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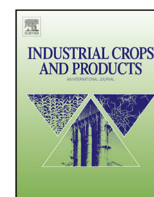

Valorization of grape stems

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ABSTRACT

The wine industry produces large amounts of grape stem byproducts, which have been described as a natural source of polyphenols. In the present study, an extract from grape stems was evaluated for its antioxidant and antimicrobial activities in model wine to determine its potential capacity to replace and/or reduce SO_2 in winemaking. Additionally, its possible effects on aroma were studied.

Grape stem extract (STE) showed high antioxidant activity and it can be proposed as an energetic antioxidant. Its antimicrobial activity was compared to that of SO_2. STE showed a lower inhibitory effect than SO_2 for *Saccharomyces cerevisiae*, *Hanseniaspora uvarum*, *Dekkera bruxellensis* and *Pediococcus damnosus* whereas STE seems to be more efficient against *Candida stellata and Botryotinia fuckeliana.* GC–olfactometry analysis of STE showed that its most important odorants are naturally present in wines, and thus olfactometric profile modifications in wine, after STE addition, could appear quantitatively but not qualitatively.

It can be concluded that grape stems are compounds with a low sourcing cost, high antioxidant activity and good antimicrobial properties.

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1. Introduction

The world wine industry produces thousands of tons of residues which represent a waste management issue both ecologically and economically (FAO, 2012). Among wine by-products, pomace and grapevine canes have been widely studied (Çetin et al., 2011; Rayne et al., 2008). However, less attention has been focused on stems. Grape stems are removed before winemaking and represent around 5% of wine byproducts. They are frequently used for producing compost or for feeding ruminants (Anastasiadi et al., 2012). The possibility of increasing added value in stem residues generated by wineries around the world promotes studies on this byproduct.

Extracts from grape byproducts have been proposed as preservatives in the food industry due to their antioxidant and antimicrobial properties. For example, phenolic extracts from grape and grape derivatives have been shown to be useful preservatives in chicken meat, horse mackerel and fruit juices (Pazos et al., 2006; Selani et al., 2011).

The most used preservative in the wine industry is sulphur dioxide (SO_2). In wine, SO_2 exhibits an important antioxidant function that helps to reduce the effects of dissolved oxygen and inhibit oxidase enzymes, which are endogenous to grapes and also come from fungal infections (Izquierdo-Cañas et al., 2012). Moreover, SO_2 inhibits the development of all types of microorganisms, such as yeasts, lactic acid bacteria and, to a lesser extent, acetic acid bacteria (Santos et al., 2012). However, several human health risks, including dermatitis, urticaria, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated with SO_2 (Vally et al., 2009). Consequently, the International Organization of Vine and Wine (OIV) has established limits for SO_2 content in wines (OIV, 2012). Thus, there is great interest in the search for other preservatives that can replace and/or reduce SO_2 content in wines.

Another important reason that has increased the interest in searching for alternatives to SO_2 in wines is the fact that only molecular SO_2 (a percentage of free SO_2) possesses antioxidant and antimicrobial properties. The percentage of free SO_2 depends on

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the pH, a high pH decreasing its proportion, and therefore its effectiveness. In the last few years, wine pHs have increased due to the changing climate, and thus wines are becoming more vulnerable to spoilage (Sadras et al., 2013).

Some chemicals have been tested as an alternative to SO_2: colloidal silver complex (Garde-Cerdán et al., 2014; Izquierdo-Cañas et al., 2012), dimethyl dicarbonate (Costa et al., 2008), ascorbic acid, hypophosphorous acid, thiodipropionic acid, Trolox C, stannous chloride, and Sporix (Panagiotakopoulou and Morris, 1991), and even natural products (lysozyme and bacteriocins) (Bartowsky, 2009). Among these, the use of phenols is a promising alternative. Oenological tannins (Sonni et al., 2009), vegetal extract (Salaha et al., 2008), almond skin and eucalyptus leaf extracts (González-Rompinelli et al., 2013) have resulted efficient in reducing SO_2 in wines.

This study joins the two current oenological research interests mentioned above, increasing the added value of grape stem byproducts and developing other preservatives that can replace and/or reduce SO_2 content in wines. In particular, it aims to assess the antioxidant and antimicrobial activity and olfactometry profile of the grape stem extract as a potential alternative to SO_2 in winemaking. It is a preliminary study to test this extract in winemaking.

2. Experimental

2.1. Chemicals and reagents

Analytical grade methanol, acetic acid, diethyl ether, ethyl acetate and ethanol were supplied by Panreac (Barcelona, Spain). Chemical standards: ethyl butyrate, hexanal, isoamyl acetate, 2-hydroxy-3-pentanone, hexyl acetate, 6-methyl-5-hetpten-2-one, methyl octanoate, 1-octen-3-ol, *Z*-2-nonenal, 3-isobutyl-2-methoxypyrazine, phenylethanal, α-terpineol, *E*-2-undecenal, *E*,*E*-2,4-decadienal, guaiacol, eugenol, vanillin, *n*-alkanes (C7-C40), Trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,20-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein (FL), K(OH) solution and dichloromethane (LiChrosolv quality) were purchased from Sigma–Aldrich (Steinheim, Germany). Anhydrous sodium sulfate, tartaric acid and absolute ethanol (99.9% HPLC quality) were obtained from Panreac (Barcelona, Spain). Solfosol (water solution of SO_2) was supplied by Sepsa-Enartis (Penedès, Spain). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

2.2. Grape stem extract (STE)

Stems from the Syrah grape variety harvested in 2011 at IFAPA-Rancho de la Merced Centre in Jerez de la Frontera (Spain) were used for this research. The Syrah variety was chosen for being widely spread worldwide (35.000 ha).

