EXTENDED ABSTRACT

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Recovery of polyphenols having beneficial biological activities from red and white grape pomace

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Abstract

Grape is the world's largest fruit crop mostly used in wine making, a process during which 20-30% of the fruit weight ends up as pomace that represents a rich but yet underutilized source of valuable compounds. Polyphenolic compounds were extracted from red and white grape pomace and the extracts assayed for several beneficial bioactivities. Phenolics of wet (WP) and dried (DP) grinded pomace were recovered by enzymatic digestions and ethanol-based extractions. In general, total phenolic extracts from white pomace were 1.4-fold (WP) and 7.8-fold (DP) more abundant than those from red samples. Large concentrations of anthocyanins and flavanols were found respectively in red and white samples. All the extracts showed antioxidant activity; the best red pomace samples exerted cholesterol-lowering capacity; anti-tyrosinase effects were detected in white extracts while anti-inflammatory activity only in aqueous white samples. The results support the possibility of exploiting grape pomace extracts as ingredients for functional and innovative products in the food, nutraceutical, cosmetic or bioplastic fields.

Introduction

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 (Djilas et al., 2009). These large amounts of by-products constitute a serious environmental and disposal problem for wineries. However, they also represent a rich but yet underutilized source of valuable compounds, which may find applications in the food, feed, cosmetic and pharmaceutical fields (Djilas et al., 2009; Yu et al., 2013). Grape pomace is characterized by a high content of phenolic compounds that are only partially extracted during the wine making process and whose range and extractability mainly depends on the applied oenological parameters. Flavonoids, phenolic acids and stilbenes are among the main constituents of grape pomace and the beneficial influence on human health of grape and wine phytochemicals has been increasingly investigated (Yu et al., 2013; Ferri et al., 2016). Available studies regarding phenolic compositions, extractions and applications are mainly focused on pomace from red grape varieties, whereas little attention has been devoted to white grape pomace, which also contains a wide spectrum of potentially bioactive phenols. In the present study, the recovery and bioactivities of phytochemical compounds were studied on wet (WP) and dried pomace (DP) from red and white grapes (*Vitis vinifera* L.), by means of enzyme-assisted and ethanol-based extractions. Results of the treatments leading to the highest phenolic yields were compared and discussed in view of their possible future industrial

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applications.

Materials and Methods

Grape pomace materials

Red (a mix of *Vitis vinifera* cv. Sangiovese and Montepulciano) and white (a mix of *Vitis vinifera* cv. Trebbiano and Verdicchio) wine pomace, was supplied by the Cantine Moncaro wineries (Jesi, Ancona, Italy) immediately after wine production. Pomace was either frozen (wet pomace, WP) or dried (dried pomace, DP) in an industrial vented oven (60°C for 24h) and stored at -20°C until analyses. All the types of pomace contained berry skins, seeds, petioles and stalks.

Enzyme and ethanol-based extractions

Red and white WP and DP were treated as previously described (Ferri et al., 2016). Six commercial formulations (Pectinex 3XL, Pectinex Ultra SPL, Termamyl, Fungamyl, Pentopan 500BG, Celluclast; all purchased from Sigma-Aldrich, Milan, Italy) were tested for enzyme-assisted extraction. Concentration (0, 0.5, 1 or 2% enzyme volume/pomace DW), temperature (24, 30 or 37°C) and time (2, 6 or 24h) of incubation were optimized in 20 mL scale. After each treatment, water extract was removed by centrifugation, the pellet was incubated with 30 mL of 95% v/v ethanol at 24°C overnight and the resulting ethanol extract was collected.

Results and Discussion

The four types of grape pomace were treated for phytochemical recovery by a two-step processes: an enzyme-assisted extraction followed by an ethanol-based incubation. Based on the phenolic yield, two best treatments were selected for each starting material. Red wet pomace (WP): no enzyme, 24°C, 2h; 1% Celluclast, 37°C, 2h; red dry pomace (DP): no enzyme, 30°C, 24h; no enzyme, 37°C, 24h; white WP: no enzyme, 30°C, 2h; 2% Pentopan, 30°C, 6h; white DP: no enzyme, 37°C, 2h; 2% Celluclast, 37°C, 2h. Figure 1 shows the average amounts of total phenolics, total flavonoids and tannins of the two selected treatments.

