

Grape stalks: From wastes to source of antioxidants and nutraceuticals

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Abstract. Wine production is one of the most significant agricultural activities worldwide. The winemaking process generates large amounts of by-products: grape marc, stalks, and exhausted grape marc. Until now, many studies have been focused on marc valorization, very few on stalks. The aim of this research was to deeply explore the potential of residual stalks in the wine industry from a circular economy perspective.

Polyphenols were extracted from stalks with new sustainable technologies in the frame of “green chemistry” without the use of hazardous solvents. Extracts were analyzed for total phenolic compound content (TPC) and their antioxidant activity was determined.

As polyphenols can have antimicrobial activity, the effect of the extracted polyphenols against wine-spoiling yeast *Brettanomyces bruxellensis* was determined. The percent reduction of the culture optical density, in the presence or absence of polyphenols, was compared to assess the antimicrobial activity of the samples.

The results obtained underline the importance of winemaking by-products (stalks) and their eco-friendly valorization to obtain molecules for food, nutraceutical and cosmetic industries.

1 Introduction

It is well known that food processing industries generate large amounts of wastes and residues which threaten the environment. This aspect is one of the main social challenges, which must be addressed to minimize the environmental impact.

Wine production is one of the most significant agricultural activities worldwide. The winemaking process generates large amounts of by-products: grape marc, stalks, and exhausted grape marc. All these products impact waste management both from an ecological and an economic point of view. Until now, most studies have been focused on marcvalorisation, but very few concern stalks.

In the last years, plant extracts from different wastes of agri-food and wine industries have received consideration due to their importance in different fields, such as nutraceuticals, functional foods, food additives and cosmetics [1,2]. These extracts are rich in phenolic compounds with recognized antioxidant and

antimicrobial action, and therefore they can be applied in the winemaking industry and in nutraceutical formulations, according to a circular economy approach.

The current study was aimed to investigate these positive activities on polyphenols extracted from grape stalks and to deeply explore their potential in the wine industry in a circular economy perspective.

2 Materials and methods

2.1 Polyphenol extractions

Stalks were collected from Nebbiolo grape cultivar. They have been milled and extracted with Subcritical water extraction (SWE). This was performed according to Mikukaet al. [3]. Distilled water was used as solvent. All of the aqueous extracts were dried in a freeze-dried for 24 hours.

2.2 Polyphenol analysis

Extracts were diluted in deionized water to obtain a 1 mg/ml mixture.

This solution was analysed using CDRwine Lab ® (Orsell, Italy) and the following compounds were quantified:

Catechins, Tannins, Polyphenol Index (absorbance 280 nm), Total Polyphenol Content (Folin Ciocalteu).

2.3 Antioxidant activity

The DPPH• radical scavenging assay was assessed using the method described by Salgado-Ramos et al. [4].

The phenolic extracts were diluted in water. 30 µL of diluted samples was added to 300 µL of 108 µM DPPH• methanolic solution, the mixture was diluted with 570 µL of 80% (v/v) methanol. The absorbance at 517 nm was measured, using a spectrophotometer, after 30 min of incubation in the dark at room temperature. Trolox was used as a standard. Concentrations were calculated from a calibration curve in the range between 0.025 and 0.3 mM Trolox, and the results were expressed as Trolox equivalents (TE)/g of dry matter for solid samples, and as Trolox equivalents (TE)/L of the sample, for liquid samples.

The ferric reducing antioxidant power (FRAP) assay was used to quantify the reducing power of phenolic extracts, using the colorimetric method proposed by Santos et al. [5]. FRAP reagent was freshly prepared by mixing sodium acetate buffer (300 mM pH 3.6), 10 mM TPTZ solution prepared in 40 mM HCl, and 20 mM FeCl₃ 6H₂O, using the proportion 10:1:1 (v:v:v). The assay was carried out using 96-well microplates: 280 µL of FRAP mixture and 20 µL of properly diluted sample were added in each well, and absorbance at 593 nm was measured after 30 minutes incubation at room temperature. Ascorbic acid was used as a standard (linearity of calibration curve: 15-60 mg/l), and results were expressed in mg ascorbic acid equivalents/g extracts (mg AAE/g extr).

2.4 Antimicrobial activity assays

Brettanomyces bruxellensis ISE373, belonging to the CREA Culture collection named CMVE, was pre-cultured in YEPG medium (1% peptone, 1% yeast extract, 2% glucose) and grown for three days at 25 °C.

Subsequently assays were prepared according to Garcia- Ruiz et al. [6] and four different concentrations of extracts were tested 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml. The assays were performed in a microplate in a final volume of 200 µl. Optical density at 600 nm was read and recorded using the 800 TS plate reader (Bioteck, Italy).

3 Results and Discussion

3.1 Polyphenol analysis

Some compounds have been quantified from the grape stalk extracts. The results are shown in Table 1.

