Monitoring the process to obtain red wine enriched in resveratrol and piceatannol without quality loss

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ABSTRACT

Stilbene-enriched wine is considered an interesting new food product with added value as a consequence of the numerous health-promoting properties ascribed to it, mainly from its trans-resveratrol content. Postharvest grapes have been treated with ultraviolet-C light to produce stilbene-enriched grapes that were later used in a conventional winemaking process to obtain a red wine enriched in stilbenes.

By measuring oenological parameters and stilbene concentration it has been possible to monitor both the quality parameters and stilbenes throughout the process. The maximum concentration in trans-resveratrol and piceatannol was obtained after pressing, but there was significant loss from grape to wine. A significant increase in both piceatannol and trans-resveratrol concentration (up to 26 times and 3.2 times higher than in control, respectively) was achieved in bottled wine. Regarding the oenological parameters, the wines obtained possessed good quality, apart from a herbaceous aroma, which could not be identified by GC–olfactometry.

1. Introduction

Numerous epidemiological studies have shown that long-term, moderate consumption of wine is linked to a lower level of cardiovascular illnesses. In 1992 a study conducted by Renaud and De Lorgeril revealed that the incidence of heart infarction in France is about 40% lower than in the rest of Europe; this was termed the "French paradox", which appeared to be related to regular consumption of red wine (Renaud & De Lorgeril, 1992). Numerous other beneficial qualities, with positive effects on health, have been attributed to wine, including anti-oxidant, anti-carcinogenic and anti-spasmodic properties, enhancement or activation of bile secretion, antibacterial and antihistaminic agents (Pignatelli et al., 2006). The findings that red wine possesses more health-promoting activity than beer or spirits have led to research attention being focused on phenolic compounds; within this group, stilbenes (in particular, trans-resveratrol) seem to show high bioactivity.

In the last few years, interest in resveratrol has increased greatly, due to its numerous health-promoting properties (according to the ISI Web of Knowledge, there have been 4768 bibliographic entries in the last 10 years). Resveratrol has been described as a compound capable of preventing or reducing a wide range of diseases, such as cancer (Jang et al., 1997), cardiovascular diseases, and ischaemic damage (Bertelli, 2007); it can also increase the resistance to stress and prolong the lifespan of diverse organisms, from yeast to vertebrates (Guerrero, García-Parrilla, Puertas, & Cantos-Villar, 2009). Studies in mice have shown that obese animals, whose diet was supplemented with resveratrol, not only lived longer, but were more active and produced fewer cases of the negative effects of a high-calorie diet (Baur et al., 2006). Regarding bioavailability, numerous studies in animals and humans have shown that resveratrol is metabolised with relative difficulty. However, a relatively low dose of resveratrol obtained regularly from red wine or other dietary sources could be therapeutic in some cases (Bertelli, Bertelli, Gozzini, & Giovannini, 1998).

Apart from resveratrol, the presence in wine of other stilbenes, such as piceid, astringin, piceatannol and viniferins, has been described. Piceatannol is rarely found in wines and its presence in wines is very interesting since it is bioactive and has a long plasma half-life. It exhibits a pronounced anti-oxidant activity and exerts immunosuppressive, anti-leukaemic, and anti-tumorigenic activities in various cell lines and animal models (Murias et al., 2005). It has also been demonstrated that viniferins present anti-inflammatory and anti-proliferative activities (Kitanaka et al., 1990). All these stilbenes show high bioactivity but they are present in concentrations even lower than that of resveratrol, and less research has been done on them.

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Dietary sources of resveratrol are rather limited, grapes and their derivatives being the main source (Guerrero et al., 2009). Resveratrol is found in the seed and skin of grapes (not in the flesh) and, hence, in grape juice and wine. Its concentration in red wine is higher than in white one because in red winemaking the must, grape skin and seed are in contact during the whole fermentation process. The amount of resveratrol in wines varies widely depending on many factors: grape variety, geographic region, agronomic factors, climate, plant stress conditions and oenological practices all have an influence (Bavaresco, 2003). Regarding optimum oenological practices for increasing resveratrol content, all the processes that maximize the extraction of phenols from skin are recommended (Vrhovsek, Wendelin, & Eder, 1997). It is difficult to predict the amount of resveratrol in wines because of many factors affecting resveratrol biosynthesis.

Different strategies can be undertaken to increase the concentration of stilbenes in grape, since these compounds are phytoalexins and, therefore, can be induced *via* different kinds of stress. In particular, postharvest treatment of grapes by ultraviolet-C light (UVC) has been suggested as suitable stress-promoting technology to obtain stilbene-enriched wine under laboratory conditions (Cantos, Espín, & Tomás-Barberán, 2003); however, the validity of the process needs to be confirmed in a standard-scale experimental winery as a prior step to industrial-scale production. Stilbene-enriched wines potentially offer added value compared to traditional wines (Barreiro, Colombo, & Cantos, 2008).

