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Extraction kinetics of anthocyanins from skin to pulp during carbonic maceration of winegrape berries with different ripeness levels

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ABSTRACT

The evolution of the content and profile of anthocyanins was studied in the skin and pulp of Gamay winegrapes during twelve days of carbonic maceration. The ripening effect was also investigated using berries belonging to two density classes (A = 1094–1100 kg/m^3 and B = 1107–1115 kg/m^3). The ripest berries showed a higher extraction yield, even though the differences among density classes tended to decline towards the end of the process, and few significant differences were found in the anthocyanin profile. The maceration time influenced strongly not only the content and extraction yield, but also the qualitative composition of anthocyanins towards the predominance of malvidin derivatives. Finally, the extraction yield of anthocyanins was positively related with the ethanol formed and the skin mechanical properties using linear regression models, which showed that the skin hardness is likely to be an important variable in modelling daily anthocyanin extraction during carbonic maceration, particularly from the sixth day.

1. Introduction

Carbonic maceration (CM) is a winemaking process exploiting the adaptability of intact grape berries to a medium saturated with carbon dioxide (Tesniere & Flanzy, 2011). Under this anaerobic atmosphere, a series of transformations inside the whole berry are induced by the activity of endogenous enzymes present in the grapes. During the CM process, the berries undergo intracellular fermentation without yeast intervention causing the transformation of a small amount of sugars into alcohol (1.5–2% v/v alcohol), the reduction of the malic acid content, and the extraction and formation of secondary metabolites, in particular phenolic and volatile compounds (Ducruet, 1984; Gómez-Míguez & Heredia, 2004; Spranger et al., 2004; Tesniere & Flanzy, 2011).

In practice, in winery, the intact grape clusters are put into a closed tank and kept under CO_2 atmosphere for seven or more days depending on temperature, grape ripeness and cultivar (Chinnici, Sonni, Natali, Galassi, & Riponi, 2009). Inside the tank, the grapes respiring also consume oxygen and the anaerobic metabolism occurs whenever the oxygen concentration is low in a gaseous or liquid environment, however the intensity of the phenomena decreases in liquid environment (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2000). For this reason, some importance was attributed to the physico-mechanical properties of the berries to resist the compression in carbonic maceration vinification (Giacosa et al., 2013). After CM, the grapes are pressed and the juice completes alcoholic fermentation without skin maceration (Tesniere & Flanzy, 2011). Therefore, this type of winemaking technology can significantly affect the phenolic composition of the wine. Many studies reported in the scientific literature showed the differences in the phenolic profile of CM wines with respect to other conventional young wines produced using different vinification techniques (Spranger et al., 2004; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001). A low content of tannins is generally typical of the 'young' wines produced by CM (Pellegrini et al., 2000; Sacchi, Bisson, & Adams, 2005). However, a correct extraction of anthocyanins from the skin into the pulp should be guaranteed during CM in order to assure positive chromatic characteristics because these are strongly related to consumer preference of these wines (Parpinello, Versari, Chinnici, & Galassi, 2009). Specific studies showed that CM wines are characterized by high contents of B-type vitisins and ethyl-bridged anthocyanin-flavanol adducts resulting from the high concentration of acetaldehyde produced by anaerobic metabolism (Chinnici et al., 2009), however low contents of total and monomeric anthocyanins were observed (Castillo-Sánchez, Mejuto, Garrido, & García-Falcón, 2006; Gómez-Míguez & Heredia, 2004).

The content and profile of anthocyanins in winegrapes are of considerable importance in evaluating their oenological potentiality

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because these compounds are directly associated with the wine colour. The extraction of anthocyanins from the grape into the wine depends on the tendency of the berry skin to yield them (González-Neves et al., 2004). Their extractability generally increases throughout grape ripening as a consequence of the degradation of the skin cell wall by pectolytic enzymes, and the changes in the polysaccharide composition, cellulose content and degree of methylation of pectins (Hernández-Hierro et al., 2014; Ortega-Regules, Ros-García, Bautista-Ortín, López-Roca, & Gómez-Plaza, 2008; Ribéreau-Gayon et al., 2000). However, while the anthocyanin extraction into the must-wine during a classical maceration process was extensively investigated (Canals, Llaudy, Valls, Canals, & Zamora, 2005; González-Neves, Gil, & Barreiro, 2008; Rolle, Torchio, Ferrandino, & Guidoni, 2012; Romero-Cascales, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2005), to our knowledge very scarce data were reported in the scientific literature about the release of anthocyanins from the berry skin to the pulp during CM.

Therefore, in this work, we investigated the evolution of anthocyanins in the skin and pulp of berries undergoing CM with the aims of: (i) determining the extraction kinetics of these compounds during the process; (ii) evaluating the impact of two different levels of berry maturity on the content and composition of anthocyanins released; and (iii) relating the anthocyanin extraction with the physico-mechanical properties of the berry skin.

