Food Chemistry 197 (2016) 1102–1111

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Grapevine-shoot stilbene extract as a preservative in red wine

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A R T I C L E I N F O

Article history:
Received 4 September 2015
Received in revised form 18 November 2015
Accepted 19 November 2015
Available online 26 November 2015

Keywords:
Stilbene
Sulfur dioxide
Color
Volatile compounds
Sensorial
Olfactometry

A B S T R A C T

This paper reports the use of a grapevine-shoot stilbene extract (Vineatrol®) as a preservative in red wine. Its effectiveness to preserve red wine quality under two different winemaking systems (traditional and Ganimede) was studied at bottling and after twelve months of storage in bottle. Enological parameters, color related parameters, volatile composition, sensory analysis and olfactometric profile were evaluated. At bottling wines treated with Vineatrol showed higher color related parameters and higher score in sensory analysis than those treated with SO_2. The use of SO_2 increased ester and alcohol volatile compounds in relation to the use of Vineatrol. Wine olfactometric profile was modified by Vineatrol addition. Two new odorant zones with high modified frequency appeared in wines treated with Vineatrol. After 12 months of storage in bottle, wines treated with Vineatrol showed parameters related to oxidation. The weak point of the process seemed to be the evolution during the storage in bottle.

1. Introduction

The most widely preservative used in food industry is sulfur dioxide (SO_2). This fact is extensible to wine industry. SO_2 is probably one of the most versatile and efficient additives used in winemaking due to its antiseptic and antioxidant properties. It exhibits an important antioxidant function that helps to reduce the effects of dissolved oxygen as well as to inhibit oxidase enzymes, which are endogenous in grape and also come from fungal infections. Moreover, SO_2 inhibits the development of all types of microorganisms, such as yeasts, lactic bacteria and, to a lesser extent, acetic bacteria. However, several human health risks, including dermatitis, urticaria, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated to SO_2 (reviewed in Guerrero & Cantos-Villar, 2015). Since SO_2 is widely used as preservative in many food products, and is accumulative in the organisms, its reduction in wine is a consumer's demand. Increasingly, consumers have been clamoring for natural, organic alternatives as opposed to the chemical preservatives present in wine (Comuzzo, Rauhut, Werner, Lagazio, & Zironi, 2013). In fact, there are negative perceptions of sulfites and willingness to pay for non-sulfited wines (Costanigro, Appleby, & Menke, 2014).

Consequently, there is a great interest in finding alternative technologies as well as other preservatives that can replace and/or reduce SO_2 content in wines. Physical methods, also called green technologies, include pulsed electric field, ultrasounds, ultraviolet light and high hydrostatic pressure (Santos, Nunes, Saraiva, & Coimbra, 2012). In spite of promising results, these technologies require complex and expensive equipments. Some chemicals have been also tested as an alternative to SO_2: colloidal silver complex (García-Ruiz et al., 2015; Izquierdo-Cañas, García-Romero, Huertas-Nebreda, & Gómez-Alonso, 2012), dimethyl carbamate (Costa, Barata, Malfeito-Ferreira, & Loureiro, 2008), and even natural products (lysozyme and bacteriocins) (Lasanta, Roldán, Caro, Pérez, & Palacios, 2010; Liburdi, Benucci, & Esti, 2014). Among them, the use of phenolics has been proposed as an alternative. Sonni et al. studied the effects on volatile composition of white wines by the substitution of SO_2 during fermentation with lysozyme and tannin (Sonni, Cejudo Bastante, Chinnici, Natali, & Riponi, 2009). They concluded that both volatile composition and sensory impact were importantly modified. Salaha, Kallithraka, Marmaras, Koussissi, and Tzourou (2008) tested a black radish extract and ascorbic acid as alternative to SO_2 in red winemaking. Enological parameters and anthocyanin content were strongly

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http://dx.doi.org/10.1016/j.foodchem.2015.11.102
0308-8146/

affected, showing wines commercially acceptable. González-Rompinelli et al. (2013) assayed almond skin and eucalyptus leave extracts as preservatives during Verdejo wines aging in barrels. Aromatic composition and phenolic compounds were also modified, but no significant differences were found in the global sensory score of the wines. We have recently tested the effectiveness of hydroxytyrosol as a preservative in red wine (Raposo et al., 2016). However, hydroxytyrosol was not able to avoid oxidation during the storage in bottle.

Vineatrol® is an extract from grapevine-shoot that is particularly rich in stilbenes, mainly resveratrol and its oligomers. It has been described as natural source of bioactive stilbenes and a potent antioxidant (Müller et al., 2009; Romain et al., 2012).

The preservative capacity of stilbene extracts has been recently evaluated (Ruiz-Moreno et al., 2015). The antioxidant activity, antimicrobial activity and olfactometric profile of a stilbene stem extract in a model wine were evaluated. It was concluded that the stilbene extract showed good properties to replace SO_2 but it should be tested in real wine.

In the current work, the potential use of Vineatrol, a shoot stilbene extract, to replace the SO_2 in red wine was investigated. Vineatrol was tested under two different winemaking systems: traditional and Ganimede. Enological quality parameters, color related parameters, volatile composition, olfactometric profile and sensory wine properties were evaluated, at bottling and after storage in bottle.