In this study, the winemaking process has been monitored both by oenological parameters and by content in stilbene compounds, to obtain a stilbene-enriched wine of standard quality, using grapes enriched by postharvest UVC treatment. In addition, the organoleptic characteristics of the resulting wine were also investigated using a tasting panel and GC–olfactometry.

2. Materials and Methods

2.1. Reagents

trans-Resveratrol and piceatannol were purchased from Sigma–Aldrich (Madrid, Spain). The *trans* isomers were transformed to *cis* forms under UV light. Acetic acid, dichloromethane, formic acid and methanol, all of analytical grade, were supplied by Panreac (Barcelona, Spain). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

2.2. Grapevine

The local red grape variety Jaen tinto (*Vitis vinifera)* was grown in Jerez de la Frontera (IFAPA, Rancho de la Merced). Ripeness of the grape (sugars, acids, pH and berry weight) was monitored to determine the optimum harvest date (data not shown).

2.3. UVC treatment and storage period

The UVC was carried out according to the procedure described by the patent WO/2002/085137; ES 2177465. Briefly, grapes were irradiated inside a system of 34 UV-C lamps (G30T8; Silvania, Danvers, MA) – 17 each on top and bottom panels – with a theoretical power of 510 W and an average flow velocity of 14.72 mW/cm^2 (Vilber Lourmat VLX 254 radiometer; Vilber Lourmat, Mame-la-Valle, France) for 60 s at 42 cm. The parameter "maximum day" (*Dm*) was defined as the number of days elapsed after UVC treatment to achieve the maximum *trans*-resveratrol concentration in grapes. Harvested grapes were divided into three batches (Fig. 1): the first batch (named CT–CT) was processed immediately after harvesting, starting winemaking without any prior treatment or

storage of grapes. The second batch (CT) was stored at 20 °C (without irradiation) after harvesting until *Dm* was reached. The third batch (UV) was irradiated with UVC after harvesting and stored at 20 °C until *Dm*. The temperature of 20 °C was found to be the optimum for obtaining a balance between the synthesis of stilbenes and the deterioration of grape quality (unpublished data). Each batch was processed in triplicate.

2.4. Red winemaking process

The grapes were de-stemmed, crushed and placed in a 50-l steel vessel (the CT–CT batch immediately after harvesting and the other two batches after being stored for 3 days) Pectolytic enzymes (3 g/100 kg, Vinozym Vintage FCE, Novozymes, Bordeauz, France) and sulfur dioxide (70 mg/kg) were added to maximize extraction and to protect the must. One day later, fermentation was started after yeasting (Actiflore F5, Laffort, Spain), and temperature was maintained at 27 ± 1 °C during alcoholic fermentation (AF). As soon as the tumultuous fermentation had finished (density 999 g/l), the wine was vatted: the free-run wine was decanted and the solid parts were placed in a pneumatic press (Willmes, Germany) to obtain the press-run wine, which was mixed with the free-run wine to form the press wine. For the malolactic fermentation, lactic bacteria *Oenococcus lacti* (1 g/hl, Challenge Easy ML, Sepsa-Enartis, Spain) and nutrients (20 g/hl Nutriferm ML, Sepsa-Enartis, Spain) were used. When this stage of the process was finished, the wine was decanted into another vessel, eliminating the first lees (racking), and wines were rendered inert (using N$_2$) and stored in a cold chamber (at 0 °C) until clarification (using 10 g/hl egg white albumin, Laffort, Bordeaux, France). Finally, the wine of each batch was bottled. A complete diagram of the process is shown in Fig. 1. During each step of the process, both the oenological parameters and the content in stilbenes were determined. In addition, samples were taken from both the skins (solid phase) and the must-wine (liquid phase) for analysis of the stilbene content on each day of the AF.

2.5. Stilbene extraction

Stilbenes were extracted from solid samples (grape skin and pomace) according to the procedure previously described by authors Guerrero, Puertas, Fernández, Palma, and Cantos (2010). Regarding liquid samples (must, lees and wines) stilbene extraction was performed following other authors (Bavaresco, Pezzutto, Ragga, Ferrari, & Trevisan, 2001). All extractions were conducted in triplicate, in the dark and at low temperature to avoid the occurrence of oxidation and isomerisation processes.

2.6. UPLC and HPLC analysis

Stilbenes were identified using an ACQUITY Ultra Performance LC^{TM} system and were quantified by HPLC-DAD. The methods followed have recently been described in detail by Guerrero et al. (2010). Briefly, stilbenes were identified using an ACQUITY Ultra Performance LC^{TM} system (Waters, Milford, MA) linked simultaneously to both a PDA 2996 diode array detector (Waters) and an ACQUITY triple quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK), equipped with an electrospray ionisation (ESI) source operating in negative mode. MassLynx^{TM} software (Version 4.0, Waters) was used to control the instruments, and for data acquisition and processing. In order to quantify stilbenes, a Waters HPLC system with a Model 1525 pump, a Waters 996 diode array detector and a Waters 474 fluorescence detector was used.