Food Chemistry 206 (2016) 239–248

Contents lists available at ScienceDirect

Food Chemistry

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

Anthocyanins influence tannin–cell wall interactions

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ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 13 March 2016
Accepted 14 March 2016
Available online 15 March 2016

Keywords:
Anthocyanins
Proanthocyanidins
Polysaccharides
Adsorption
Extractability
Phloroglucinolysis
Size exclusion chromatography

ABSTRACT

The rate of tannin extraction was studied in a vinification of red grapes and the results compared with another vinification made with white grapes fermented as for typical red wine, in the presence of skins and seeds. Even though the grapes presented a quite similar skin and seed tannin content, the differences in tannin concentration between both vinifications was very large, despite the fact that the only apparent difference between the phenolic composition of both wines was the anthocyanin content. This suggests that anthocyanins play an important role in tannin extractability, perhaps because they affect the extent of the tannin–cell wall interaction, a factor that largely controls the resulting quantity of tannins in wines. To confirm this observation, the effect of anthocyanins on the tannin extractability from grape seeds and skin and on the interaction between tannins and grape cell walls suspended in model solutions were studied. The results indicated that anthocyanins favored skin and seed tannin extraction and that there is a competition for the adsorption sites between anthocyanins and tannins that increases the tannin content when anthocyanins are present.

1. Introduction

Anthocyanins and tannins are the compounds most directly responsible for the color and mouthfeel of red wine. Although tannins are not red in color, they participate in the stabilization and enhancement of wine color through the copigmentation process involving a physicochemical association between an anthocyanin and colorless copigment without the formation of a covalent bond (Lambert, Asenstorfer, Williamson, Iland, & Jones, 2011) and the formation of new compounds such as those formed by direct link of anthocyanins and proanthocyanidins, ethyl-linked anthocya nin–proanthocyanidin adducts and flavanyl pyranoanthocyanins (He et al., 2012).

Anthocyanins and tannins are located in the grape skin while the seeds only content tannins. In the skins, the phenolic compounds are located mainly in the vacuoles, inside the cells, although tannins can be also found bound to the cell wall (Amrani Joutei & Glories, 1995; Geny, Saucier, Bracco, Daviaud, & Glories, 2003). During the process of maceration, anthocyanins and tannins from skins and seeds diffuse into the wine (Busse-Valverde et al., 2011).

Because of the strong influence of phenolic compounds on red wine quality, many studies have been made to determine how exactly they are extracted. It seems that during the maceration process the cell walls need to be broken to allow their vacuole contents to be extracted, and so the content of these phenolic compounds in red wines will depend on the amount of these pigments in the skin of grapes at harvest time, on the ease of their extraction and on the winemaking techniques used (Busse-Valverde et al., 2010).

It has been demonstrated that knowing the quantities of anthocyanins in grape skins is not sufficient for estimating wine anthocyanin concentrations and this lack of correlation has been commonly attributed to the partial retention of these anthocyanins in the skin cells due to the barrier effect of the cell walls (Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006; Rolle, Torchio, Ferrandino, & Guidoni, 2012; Rolle, Torchio, Zeppa, & Gerbaux, 2009). Similarly, previous studies have shown that the quantities of grape tannins do not correlate well with the quantity of these compounds that will be detected in the corresponding wines (Adams & Scholz, 2007; Busse-Valverde et al., 2010; Harbertson, Kennedy, & Adams, 2002), the quantities found frequently being much lower than expected (Busse-Valverde, Bautista-Ortín, Gómez-Plaza, Fernández-Fernández, & Gil-Muñoz, 2012).

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http://dx.doi.org/10.1016/j.foodchem.2016.03.045
0308-8146/

In the case of tannins, some studies have not only shown that the cell walls act as a barrier but that tannin–cell wall interactions occur during winemaking as one of the reasons for such observations. Cell walls (CW) are mainly constituted of proteins and polysaccharides that contain hydroxyl groups as well as aromatic and glycosidic oxygen atoms that have the ability to form hydrogen bonds and hydrophobic interactions with some molecules (Le Bourvellec, Guyot, & Renard, 2004; McManus et al., 1985), including some phenolic compounds. Such interactions reduce the extractability of tannins during fermentative maceration since a substantial proportion may be adsorbed by the CW in suspension in the must during vinification (Bautista-Ortin et al., 2015) and finally precipitated during settling.

One question that also arises is whether anthocyanins and tannins influence their mutual extractability. Previous studies showed that a reduction in the sources of tannins during winemaking (by eliminating the seeds) led to a wine with a proanthocyanidin concentration 40% lower than in a control wine but the concentration of anthocyanins was not affected (Bautista-Ortin, Busse-Valverde, Lopez-Roca, Gil-Muñoz, & Gómez-Plaza, 2014). However, white grapes fermented as for a typical red wine in the presence of skins and seeds resulted in wines with quite different astringent sensory profiles from those of red wines and they tend to be coarser and with lower astringency (Singleton & Trouslade, 1992). The only apparent difference between the phenolic composition of pomace-fermented white wines and red wines is the presence of anthocyanins (Singleton & Trouslade, 1992). These authors suggested that the incorporation of anthocyanins in polymeric procyanidins seems to increase the amount of tannin retained in wine, thus explaining the larger quantities of tannins found in red wines and their higher astringency.

