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Antioxidant properties of different products and additives in white wine

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ABSTRACT

Different winemaking products (ascorbic acid, glutathione, yeast lees and a yeast autolysate) were tested in comparison with sulphur dioxide, concerning radical scavenging activity (measured by DPPH· assay), oxygen consumption capacity and ability to reduce wine colour and predisposition to browning. Trials were performed in white wines and model solution. SO_2 was the most active in reducing wine colour development. Fresh lees and ascorbic acid were very effective in oxygen and free radical scavenging, but they both induced browning during wine storage, the former, by releasing phenolic compounds. Glutathione was also able to scavenge DPPH· in wine, but less effective against oxygen, and it induced browning during storage. Surprisingly, the yeast derivative preparation was the treatment that behave more similarly to sulphiting; it was very active in scavenging DPPH·, and, even without modifying oxygen consumption rate, it protected quite well wine colour over an 8 months storage time.

1. Introduction

Despite the mechanisms involved in wine oxidation have been extensively reviewed (du Toit, Marais, Pretorius, & du Toit, 2006; Oliveira, Ferreira, De Freitas, & Silva, 2011; Singleton, 1987; Waterhouse & Laurie, 2006), the protection of wine against oxidative spoilage remains one of the main goals of modern winemaking, becoming particularly critical when low levels of sulphur dioxide are used. The chemistry of this additive in wine has been recently re-written by Danilewicz (2007, 2011), Danilewicz, Seccombe, and Whelan (2008): they clearly demonstrated that SO_2 does not react directly with oxygen, as previously thought (Ribéreau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006), but, in presence of metal ions, it is able to scavenge hydrogen peroxide and the quinones formed from the oxidation of polyphenols (Danilewicz et al., 2008). Due to the toxicity and allergenic potential of sulphites, different compounds have been proposed for reducing their final concentration in wine, even if, none of them is likewise effective in protecting wine against oxidations.

Ascorbic acid (ASC) is the most known among these products; it is able to scavenge hydroxyl radicals (Bradshaw, Barril, Clark, Prenzler, & Scollary, 2011) and quinones (Bradshaw et al., 2011; Waterhouse & Laurie, 2006), but its metal catalyzed oxidation produces hydrogen peroxide (Bradshaw et al., 2011; Ribéreau-Gayon et al., 2006; Zoecklein, Fugelsang, Gump, & Nury, 1995) and this may trigger browning reactions if sulphites are not present (Bradshaw, Cheynier, Scollary, & Prenzler, 2003; Bradshaw, Prenzler, & Scollary, 2001). The capacity of ascorbic acid to act both as antioxidant and free-radical initiator is known as "crossover effect" (Bradshaw et al., 2001, 2003; Buettner & Jurkiewicz, 1996) and explains the reason why ASC is normally used in wine in combination with sulphites (Ribéreau-Gayon et al., 2006; Zoecklein et al., 1995; Bradshaw et al., 2011).

Another traditional system to protect wine against oxidations is the use of yeast lees (Pérez-Serradilla & Luque de Castro, 2008). Fresh lees have a high oxygen consuming capacity (Fornairon-Bonnefond & Salmon, 2003), due to the presence of yeast membrane lipids and sterols (Fornairon-Bonnefond & Salmon, 2003; Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000); adsorbed polyphenols (Gallardo-Chacón, Vichi, Urpí, López-Tamames, & Buxaderas, 2010), thiol groups of cell wall proteins (Jaehrig, Rohn, Kroh, Fleischer, & Kurz, 2007; Gallardo-Chacón et al., 2010) and β-glucans from yeast cell walls (Jaehrig et al., 2007) also contributes to their antioxidant properties. However, ageing on the lees can modify wine sensory characters, and for this reason it is not suitable for all the wine typologies; moreover, lees alone do not protect wine against microbial pollution and sulphiting is always required.

The possibility to use glutathione (GSH) as wine antioxidant has been considered since the role of this tripeptide in preventing must browning has been highlighted (Singleton, Salgues, Zaya, &

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Trousdale, 1985); nevertheless, very few studies are currently available concerning GSH addition in wine. It has been reported that GSH supplementation could have positive effects on wine colour and aroma (Dubourdieu & Lavigne-Cruege, 2003; Papadopoulou & Roussis, 2008), but high amounts of GSH in oxidative conditions can lead to colour formation (Sonni, Clark, Prenzler, Riponi, & Scollary, 2011).

GSH can be supplemented also in form of yeast derivatives (YD): the ability of "glutathione-enriched" inactive dry yeast preparations (IDY) in reducing the loss of volatile compounds during wine storage has been recently reported by Andújar-Ortiz, Rodríguez-Bencomo, Moreno-Arribas, Martin-Alvarez, and Pozo-Bayón (2010), Rodríguez-Bencomo et al. (2014): they hypothesized that this may be due to the antioxidant capacity of GSH, but also other components of the IDY preparation might be involved (Andújar-Ortiz et al., 2010), such as some peptides containing methionine, tryptophan, and tyrosine (Rodríguez-Bencomo et al., 2014).

Despite none of these alternatives has antimicrobial activity (as instead SO_2 has), the opportunity to reduce sulphur dioxide by their utilization, is arousing more and more interest, among winemakers. Nevertheless, despite the amount of works reporting the antioxidant effects of these substances, the most of the papers regards model solutions and moreover, it is currently difficult to foresee in which extent it is possible to replace sulphites with each of these alternatives, preserving wine quality, because of the lack of scientifically-based direct comparisons, among their effects and those of sulphur dioxide.

