extract	WP control 24 °C 2 h	WP 2% fungamyl 24 °C 2 h	WP control 37 °C 2 h	WP 1% celluclast 37 °C 2 h	DP control 30 °C 24 h	DP control 37 °C 24 h
Quercetin	Water	0.04 ± 0.01	0.11 ± 0.04*	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
	Ethanol	1.90 ± 0.38	2.42 ± 0.80	1.80 ± 0.36	2.09 ± 0.77	2.44 ± 0.80	2.19 ± 0.77
Rutin	Water	2.72 ± 0.54	1.85 ± 0.13*	3.18 ± 0.64	3.40 ± 0.12	2.96 ± 0.38	3.51 ± 0.06*
	Ethanol	11.21 ± 2.24	7.58 ± 0.86*	9.24 ± 1.85	9.93 ± 3.50	8.70 ± 2.11	6.67 ± 1.81
***Cis* Piceid**	Water						
	Ethanol	41.59 ± 8.32	35.43 ± 6.38	44.17 ± 8.83	47.80 ± 17.60	30.14 ± 8.86	23.44 ± 6.37
Catechin	Water	0.69 ± 0.14	0.71 ± 0.01	1.78 ± 0.36	1.10 ± 0.48	4.05 ± 0.76	3.71 ± 0.94
	Ethanol	8.06 ± 1.61	6.45 ± 0.84	6.84 ± 1.37	6.36 ± 2.19	2.50 ± 0.19	2.27 ± 0.57
Epicatechin	Water	0.61 ± 0.12	0.85 ± 0.01*	0.33 ± 0.07	0.55 ± 0.36	1.29 ± 0.24	1.07 ± 0.96
	Ethanol	0.72 ± 0.14	0.95 ± 0.69	0.87 ± 0.17	0.99 ± 0.38	0.96 ± 0.05	0.91 ± 0.18
Epigallocatechin gallate	Water	0.69 ± 0.14	0.43 ± 0.06*	0.66 ± 0.13	0.34 ± 0.14*	0.66 ± 0.09	0.87 ± 0.03*
	Ethanol	0.98 ± 0.20	1.04 ± 0.05	0.82 ± 0.16	1.07 ± 0.31	0.46 ± 0.17	0.30 ± 0.13
Epicatechin gallate	Water	0.64 ± 0.13	0.63 ± 0.15	0.60 ± 0.12	0.67 ± 0.17	1.07 ± 0.13	1.33 ± 0.04*
	Ethanol						
Epigallocatechin	Water					3.64 ± 0.64	2.95 ± 0.65
	Ethanol						
Gallic acid	Water	1.68 ± 0.34	1.65 ± 0.05	2.08 ± 0.42	1.85 ± 0.01	9.38 ± 1.02	14.44 ± 5.28
	Ethanol						
Vanillin	Water	0.78 ± 0.16	0.75 ± 0.12	0.81 ± 0.16	0.77 ± 0.09	0.44 ± 0.09	0.40 ± 0.13
	Ethanol						
Vanillic acid	Water	0.77 ± 0.15	0.68 ± 0.05	0.85 ± 0.17	1.28 ± 0.02*	4.00 ± 0.43	4.43 ± 0.54
	Ethanol						

which are generally resistant to enzymatic breakdown. In addition, the accessibility of hydrolytic enzymes may be hindered by lignin and low molecular weight phenolic compounds forming covalent linkages with sugar residues, thus 'shielding' the sub strate from enzymatic degradation. These factors have been suggested to explain the low activity of hydrolytic enzymes, such as Celluclast, on grape pomace [10]. Moreover, phenolics them selves have an inhibitory effect on enzymatic digestion [3].

HPLC analyses of extracts

Water and ethanol extracts of the six best treatments (Fig. 2) were analysed by HPLC DAD to quantify specific polyphenolic compounds (Table 1). In WP samples, the detected polyphenols were more abundant in ethanol than in water extracts (e.g., a 47.5 fold difference for quercetin in control 24 °C samples, Table 1). Most of the compounds were extracted at higher concentrations as a consequence of the enzymatic digestions and a particularly significant increase was detected in 2% Fungamyl treated WP sample when compared to the respective control 24°C (Table 1). Water was generally able to extract phenolics more efficiently from DP than from WP. Incubation temperature only determined minor differences in extract composition (Table 1). While gallic acid, catechins, quercetin and its derivatives have been found in pomace extracts [7,8,11], in this paper we also found the presence of rutin, vanillin, vanillic acid, epigallocatechin and epigallocatechin gallate. Phenolic acids (hydroxycinnamic and hydroxybenzoic acids) and stilbenes have been reported previously in pomace extracts also obtained after enzymatic digestion [7,11]. However, these compounds were almost absent from the present extracts, with the exception of gallic acid and *cis* piceid (Table 1), probably due to differences in grape pomace cultivars and the winemaking process.