In light of all these observations, our objective in this study is to observe the differences in wine tannin content between a wine made from white grapes of the Macabeo variety fermented like a typical red wine and a wine made from Monastrell red grapes. If these differences exist, and since the adsorption of tannins in suspended cell walls has been confirmed to be one of the most important mechanisms controlling wine tannin content, attention will be turned to how the presence of anthocyanins affects the extractability of tannins from grape seed and skins and the extent of tannin–CW interactions and whether these changes help to explain the differences between tannin concentration in wines made with red or white grapes.

2. Material and methods

2.1. Grape and vinifications

Grapes from cv. Monastrell (red grapes) and Macabeo (white grapes) grown in the same area of Jumilla (Spain) were used for the study. Monastrell represents 80% of the cultivated grapes in this area, and Macabeo, although cultivated in a much lower extension than Monastrell, is one of the white varieties most cultivated in Southeast Spain.

When the grapes reached optimum maturity, grapes from 150 selected plants were harvested and transported to the winery in 20 kg boxes. For chemical analysis of polyphenolic compounds, five to six berries from different clusters were randomly collected and frozen at −20 °C until analysis.

The grapes from Monastrell grapes (90 kg per triplicate) were crushed and destemmed and sulphited (8 g of SO_2/100 kg of grapes). Total acidity was corrected to 5.5 g/L and selected yeasts were added (Laffort, DSM, Servian, France, 10 g of dry yeast/100 kg of grapes). The vinification was conducted in triplicate, in 100 L tanks, at 25 ± 1 °C. Throughout the fermentative pomace contact period (15 days), the cap was punched down twice a day and the temperature and must density were recorded. At the end of this period, the wines were pressed at 1.5 bars in a 75 L tank membrane press. Free-run and press wines were combined and stored at 2 °C until analysis.

The Macabeo grapes (90 kg per triplicate) were also crushed and destemmed and sulphited (8 g of SO_2/100 kg of grapes). The must was then pressed in a tank membrane press and the must was treated with pectolytic enzymes for static cold racking. After 24 h, clear must was separated, total acidity was also corrected to 5.5 g/L and selected yeast were added. When these grapes were vinified in the same way as red grapes, the experimental conditions were the same as those described for Monastrell grapes, with a skin contact period of 15 days. All vinifications were conducted in triplicate, in 100 L tanks, at 25 ± 1 °C. At the end of alcoholic fermentation the wines were stored at 2 °C. Must and wine were sampled every two days until the end of the skin maceration period for the analysis of tannins.

2.2. Extraction and isolation of anthocyanins

Berries from the frozen clusters were randomly chosen and peeled while still frozen. Grape skins (75 g) of the variety Monastrell were placed in four covered Erlenmeyer and treated with 300 mL of methanol. The samples were shaken at 200 rpm in an orbital shaker at room temperature for 24 h. To minimize anthocyanins oxidation, solutions were sparged with nitrogen and the extraction was carried out in the dark. After the extraction time, the samples were centrifuged at 13,000 rpm and the supernatant was dried in a rotary evaporator under vacuum at 35 °C. For the purification of anthocyanins the methodology proposed by Castillo-Muñoz, Fernández-Gonzalez, Gómez-Alonso, García-Romero, and Hermosin-Gutierrez (2009) was used with some modifications. For this, the extracts were dissolved in 0.1 N HCl and passed through an Oasis MCX cartridge (Waters Corp., Milford, MA; cartridges of 6 cm^3 capacity filled with 500 mg of adsorbent) previously conditioned with 5 mL methanol and 5 mL of HPLC grade water. The cartridges were washed with 10 mL of 0.1 N HCl and 10 mL of water. Flavonols were eluted with 30 mL of methanol and then anthocyanins with 20 mL of 0.2% HCl solution in methanol. The anthocyanin extract was concentrated to dryness on a rotary evaporator and redissolved in 5 mL of acetone. Then, the ketonic extract was centrifuged and the supernatant was again concentrated to dryness, redissolved in 2 mL of 0.1 N HCl and passed through Sep-Pak C18 cartridges (1 g, Waters, Milford, USA) previously conditioned with 10 mL of methanol and 10 mL of water. Then the cartridges were washed with 15 mL of water and the compounds of interest were eluted with 20 mL of 0.2% HCl in methanol. The methanol extracts were concentrated and lyophilized to a dry powder.

2.3. Tannins used in the experiment

Two purified tannin fractions from ripened Monastrell grape skins and seeds were selected for the experiment as well as a seed-derived enological tannin (Agrovin S.A., Alcazar de San Juan, Spain).

For the obtention of the purified fractions, *V. vinifera* L. cv. Monastrell grape berries were used as the source material for grape skin and seed tannins. The skins and seeds from 1 kg of frozen grapes were separated from the berry mesocarp and rinsed with distilled−deionized water, and these intact tissues were extracted in covered Erlenmeyer flasks with 2:1 acetone/water at room temperature for 24 h. To minimize tannin oxidation, solutions were sparged with nitrogen and the extraction was carried out in the dark. Following extraction, the extract was concentrated