The grape stem extract was obtained by means of ultrasound (model UP 200S, from Dr. Hielscher GmbH, Teltow, Germany) as previously described (Piñeiro et al., 2013). This method has been developed in our laboratory. It is a simple, rapid, and reliable UAE method recently developed and validated for stilbenoid analysis in grape stem samples. Briefly, ground freeze-dried grape stems were extracted under the following conditions: distilled alcohol/water (80:20, v/v) as extraction solvent, 75 °C as extraction temperature, 70% amplitude, cycle 0.7 s, 7 mm probe tip and 250 ml as extraction volume (ratio 1:30). The extraction time was 15 min. Subsequently, a rinsing step was applied to the extracted sample with 25 ml of fresh solvent. The obtained extracts were centrifuged at 1469 × *g* for 5 min in a Digicen 20-R centrifuge (Orto Alresa, Spain), paper filtered, filtered through a 0.22 μm filter (PVDF Teknokroma, Barcelona, Spain) and kept at −18 °C until analysis. This produced a stilbene-rich extract. Piñeiro et al. (2013) characterized this grape stem extract as follows: piceatannol (21.1 ± 0.1 mg/kg dw), *trans*-resveratrol (139.1 ± 0.8 mg/kg dw), ε-viniferin (65.1 ± 1.1 mg/kg dw) and vitisin B (13.0 ± 1.9 mg/kg dw).

Finally, the grape stem extract was adjusted to 50 ppm and 80 ppm in a model wine system (12%, v/v, ethanol, 4 g/l tartaric acid and adjusted to pH 3.4 with NaOH). These concentrations were chosen as they are recommended for SO_2 in winemaking in warm climates (50 ppm for red wine, and 80 ppm for white wine) (Puertas et al., 2013).

2.3. Antioxidant activity

2.3.1. DPPH assay

The samples were analyzed using the technique reported by Brand-Williams et al. (1995). The stock solution was prepared by dissolving 24 mg of DPPH in 100 ml of MeOH, and then stored at 20 °C in the dark until needed. The working solution was obtained by diluting 10 ml of the stock solution with 45 ml MeOH, to obtain an absorbance of 1.1 ± 0.1 units at 515 nm, using a Shimadzu UV-1063 spectrophotometer. A volume of 10 μl of different STE concentrations was added to 990 μl of 0.094 mM DPPH in MeOH, to reach 1 ml. To determine the reaction kinetics, the assays were continuously monitored at 515 nm over a 1 h period at 25 °C. Each sample was analyzed in triplicate. The antioxidant activities were expressed as μM Trolox equivalents/mg extract.

2.3.2. ORAC assay

The oxygen radical absorbance capacity was determined as previously described (Dávalos et al., 2004). The ORAC analyses were conducted on a Synergy HT multi-detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, VT, USA), using 96-well polystyrene microplates with black sides and a clear bottom, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by KC4 software, version 3.4. The reaction was performed in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 μl. FL (100 μl; 3 nM, final concentration) and 20 μl stem extracts, were placed in the wells of the microplate. The mixture was preincubated for 30 min at 37 °C, before rapidly adding the AAPH solution (30 μl; 19 mM, final concentration). Reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The antioxidant activities were expressed as μM Trolox equivalents/mg extract.

2.4. Antimicrobial assay

Pure cultures were obtained from the CECT (Spanish Collection of Type Cultures, Valencia, Spain). Yeasts: *Saccharomyces cerevisiae* (CECT 1942), *Candida stellata* (CECT 11969), *Hanseniaspora uvarum* (CECT 1118), *Dekkera bruxellensis* (CECT 11045). Bacteria: *Lactobacillus plantarum* (CECT 5956), *Acetobacter aceti* (CECT-298T), *Pediococcus damnosus* (CECT 793), *Oenococcus oeni* (CECT 218). Fungus: *Botryotinia fuckeliana* (CECT 20518). All the microorganisms were grown in optimal conditions. Subsequently, the extract and/or SO_2 were added to determinate its antimicrobial properties. Yeasts were cultivated on 21 g/l malt broth (Sigma–Aldrich, Germany) with a final pH of 6.2. Bacteria were cultivated on 51 g/l MRS broth (Fluka, Spain) and fungus on 39 g/l potato dextrose broth (Fluka, Spain) with a final pH of 6.2 and 5.6, respectively.

For the determination of microbial growth kinetics, indirect impedance measurements were carried out by the BacTrac measuring system ("Bacteria Tracer", SY-LAB, Vienna, Austria). This

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