Biological activities of the extracts

The antioxidant activity of both water and ethanol extracts was determined using the ABTS method. On average, DP samples showed an about 1.8 fold lower total antioxidant activity (water plus ethanol extracts) than WP samples, in accordance with the lower amount of polyphenols released from DP (Figs. 1 and 2). As expected, ethanol extracts of both WP and DP samples showed a higher antioxidant capacity compared to water extracts (Fig. 3). In agreement with the results on total polyphenol and flavonoid levels (Fig. 2a,b), the highest total antioxidant levels were detected in 2 h control 24°C (2930 mg AA eq/L) and 2 h 1% Celluclast (2922 mg AA eq/L) WP samples (Fig. 3). The increase of 1%Celluclast antioxidant activity with respect to its control (Fig. 3) can be due to the fact that this commercial mixture contains several enzyme activities that may selectively release antioxidant phenols (such as ferulic acid esterase) or modify polyphenols thereby promoting their antioxidant activity (such as glycosidase that decreases the glycosylation level of phenolic glycosides) [10]. The antioxidant capacity of different grape pomace extracts has been measured in the past by using several *in vitro* methods [3,18,19]. In particular, using the ABTS method, a maximum of

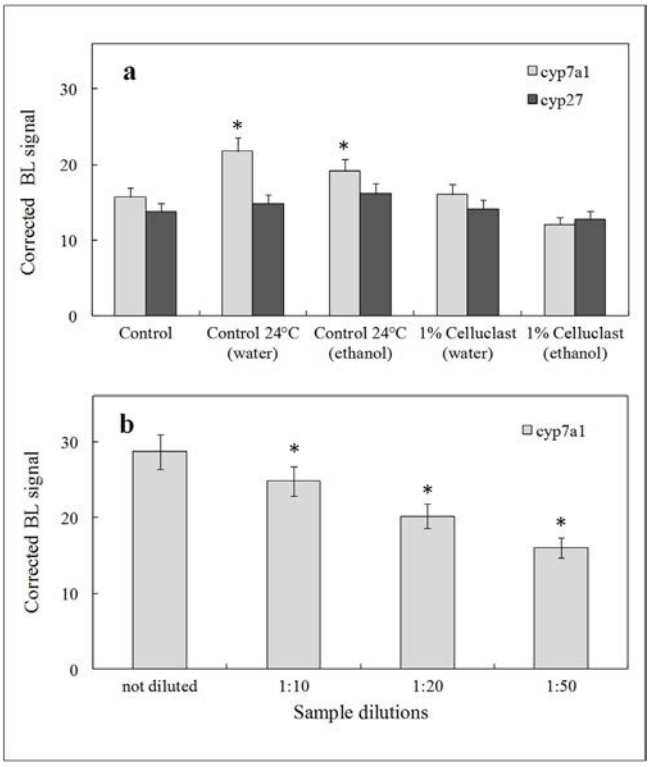

FIGURE 4

Effect of water and ethanol extracts of WP control 24 °C and 1% Celluclast 37°C samples (2 h incubation) on cyp7a1 and cyp27a1 transcription **(a)**; cyp7a1 transcription corrected dose response curve using 24 °C water extract **(b)**. Bioluminescent (BL) emissions were corrected according to an internal viability control (a constitutively expressed red or green emitting luciferase). The star symbol indicates a statistically significant difference (Student's *t* test, $p < 0.05$) between: (a) the data and the initial control (first set of columns on the left) or (b) between the data and the previous dilution. The results are expressed as the mean of nine data ± SD.

603 and 464 μM Trolox eq/gDW was observed, respectively, in seeds and crude skin extracts from several grape cultivars, which was positively correlated with total polyphenol, proanthocyanidin and anthocyanin contents [18,19]. Similar results were also obtained in the present study in which the highest antioxidant activity levels (Fig. 3) correlated with total phenols and total flavonoids (Fig. 2a,b). The effect of the two best WP samples (2 h control 24°C and 2 h 1% Celluclast, water and ethanol extracts) on transcriptional regulation of cholesterol 7α hydroxylase (cyp7a1) and sterol 27 hydroxylase (cyp27a1) was evaluated with dual color cell based assays (Fig. 4). Since these two enzymes are responsible for bile acid biosynthesis, a key step in intracellular cholesterol homeostasis, analysis of their transcriptional regulation allowed us to preliminary investigate cholesterol lowering activity of the samples. The water extract of the control 24°C was able to significantly induce transcriptional activity of cyp7a1 (Fig. 4a), the rate limiting enzyme in the classic pathway of bile acid formation. The induction of cholesterol lowering activity was concentration dependent as confirmed by testing different dilutions of the control 24°C water extract (Fig. 4b). Neither of the samples showed any effect on transcriptional activity of cyp27a1 (Fig. 4a). The present results seem to be in accordance with previous data obtained in rats fed for 4 weeks with grape pomace extracts as drinking fluids instead of water [21]. More recently, hamsters fed for 3 weeks with pomace grape seed flours had significantly lowered plasma total , VLDL , and LDL cholesterol concentrations com pared to the controls [22]. The improved plasma cholesterol was correlated with the up regulation of hepatic genes related to cholesterol (cyp51) and bile acid (cyp7a1) synthesis as well as LDL cholesterol uptake. In addition several studies performed on isolated phenolic molecules (such as quercetin and catechins and their metabolic derivatives) also indicated a dose depen dent relation between the amount of the compounds and the cholesterol lowering general effect (in particular the increase of HDL) [5].

Conclusions

Different extraction processes were tested for wet (WP) and dried (DP) pomace aiming at the production of bioactive phenol extracts from red grape pomace, and a water extraction (with or without enzymes) followed by a second ethanol extraction was found to be most efficient. WP samples showed the highest capacity of releasing biochemicals and, overall, 2 h control 24°C and 2 h 1% Cellu clast resulted to be the best treatments. The obtained extracts contained high amounts of total polyphenols, flavonoids, tannins and anthocyanins and exerted antioxidant and cholesterol lower ing activities. The present results support the possibility to exploit fruit processing by products as a source of bioactive compounds in view of their application as ingredients of healthy, functional and innovative products in the nutraceutical, pharmaceutical or cosmetic fields.

Acknowledgment

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