Composition and cellular localization of tannins in grape skin during growth. Comparison between white and red cultivars

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Introduction

Tannins from grape skins are essential compounds for grape and wine quality as regards to their biological and organoleptic properties.

They are precociously synthesized in the cytoplasm and then stored in the vacuole. They can also be associated with cell walls, thus ensuring the integrity and structure of the skin. Consequently, they are easily released into the must and can limit the diffusion of polyphenols and aromatic compounds.

Microscopic observations have shown that the organization of skin tannins varied according to their localization and to the stage of development. Aggregated structures are always present near the cell walls, while tannins from the vacuoles become condensed throughout growth. However, detailed data are lacking as to their cellular and distribution and organization.

In the present work, we separated the cell walls from the inner part of the skin cells from Cabernet Sauvignon and Semillon berries and focused on tannins during development, from berry set to maturity.

Materials and methods

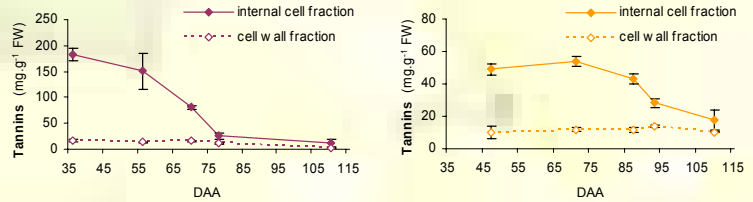
Experiments were conducted in the skins of grape berries *Vitis vinifera* L. cv. Cabernet Sauvignon and Semillon, grown in a Bordeaux vineyard in 2005. Berries were randomly collected at different stages of development: before "véraison", at two green stages (pea-sized berries and berry touch), in the middle and at the end of the color change period (50% and 100% red ripe berry, respectively) and at maturity (harvest). For Semillon, "véraison" was estimated with the decrease of chlorophyllian pigments and the loss of firmness.

Cell walls was separated from the inner cell using the procedure of Harris *et al.* (1983). Fraction purity was confirmed by light microscopy after appropriate coloration.

Tannins were extracted according to Gény *et al.* (2003) for the inner cell fraction and to Gagné *et al.* (2006) for cell walls. Total contents were then measured with the Ribéreau-Gayon and Stonestreet method (1966). After purification, tannins were submitted to thioacidolysis and HPLC analysis (Atanasova *et al.*, 2002).

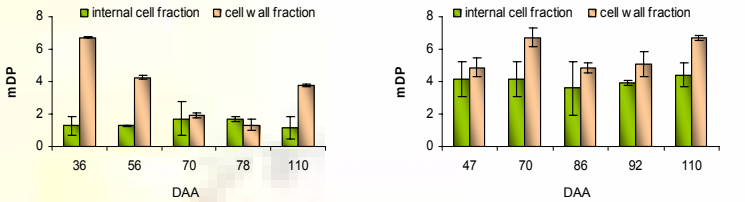
Data represent the mean ± standard deviation from triplicate samples each comprising the skins of 10 berries and are expressed in function of days after anthesis (DAA).

Accumulation and localization of tannins during ripening



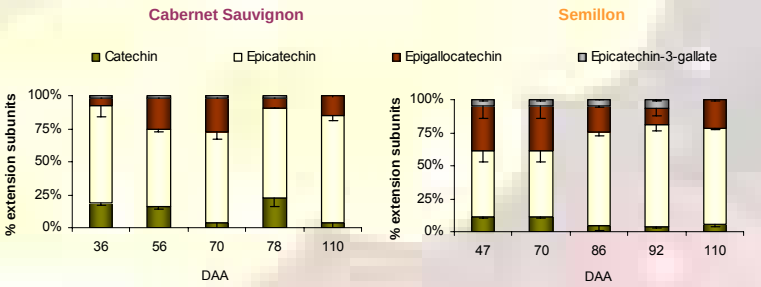
- The accumulation and localization of tannins are similar for both cultivars. As expected, contents in Cabernet Sauvignon skins are much higher than for Semillon.
- Tannin contents exhibit different evolution in the two parts of the skin cells. They are mainly localized in the inner cell at berry set and then continuoulsy decrease. Near the cell walls, tannin contents remain constant.