Table 1. Characterization of extracts.

Polyphenolic Index (mg/L gallic ac)	77
Total Polyphenol (Folin Ciocalteu) (mg/L gallic ac)	398
Catechins (mg/L)	<1
Tannins (g/L)	<0.3

As reported, the amount of total polyphenols recovered is high, confirming the good yields of the water extraction procedure.

Regarding catechins, their content in our extract resulted to be very low, in agreement with Alonso et al. [7], but in contrast with Nieto et al. [8], who recovered 2.4 mg/g dry extract of catechins in their grape stem extracts.

Tannins are under the detection limit, and this is in line with the results of Blackford et al. [9] who found that in grape stems hydrolyzable tannins are absent.

In general, the polyphenol composition is related to the type of extraction and to the conditions used in this initial process, as referred by other authors [8-10].

3.2. Antioxidant analysis

The antioxidant potential (radical scavenging activity) of the extracts was lower than other literature data (570.35 µmol/gEXT), that were however obtained using hydro-alcoholic solutions as solvent.

As the reducing power indirectly reflects the antioxidant capacity of polyphenols, it was measured by their ability to convert Fe³⁺ complexes into Fe²⁺ complexes: the calculated reducing power was 15.93 ± 0.55 mgAAE/mg extract. This result is similar to other data reported in literature [9,11], also for extracts obtained using hydro-alcoholic solutions.

3.3. Antimicrobial activity assays

The results obtained with this test showed that polyphenolic compounds extracted from stalks can have some antimicrobial effect on the wine spoilage yeast *Brettanomyces bruxellensis*. As the initial low polyphenol concentrations (0.25 mg/ml and 0.5 mg/ml) tested did not influence yeast growth (data not shown), higher concentrations (1 mg/ml and 2 mg/ml) were used.

Results are shown in Fig. 1, optical density was monitored every two hours for a total of 72 hours. It is

possible to observe that the presence of the extracts in the medium alters the kinetic of the yeast. In the control without extract the optical density (OD600) was higher than 1, whereas in the samples containing the extracts only reached with 1 mg/ml and 0.23 with 2 mg/ml, respectively.

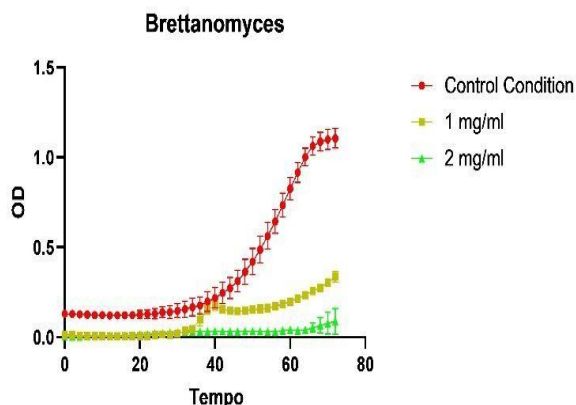


Figure 1. Kinetic of the yeast growth (72 hours) without (control 373) and with polyphenol extracts (1 mg/ml and 2 mg/ml).

Several studies highlighted the anti-proliferative and antimicrobial activity of polyphenols.

Jara-Palacios et al. [12] investigated the anti-proliferative activity of grape pomace extract on colon cancer cells and they found that they inhibited cell proliferation by 52.1% using extracts at a concentration of 100 µg/ml. In their review, Takò et al. [13] listed a great number of microorganisms, including food-related pathogens, sensitive to the antibiotic effect of phenolic-enriched extracts.

Regarding enological species, Garcia-Ruiz et al. [6] tested the antimicrobial activity of some phenolic compounds on *Oenococcus oeni*, *Lactobacillus hilgardii* and *Pediococcus pentosaceus* and they found that flavonols greatly inhibit bacterial growth. Pastorkova et al. [14] studied on *Acetobacter* species, *Brettanomyces bruxellensis*, *Hanseniaspora uvarum* and *Zygosaccharomyces* some of phenolic compounds naturally occurring in grapes, and they reported that certain grape-derived compounds, in particular p-coumaric and ferulic acids, luteolin, myricetin, pterostilbene and resveratrol, can inhibit wine spoilage microorganism. Recently, Gutiérrez-Escobar et al. [15] characterized pure stilbene extract from grapevine shoots and found that their antimicrobial activity was more efficient against yeasts than against bacteria related to the enological field.

To the best of our knowledge, this is the first report in which polyphenols extracted from grape stalks have been tested on *Brettanomyces bruxellensis*. However, this is only a preliminary study that deserves further investigations.

In conclusion, this study underlines the importance of valorizing wine-making by-products, in particular stalks. Some interesting parameters and some applications have been investigated. The study must be deepened to find other useful products and identify further applications of polyphenolic extracts.

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