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ANTIOXIDANT AND PROOXIDANT PROPERTIES OF POLYPHENOLS EXTRACTED FROM GRAPEVINE POMACES BY "GREEN" SUSTAINABLE METHODOLOGY

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SPOKE, WP E TASK DI APPARTENENZA

SPOKE 8 - WP8.1 - Task 8.1.1 Valorization of the waste by green chemistry to obtain high value molecules or new products

INTRODUZIONE

Natural polyphenols are secondary metabolites in plants that play a crucial role in defense against various types of stress. They influence multiple targets in pathways and mechanisms associated with carcinogenesis, tumor cell proliferation, metastatic spread, and drug resistance [1]. For thousands of years, extracts containing polyphenols have been utilized in traditional Eastern medicine. Evidence suggests that the long-term consumption of moderate quantities of polyphenols found in red grapes and red wine may reduce the incidence of certain cancers [2]. Pomaces, which are byproducts of the winery and grape juice industry, pose a significant environmental challenge [3]. These waste materials have garnered significant attention because they contain high levels of health-promoting compounds, including fiber and polyphenols that remain after the winemaking process [4]. The most abundant phenolic compounds in red wine pomaces are anthocyanins, concentrated in the skins, while flavonols are predominantly found in the seeds (comprising 56–65% of total flavonols) [5]. In Chardonnay (a white variety) pomaces, phenolic acids, flavan-3-ols, flavonoids, and oligomeric procyanidins have been identified (4). Grape seed extracts primarily contain the flavan-3-ols catechin and epicatechin, as well as procyanidins [6], although these compounds have also been found in the skins. The total phenolic content of grape pomace extracts is typically well-correlated with their antioxidant activity [7]. A strong and significant correlation has been observed between antioxidant activity and total phenolic content in grape pomace samples [7,8]. The phenolic composition of Syrah and Chardonnay grape pomaces was studied to evaluate their antioxidant and prooxidant properties [9]. Polyphenols were extracted using a "green" hydroalcoholic solvent (ethanol/water 1:1 v/v), followed by a detailed chemical and electrochemical characterization of the phenolic compounds. The antioxidant and prooxidant capacities of the pomace were first analyzed using cyclic voltammetry (CV) and other standard analytical assays, and then through biological tests on B16F10 metastatic melanoma cancer cells.

MATERIALI E METODI

Hydroalcoholic Extraction of Polyphenols: Lyophilized pomace samples (1 g) were transferred in 75 mL plastic tubes, added with 25 mL of solvent (ethanol/water 1:1 v/v) and shaken at 50 rpm for 5 h at room temperature in the dark (Figure 1). The liquid and solid phases were separated by centrifugation at 3220 x g for 15 minutes and, subsequently, filtrated through a filter paper under vacuum. The filtrate was transferred in a vacuum roto-evaporator, where the ethanol evaporated at 40–45 °C. Water was removed by freeze-drying and the dry residue stored at-80 °C until analyses.

Identification and Quantification of Polyphenols in the Extracts: The total polyphenol content (TPC) and the quantitative analysis of targeted phenolic compounds were determined as described by [9]. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry residue (dr).