2. Material and methods

2.1. Grapes

Vitis vinifera L. cv. Gamay grapes were collected on September 30th, 2012 at technological maturity from a vineyard (45°7′28.29″N 6°58′59.51″E) located at Chiomonte (TO) in the Susa Valley (Piedmont, northwest Italy). A 10-kg set of Gamay grape berries were randomly picked in the vineyard with attached pedicels from different parts of the bunch, transported into boxes to the laboratory and immediately processed. The study was carried out separately on the berries belonging to two different ripening stages (i.e. density classes; Singleton, Ough, & Nelson, 1966) that were: A = 1094–1100 kg/m^3 and B = 1107–1115 kg/m^3. Density separation was carried out by flotation in different saline solutions (from 100 to 190 g/l sodium chloride) as described by Rolle et al. (2011) in order to obtain a homogeneous sample of berries. The floating berries were washed with water, visually inspected before the CM process and those with damaged skins were discarded.

2.2. Carbonic maceration process

Three groups of 120 intact whole berries for each ripening stage were placed into a 500 ml glass jar (Bormioli S.p.A., Fidenza, Italy), which was previously saturated with food grade carbon dioxide (Rivoira S.p.A., Milano, Italy) and hermetically closed. The jars were stored in a chamber at controlled temperature of 28 °C for 12 days. Approximately every 12 h, the jars were saturated with carbon dioxide. The CM process was chemically monitored by sampling at 0 (fresh grape), 3, 6, 9 and 12 days.

2.3. Chemical analysis

2.3.1. Reagents and standards

Solvents (of HPLC-gradient grade) and all other chemicals (of analytical-reagent grade) were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Anthocyanin standards (delphinidin-3-O-glucoside chloride, malvidin-3-O-glucoside chloride, petunidin chloride, peonidin-3-O-glucoside chloride and cyanidin-3-O-glucoside chloride) were supplied from Extrasynthèse (Genay, France).

2.3.2. Anthocyanin extraction and determination

Ten berries for both trial replicates and sampling point were weighed before anthocyanin extraction. The berry skins, removed manually from the pulp, were quickly immersed in 25 ml of a buffer solution containing 12% v/v ethanol, 600 mg/l $Na_2S_2O_5$, 5 g/l tartaric acid and adjusted to pH 3.20 by the addition of 1 M NaOH (Torchio, Cagnasso, Gerbi, & Rolle, 2010). Afterwards, the skins were homogenized at 8000 rpm for 1 min with an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) and centrifuged for 15 min at 3000×*g* at 20 °C. The pulp was collected in a beaker containing $Na_2S_2O_5$ (100 mg) and subsequently diluted (9:1, m/m) with 5 mol/l sulphuric acid (Di Stefano & Cravero, 1991). Afterwards, the pulp was homogenized at 9500 rpm for 30 s with an Ultraturrax T10 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) and centrifuged in a PK 131 centrifuge (ALC International, MI, Italy) for 15 min at 3000×*g* at 20 °C. The supernatant was then used for analysis.

The content of total anthocyanins in skin and pulp samples was determined by a spectrophotometric method using a UV-1601PC spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA) (Di Stefano & Cravero, 1991; Torchio et al., 2010). The total anthocyanins index (TAI) was expressed as malvidin-3-glucoside chloride. The relative standard deviation of TAI, based on repeated analyses (n = 20) of ten sample extracts, was 1.14% (Torchio et al., 2010).

The determination of individual anthocyanins was performed after concentration of the berry skins or pulp extract using a SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) with methanol as eluent. The chromatographic system employed was a P100 pump equipped with an AS3000 autosampler (Spectra Physics Analytical, Inc., San Jose, CA, USA), a 20 µl Reodyne sample loop, a LiChroCART column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany) and packed with LiChrospher 100 RP-18 (5 µm) particles supplied by Alltech (Deerfield, IL, USA), and a Spectra Focus Diode Array Detector (DAD, Spectra Physics Analytical, Inc., San Jose, CA, USA) operating at 520 nm. The mobile phases consisted of formic acid/water (10:90, v/v) (mobile phase A) and a mixture of formic acid/methanol/water (10:50:40, v/v) (mobile phase B). The two mobile phases were filtered through a 0.20 µm PTFE membrane filter (Whatman International Ltd., Maidstone, Kent, UK). The mobile phase flow-rate was 1 ml/min. A linear gradient was used for the separation of anthocyanins starting at 72% A, and decreasing to 55% A in 15 min, to 30% A in 20 min, to 10% A in 10 min and to 1% A in 5 min, and then back to 72% A in 3 min. The column was then equilibrated for 10 min prior to each analysis. The data treatment was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA, USA). The identification of the free forms of anthocyanins was achieved by comparing their retention times with those of pure standards. The acylated forms of anthocyanins were identified by matching the DAD spectrum and retention time of each chromatographic peak, and by comparing these data with those available in the literature (Pomar, Novo, & Masa, 2005; Rolle & Guidoni, 2007). Individual anthocyanins were determined by comparing the area of the appropriate peak with the total peak area and were expressed in percentages. All of the analyses were performed in duplicate and then averaged.

2.3.3. Technological parameters of pulp

Ten other berries, for both trial replicates and sampling point, were manually crushed and the must obtained was centrifuged for 10 min at 3000×*g* and analyzed. Organic acids, reducing sugars