Mean degree of polymerization (mDP)

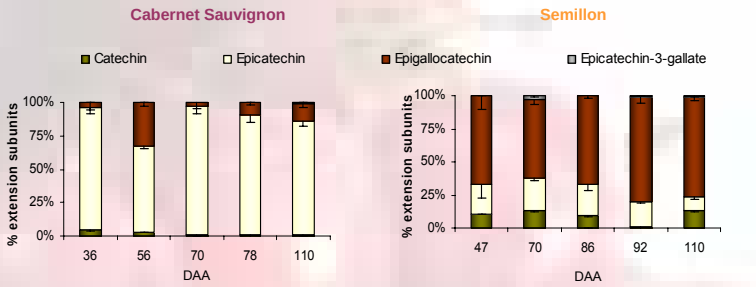


- The mean degree of polymerization (mDP) evolves differently in both cultivars when considering the cell wall fraction. In Cabernet Sauvignon skins, it is variable and significantly higher than the mDP of the inner cell tannins. In Semillon, little variation is observed and the gap between fractions is not signifiant.
- The mDP of the internal cell fraction is not modified throughout development. In Semillon skins, it is twice the value measured for Cabernet Sauvigon.

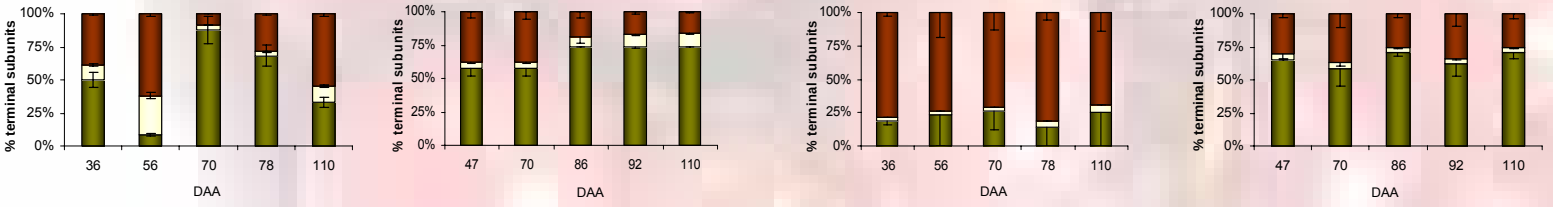
Tannin composition of the inner cell fraction during ripening



Tannin composition of the cell wall fraction during ripening



- Composition is close between the two fractions of the Cabernet Sauvignon skins, whatever the stage of development. Epicatechin is always predominant. Catechin and epigallocatechin are present in equivalent each fraction but more abundant in the inner cell.
- Both fractions of Semillon skins exhibit different composition. For the inner cell, it is identical to the same fraction of Cabernet Sauvigon. For tannins associated with cell walls, epigallocatechin is in the majority.



- For both cultivars, composition in teminal subunits is different from the composition in extension subunits. Catechin and epigallocatechin are preferentially located at the end of the polymers.
- Composition in terminal subunits is similar between fractions for Semillon skins: catechin is there predominant, while epicatechin is present in negligible amounts.
- For Cabernet Sauvignon, epigallocatechin is the most abundant flavanols in the cell wall fraction. In the inner cell, composition is variable according to the stage of development.

Both cultivars exhibit similar features. Skin tannins are mainly localized in the internal part of the cell, due to their biosynthesis and storage. They continuously decrease in contents from berry set to harvest, while contents in tannins associated with cell walls are constant.

The mean degree of polymerization and the composition are cultivar-dependent parameters. Considering mDP, Cabernet Sauvignon tannins form longer polymers than Semillon tannins, which may explain their higher reactivity with proteins. The mDP tends to be smaller in the inner cell and does not significantly vary during growth. An auto-association possibly happens in the vacuole to produce aggregated tannins with no increase of mDP. .

The composition was typical of grape skins, with significant amounts of prodelphinidins and negligible contents in epicatechin-3-gallate. It remains nearly constant throughout growth and depend essentially on the localization and the position in the polymer.

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