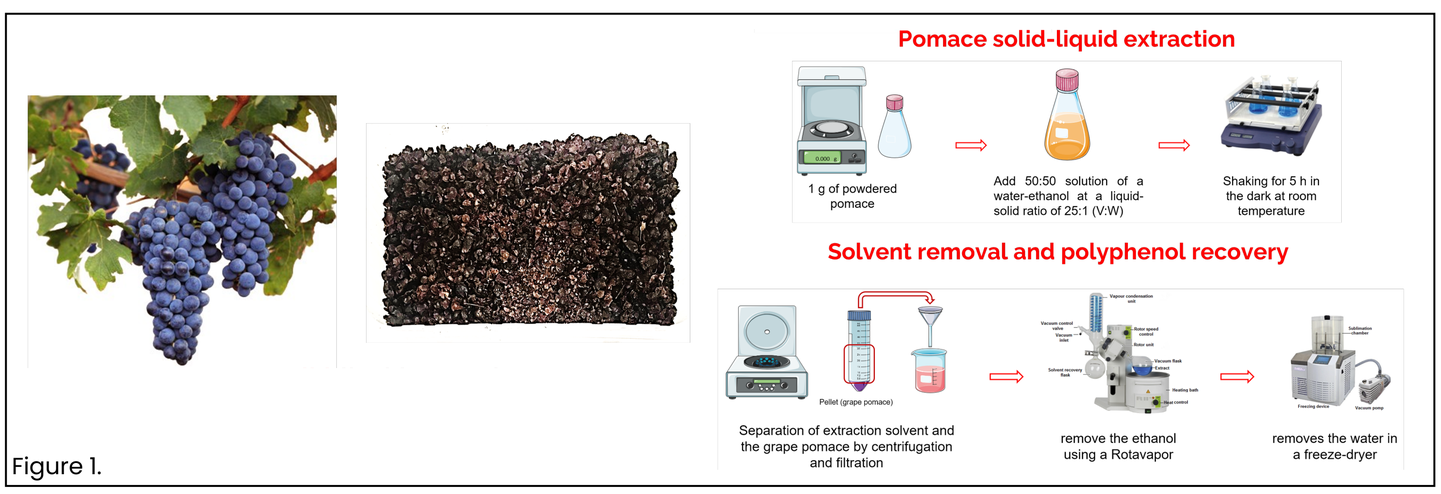


Figure 1.

Antioxidant Activity (AAox) Determination: The electrochemical characterization of the extracts and the AAox determination were performed by CV using a screen-printed sensors (GSI Technologies, Burr Ridge, IL, USA), consisting of a 5 mm carbon working electrode (WE), an Ag/AgCl pseudo reference electrode (RE), and a carbon auxiliary electrode (AE). Cyclic voltammograms (CVs) were performed from -0.2 V to +0.8 V (vs. Ag/AgCl pseudo-RE) at a scan rate of 0.1 V/s. In order to provide a quantitative comparison among the CV patterns of extracts of different origin, the voltammograms were integrated and the area under curve (AUC) was calculated at +0.5 V and +0.8 V and expressed in microcoulombs (C). The AUC values at +0.5 V refers to AAox while values at +0.8 V estimates the total polyphenol content.

Viability Assays: three different experiments were carried out on B16F10 cells (Figure 4): (1) a time-dose response test for 6, 12, and 24 h with increasing concentrations (from 1 to 500 µg/mL) of Syrah or Chardonnay pomace extract from Italy, France and Tunisia; (2) treatments with increasing concentration of H_2O_2 (from 1 to 500 µM) for 24 h; 3) a combined treatment with Syrah/ Chardonnay 250 µg/mL + H_2O_2 10-50-100 µM for 24 h. On fibroblasts two different experiments were carried out (Figure 5): (1) a dose-response test for 24 h with increasing concentrations (from 1 to 500 µg/mL) of Syrah or Chardonnay extract; (2) treatments with increasing concentration of H_2O_2 (from 1 to 500 µM) for 24 h. At the end of the experiments, cells were incubated with 100 µL of MTT (0.5 mg/mL), and the cultures were allowed to incubate at 37°C for 3 h. The MTT was removed and the formazan crystals were dissolved in 100 µL of 2-propanol. The color was read at 570 nm using a microplate reader.