2. Materials and methods

2.1. Chemicals

Analytical grade methanol and formic acid were supplied by Panreac (Barcelona, Spain). Chemical standards: resveratrol, piceatannol, dichloromethane (LiChrosolv quality), aroma standards and alkane solution (C7-C40) used for identification were purchased from Sigma–Aldrich (Steinheim, Germany). Anhydrous sodium sulfate was obtained from Panreac (Barcelona, Spain). ε-Viniferin, ampelopsin A, r-viniferin, r2-viniferin, hopeaphenol, isohopeaphenol, pallidol, miyabenol C and ω-viniferin were kindly provided by the GESVAB (Groupe D'Étude des Substances Végétales à Activité Biologique) from University of Bordeaux II. Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

2.2. Grapevine-shoot extract

Vineatrol® is a grapevine-shoot extract. Due to Vineatrol has low solubility in aqueous media, the extract was dissolved in a mix composed by: wine matrix (12% ethanol, pH = 3.6) and wine alcohol (96%), at 50:50 proportions. Samples (20 μL) of the above solution were analyzed by using a Waters HPLC system with a model 1525 pump and a Waters 996 Photodiode Array Detector. Separations were performed on a Mediterranea Sea18 column (Tecknokroma, Barcelona, Spain) (RP-18, 25 × 0.46 cm; 5 μm particle size) and a guard column of the same material, at 30 °C. The mobile phases consisted of a water:methanol:acetic acid mixture, solvent A 88:10:2 and solvent B 8:90:2 at a flow rate of 1 mL min^{-1}. Vineatrol contained: 5.66% trans-resveratrol, 13.25% ε-viniferin, 3.76% ampelopsin A, 1.44% r-viniferin, 1.22% hopheaphenol, 1.04% pallidol, 1.07% ω-viniferin, 0.97% piceatannol, 0.78% isohopeaphenol and 0.30% r2-viniferin. The total stilbene Vineatrol richness was 29%.

Vineatrol was kindly supplied by Actichem (Montauban, France).

2.3. Winemaking

The study was designed to evaluate the capacity of Vineatrol as alternative to SO_2 under different winemaking conditions (traditional and Ganimede). A complete diagram of the processes is shown in Fig. 1. Syrah grapes (560 kg) were harvested, destemmed, crushed and divided in two batches. The first batch (150 kg) was divided again in two batches (25 kg each, in triplicate): one batch was treated with SO_2 (CT), and the other batch was treated with Vineatrol dissolved as previously commented in Section 2.2 (VIN). These batches followed traditional winemaking (Fig. 1). In parallel, another winemaking system was conducted. 360 kg of crushed grapes were placed into a Ganimede fermenter (Ganimede®). The design of this type of fermenter permits the CO_2 to accumulate. Ganimede system was selected because it generates a reductive environment inside the tank, which preserves the must from oxidation. In this winemaking system, press was conducted after alcoholic fermentation, liquid wine was replaced in the container and divided in batches when malolactic fermentation was finished, in the first batch SO_2 was added (G-CT), and in the second one Vineatrol was added (G-VIN), each one in triplicate.

The concentrations of the preservatives in both winemaking systems were: 50 mg/L of SO_2 (Sulfosol, Sepsa-Enartis) and 86 mg/L of VIN (Vineatrol®), which meant 25 mg/L of total stilbenes (29% of richness of stilbenes in Vineatrol). Alcoholic fermentations (AF) were started after yeast addition (20 g/hL, ES488, Sepsa-Enartis, Spain). Malolactic fermentation was induced with *Oenococcus oeni* (1 g/hL, Challenge Easy ML, Sepsa-Enartis, Spain) and nutrients (20 g/hL Nutriferm ML, Sepsa-Enartis, Spain). Stabilization was performed during two months at 0 °C. Finally wines were racked, filtered (Optical XL, Millipore, France), antioxidants adjusted to the initial concentration (50 mg/L de SO_2 and 86 mg/L of VIN), and bottled. Stilbene concentration was follow in wines as previously described in the Section 2.2. Bottled wines were stored under control conditions (16 °C and 80% RH) during 12 months. Wine sampling was carried out at bottling and after 12 months of storage in bottle.

Since these two winemaking systems have already been studied (Garde-Cerdán, Jarauta, Salinas, & Ancín-Azpilicueta, 2008), the aim of the present study was to evaluate the capacity of Vineatrol as alternative to SO_2 under two different winemaking conditions rather than the evaluation of these two winemaking themselves.

2.4. Enological parameters

Relative density, ethanol, glycerin, dry extract, total and volatile acidity, pH, organic acids (acetic, citric, tartaric, malic, lactic, and succinic acids), total and free SO_2, acetaldehyde, ethyl acetate and methanol were determined at bottling following the official analytical methods established by the International Organization of Vine and Wine (OIV, 2014).

Anthocyanin, tannin, total polyphenols index (TPI), were measured following the method described by Saint-Cricq de Gaulejac, Vivas, and Glories (1998). Stilbenes were measured as previously described in the Section 2.2.

2.5. Color related parameters

Color intensity (D.O. 420 nm + D.O. 520 nm + D.O. 620 nm) and hue (D.O. 420 nm/D.O 520 nm) were determined by spectrophotometric measurements (Lambda 25, Perkin-Elmer, Massachusetts). Colorimetric measurements were registered with a Konica-Minolta CM-3600d spectrophotometer (Osaka, Japan), using 2 mm path-length glass-cells and distilled water as reference. The CIELab parameters (L^*, a^*, b^*, C^*_{ab}, h^*_{ab}) were determined by using