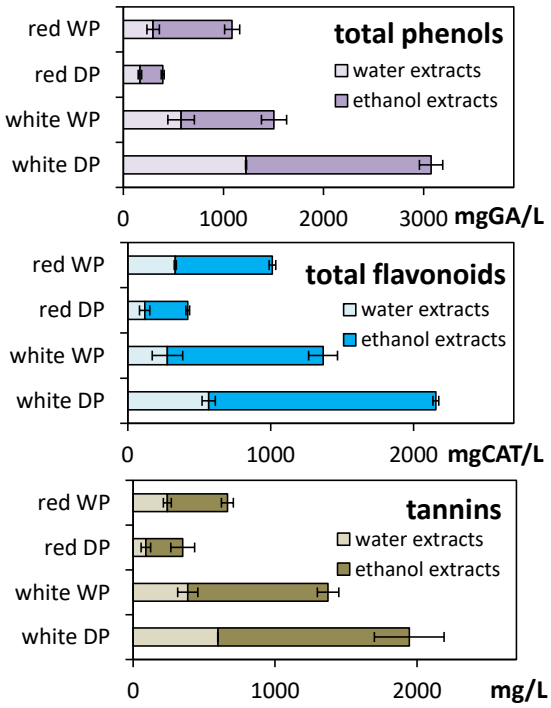

Figure 1. Total levels of phenols (expressed as mg of gallic acid (GA) equivalent per L of extract), flavonoids (in mg of catechin (CAT) equivalent per L) and tannins (in mg/L) in water and ethanol extracts. Data are the average of the two best treatments for each pomace type. WP, wet pomace; DP, dry pomace.

Similar trends were obtained for total phenolic, total flavonoid and tannin contents (Fig. 1), with higher extraction efficacy of ethanol with respect to water solvent (up to 3.9-fold for total flavonoid content in white WP). The phytochemical extractability was significantly affected by the drying processes: in red pomace this pre-treatment led to yield decreases, while in white samples DP contained 2-fold more extractable phenols than WP (Fig. 1). Previous published data indicated that the feedstock storage procedure had a significant impact on the recovery of phenolic compounds from berry pomace (Nayak et al., 2015). Total phenolic extracts from white pomace were 1.4-fold (WP) and 7.8-fold (DP) more concentrated than those from red samples (Fig. 1). Similar trends were also observed for total flavonoids and tannins (Fig. 1). Overall, large concentrations of anthocyanins were found in red ethanol samples, while flavanols were the main phenolic group present in white grape extracts

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(on average 55% and 98% of total flavonoids for WP and DP respectively). In general enzymatic treatment did not lead to a higher amount of extracted polyphenols, but it can be useful for the recovery of samples enriched in specific compounds as confirmed by HPLC-DAD analyses. Besides the obvious differences related to the used grape cultivars, the observed values may also be ascribed to a more efficient polyphenol extraction during red wine making process in which the fermentation is performed in the presence of the pomace. In contrast, white wine fermentation is generally made in the absence of berry skins and seeds, thus resulting in a higher content of phenols still present in the pomace. The present findings seem in agreement with previous studies on different grape cultivars (de la Cerda-Carrasco et al., 2015; Xu et al., 2016) in which higher polyphenol, flavonoid and proanthocyanidin concentrations were detected in white grape pomace with respect to the red ones.

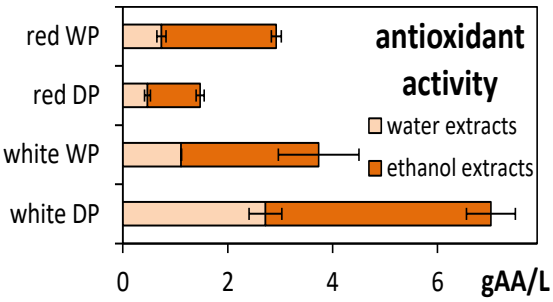

Figure 2. Antioxidant activity (expressed as g of ascorbic acid (AA) equivalent per L of extract) in water and ethanol extracts. Data are the average of the two best treatments for each pomace type. WP, wet pomace; DP, dry pomace.

All samples had a significant antioxidant activity (Fig. 2), also in agreement with characterization results (Fig. 1). Red WP without enzyme (24°C, 2h) extracts were found to possess promising dose-dependent cholesterol-lowering effects (cyp7a1 transcription significantly increased of 6.1% and 3.5% by water and ethanol extracts respect to control). Anti-inflammatory capacity was detected in white pomace water extracts, with WP and DP samples showing respectively an average 40.5% and 23% inhibition of NF-κB-RE expression in comparison to control. All white pomace samples exerted an anti-tyrosinase activity (on average 252.9 and 678.1 mg of kojic acid equivalents per L for WP and DP samples), an effect actually largely valued by industries for applications in skin whitening or in the inhibition of fruit browning during post-harvest processing (Chang, 2009).

Conclusions

Environment-friendly processes were optimized and phenolic extracts were obtained from red and white grape pomace. Significantly different phytochemical compositions were observed between water and ethanol extracts, between red and white samples and among different treatments. The present data indicate that, at least for white grape pomace, drying could be a useful technique to preserve the by-product until further industrial application, with the positive consequence of increasing phenol yields. The results strongly support a possible application of grape pomace extracts, as an inexpensive, easily available and alternative source of bioactive phytochemicals, which could be used as ingredients in the food, nutraceutical, cosmetic or bioplastic fields.

Acknowledgments

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