RISULTATI

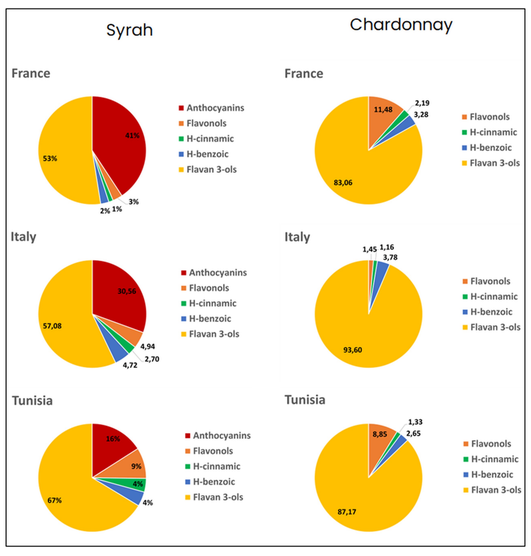
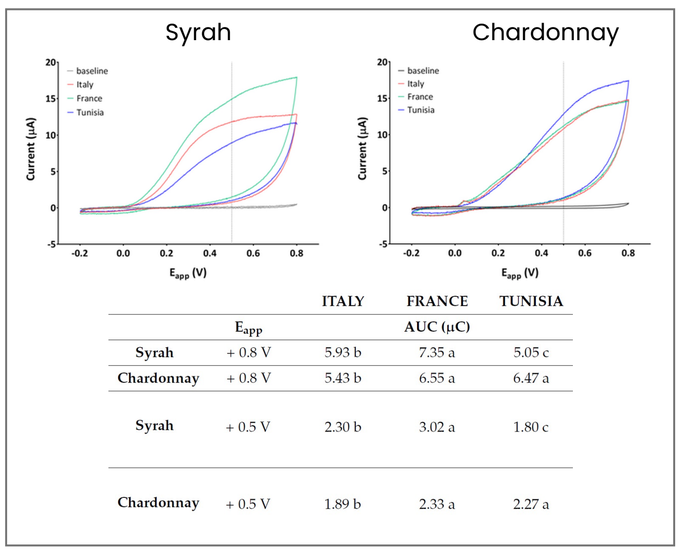


Figure 2. The Syrah pomace extracts exhibited the presence of anthocyanins, flavonols, hydroxycinnamic acids, hydroxybenzoic acids, and flavan-3-ols. All of these polyphenol classes, except for anthocyanins, were also identified in the Chardonnay pomace extracts. Syrah pomace extracts were found to be richer in phenolic compounds compared to Chardonnay varieties. The French Syrah variety was approximately 1.5 times more concentrated than the pomace extracts from the other two countries. In the case of Chardonnay, the Italian pomace extract was the richest in polyphenols, with concentrations about 1.5 times higher than those found in the French and Tunisian extracts, which contained similar amounts.



		ITALY	FRANCE	TUNISIA
	E_{app}		AUC (µC)	
Syrah	+ 0.8 V	5.93 b	7.35 a	5.05 c
Chardonnay	+ 0.8 V	5.43 b	6.55 a	6.47 a
Syrah	+ 0.5 V	2.30 b	3.02 a	1.80 c
Chardonnay	+ 0.5 V	1.89 b	2.33 a	2.27 a

Figure 3. All the voltammograms obtained using the CV technique deviated from the baseline at around +0.1 V, indicating the presence of polyphenols with low redox potential in both Syrah and Chardonnay. As the applied potential increases, the shape of the French and Italian Syrah voltammograms becomes more rounded between 0.25 and 0.4 V, suggesting a significant polyphenol component that ionizes within that potential range. This component is either absent or less prominent in the Tunisian Syrah and in all three Chardonnay extracts. Different voltammogram shapes correspond to different AUCs, which are reported at both 0.5 V and 0.8 V in the table.