For this reason, the aim of this work was to carry out a preliminary investigation on the radical scavenging activity (measured by DPPH· assay) and the oxygen consumption capacity of different enological products and additives in comparison with SO_2. Ascorbic acid (considered as reference standard), glutathione, yeast lees and a self-prepared yeast autolysate were tested. Trials were performed in model solution and in different wine typologies. Concerning oxygen consumption trials, wines were finally subjected to fast spectrophotometric measurements, for assessing the effect of the different antioxidants on colour, total phenolics and predisposition to browning.

2. Materials and methods

2.1. Chemicals

Tartaric acid, sodium hydroxide, ethanol (96% v/v), ACS grade hydrochloric acid (37%), hydrogen peroxide (30% w/w), sodium acetate and potassium metabisulphite were from Carlo Erba Reagents (Milan, Italy); ascorbic acid, glutathione, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH·) and HPLC grade methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Yeast lees and yeast derivatives (YD)

Fresh lees were supplied by Viticoltori Friulani "La Delizia" (Casarsa della Delizia, PN, Italy) and they were collected at the end of alcoholic fermentation of a white table wine, by sedimentation and racking; the percentage of solids (determined by centrifugation) was 80% (w/v). The inactive dry yeast preparation (YD) used for the trials was a thermally produced yeast autolysate, prepared as reported elsewhere (Comuzzo et al., 2012).

2.3. Evaluation of radical scavenging activity

2.3.1. Sample preparation

Trials were performed in model solution and wine. The former was a model buffer prepared by dissolving 5 g/L (33 mM) of tartaric acid in a distiled water – ethanol mixture (12% v/v); the pH was set at 3.20 by adding 4 M sodium hydroxide. Ascorbic acid (50 mg/L), glutathione (50 and 500 mg/L), potassium metabisulphite (100 and 1000 mg/L, corresponding respectively to 50 and 500 mg/L of sulphur dioxide), yeast lees (2.5% v/v) and the YD preparation (2.5% w/v) were added and the samples were immediately analysed by DPPH· assay, as reported below.

The wine was a white table wine from harvest 2010 (free sulphur dioxide 15 mg/L, alcoholic strength 12.00% v/v, pH 3.32), supplied by Viticoltori Friulani "La Delizia" (Casarsa della Delizia, PN, Italy); additives, lees and YD preparation were added in the same amounts reported above for wine-like solution; in addition a Control sample (untreated wine) was also included in the experimental design. Control wine and treated samples were subjected to DPPH· assay as reported below. All the experiments were carried out in three repetitions, for both wines and model solutions.

2.3.2. DPPH· assay

DPPH· assay was performed by a modification of the methods reported by Brand-Williams, Cuvelier, and Berset (1995) and Gallardo-Chacón et al. (2010), using a UV–vis spectrophotometer (model V-530, Jasco Co. Ltd., Tokyo, Japan). A 6×10^{-5} M DPPH· solution was prepared fresh daily, in a 60:40 mixture of methanol: acetate buffer (0.1 M sodium acetate, buffered at pH 4.50 with 6 M hydrochloric acid). 3 mL of this stock solution were introduced in a 10 mm optical path length glass cuvette (Hellma Analytics, Mülheim, Germany) and 100 μL of the wine samples or fresh prepared antioxidant model solutions were added; DPPH· discolouration was followed at 515 nm during 10 min, reading the absorbance against methanol: acetate buffer. Results were expressed as the percent diminution of the original absorbance [ΔAbs 515 nm (%)].

For the samples treated with yeast lees and YD preparation, where insoluble particles were present, the reaction with DPPH· has been carried out as suggested by Gallardo-Chacón et al. (2010): 3 mL of DPPH· and 100 μL of sample were introduced in a test tube; after 10 min, the reaction mixture was filtered on a 0.80 μm nylon membrane and immediately subjected to spectrophotometric measurement. The initial value of the absorbance was read by adding 100 μL of methanol: acetate buffer, to 3 mL of DPPH· stock solution.

Concerning model solutions, for taking into account the effect of the solvent, a blank was also prepared, performing the DPPH· assay on the model buffer alone (tartaric acid in hydroalcoholic solution 12% v/v, pH 3.20); the percent values measured for the ΔAbs 515 nm were used to correct the analytical results.

2.4. Oxygen consumption capacity

2.4.1. Equipment

The system used for oxygen measurements was an OxySense® fluorimeter (OxySense Inc., Dallas, TX, USA); O2xyDot® oxygen sensitive sensors (OxySense Inc., Dallas, TX, USA), were glued, by a specific silicon based oxygen permeable adhesive (OxySense Inc.), to the inner surface of each of the 750 mL colourless glass bottles used for the experiments. When O2xyDot® sensors are illuminated by a pulsed blue light, they emits a red fluorescent light, that is monitored by OxySense® fluorimeter. Dynamic quenching by oxygen molecules determines a decrease of the O2xyDot® fluorescence lifetime, that is proportional to the oxygen concentration in the bottles; the temperature is measured simultaneously, by an infrared sensor positioned in the reader pen (Li et al., 2008).

2.4.2. Sample preparation

Two different white wines were used in two different sets of experiments. In the first one, a base wine for Prosecco D.O.C.G. Conegliano Valdobbiadene (harvest 2012), produced from the