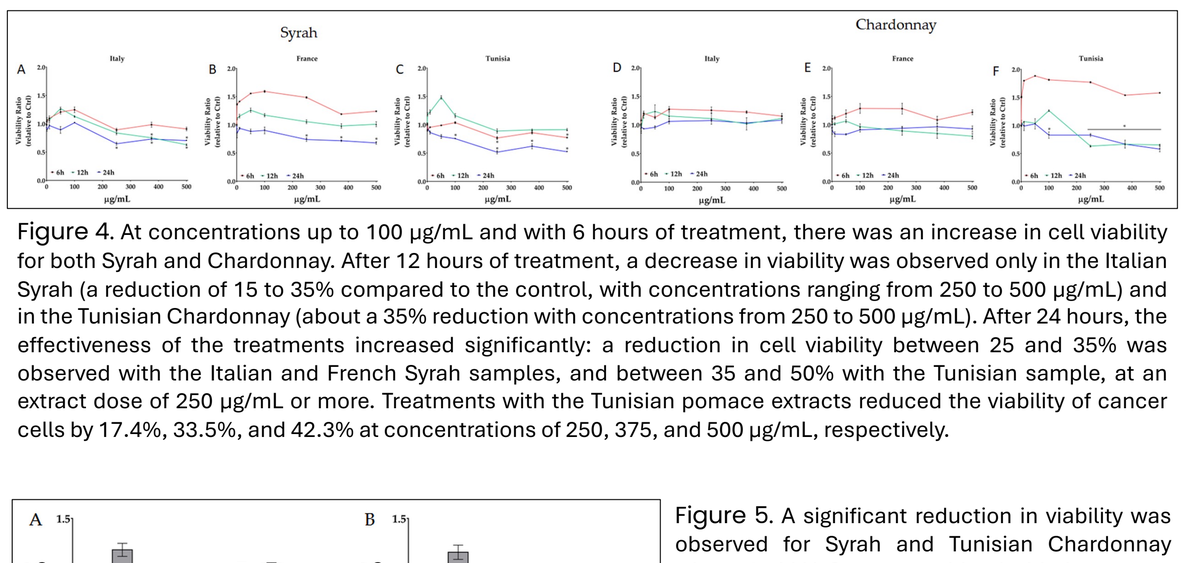


Figure 4. At concentrations up to 100 µg/mL and with 6 hours of treatment, there was an increase in cell viability for both Syrah and Chardonnay. After 12 hours of treatment, a decrease in viability was observed only in the Italian Syrah (a reduction of 15 to 35% compared to the control, with concentrations ranging from 250 to 500 µg/mL) and in the Tunisian Chardonnay (about a 35% reduction with concentrations from 250 to 500 µg/mL). After 24 hours, the effectiveness of the treatments increased significantly: a reduction in cell viability between 25 and 35% was observed with the Italian and French Syrah samples, and between 35 and 50% with the Tunisian sample, at an extract dose of 250 µg/mL or more. Treatments with the Tunisian pomace extracts reduced the viability of cancer cells by 17.4%, 33.5%, and 42.3% at concentrations of 250, 375, and 500 µg/mL, respectively.

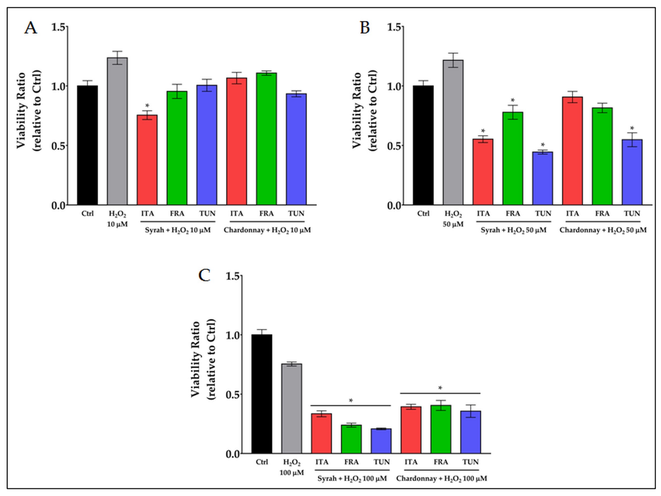


Figure 5. A significant reduction in viability was observed for Syrah and Tunisian Chardonnay when 50 µM H_2O_2 was combined with the extract. When the H_2O_2 dose was increased to 100 µM, the reduction in viability remained significant for both Syrah and Chardonnay. The reduction in viability induced by the combined treatments ranged from 45% to 80% with Syrah extracts and from 45% to 65% with Chardonnay extracts. This prooxidant effect on cancer cells was achieved with doses of H_2O_2 that did not have a toxic effect on fibroblasts. The same effect, using only hydrogen peroxide, could have been achieved only with cytotoxic doses for healthy cells (over 100 µM).

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[1] Asensi M et al., 2011, doi: 10.3109/10408363.2011.631268; [2] Russo G et al., doi: 10.1007/978-3-642-38007-5_11; [3] Antoni´c B et al., 2020, doi: 10.3390/foods9111627; [4] Chedea VS et al., 2010, doi: 10.1016/j.foodchem.2009.12.020; [5] Beres C et al., 2017, doi: 10.1016/j.wasman.2017.07.017; [6] Rockenbach I.I. et al., 2012, doi: 10.1016/j.foodres.2012.07.001; [7] Spigno G et al., 2007, doi: 10.1016/j.jfoodeng.2006.10.021; [8] José Jara-Palacios M. et al., 2014, doi: 10.1016/j.foodres.2014.09.009; [9] Spissu Y et al., 2023, doi: 10.3390/antiox12010080.

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