Characterisation of proanthocyanidins from grapes and from the heartwood of Quebracho the tailored use of tannins

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Introduction

Oenological tannins are phenolic compounds that can be used to protein stabilise wine and to facilitate fining. They can also associate with wine compounds, such as anthocyanins and polysaccharides. In this way, they play an essential role in wine colour and colloidal stability. Some tannins bind health-harming free radicals, significantly limiting wine oxidation (atypical ageing). They also react with free metals such as iron. Finally, they help to remove sulphur aromas by binding odorous sulphur-containing molecules.

To identify the principal oenological tannins with confidence, a specific approach based on the characterisation of proanthocyanidin (PA) composition was devised. Different types of polymeric PA were quantified by hydrochloric acid butanolysis (the Bate-Smith reaction). Using thioacidolysis/liquid chromatography/electrospray ionisation mass spectrometry (thioacidolysis/LC/ESI), it was possible to identify the nature of the constituent flavan-3-ols (catechin and epicatechin in procyanidin tannins; gallocatechin and epigallocatechin in prodelphinidin tannins), the degree of galloylation, and the mean degree of polymerisation (mDP). A complete structural study by MALDI-TOF mass spectrometry was also performed.

A study of grape seed tannins used in enology, by high performance liquid chromatography, allowed separation of molecules according to molecular weight. Three characteristic profiles were obtained among commercial grape seed tannins.

Knowledge of the composition of the proanthocyanidin tannins allows the winemaker to use them for specific purposes, for instance to stabilise the colour, to smooth astringency, to improve the mouthfeel due to the phenolics. Most of these effects could be obtained only by tannins from grapes; tannins from quebracho or mimosa wood cannot react with wine phenolics, they act only as additives.

The concept of a "tailored use of tannins" should be implemented from the start of alcoholic fermentation and continued during wine ageing. Taste and colour reveal the phenolic maturity of the grapes, and indicate which physicochemical treatments should be implemented. Regular sensory and chemical analysis can expose a polyphenolic imbalance, linked either to the grape variety or the vintage, and which may be a deficiency (lack of maturity, …) or an excess of tannins or anthocyanins (long maceration, …). This imbalance produces wines with harsh, bitter or unstructured tannins, wines with weak, sustained or unstable colour, and wines leaning towards oxidative notes.

Understanding tannins to tailor their addition

Depending on their origin, commercial tannin preparations contain either gallotannins (derived from gallnuts or tara), ellagitannins (from oak or chestnut), or proanthocyanidins (PA), also called condensed tannins (from grapes or exotic woods). The quality of the tannins depends on their purity, which depends upon the botanical origin of the tannins, the maturity of the originating plant, and the extraction and production process.

Condensed tannins (or proanthocyanidins) are polymers of flavan-3-ol units. These flavan-3-ol units have either a phloroglucinol or resorcinol-type A ring. The phloroglucinol ring system is found in catechin and epicatechin, the flavan-3-ol units of the procyanidins (PA*c*), and in gallocatechin and epigallocatechin, the flavan-3-ol units for the prodelphinidins (PA*d*). A resorcinol ring is found in fisetinidol and epifisetinidol, the flavan-3-ol units for the profisetinidins (PA*f*), and in robinetinidol and epirobinetinidol, the flavan-3-ol units for the prorobinetinidins (PA*r*). Procyanidins are found in grape seeds and grape skins, prodelphinidins in grape skins only, and profisetinidins and prorobinetinidins in quebracho and mimosa woods. This study focuses on the main commercial products made from grape skins and seeds, of *Vitis vinifera sp.,* and from quebracho (*Schinopsis balansa)* heartwood.

Procyanidin characterisation methods

The monomeric constituents of polymeric PA were identified, and different types of polymeric PA were differentiated, by means of depolymerisation processes (the Bate-Smith reaction, and thioacidolysis) and by mass spectrometry (MS). The Bate-Smith reaction is an oxidative depolymerisation of proanthocyanidins that occurs with heating in a mineral acidic medium. It forms anthocyanins, absorbing at 550 nm (red colour) [1]. From the types of anthocyanins produced, structural information about the initial proanthocyanidins is gained. Samples of the skins and seeds of grapes and of quebracho heartwood showed a positive reaction, which revealed the presence of proanthocyanidins. Thioacidolysis is a selective acidic depolymerisation method using a thiol as a nucleophilic agent (Vivas et al. 2004). Finally, to confirm the identification and to complete the structural elucidation, Matrix-Assisted Laser Desorption Ionization (MALDI) Time of Flight (TOF) mass spectrometry was used, a technique particularly well adapted for analysis of large molecules (Vivas et al. 2004). This provided interesting information on the nature of the PA molecules (Figures 1 and 2).

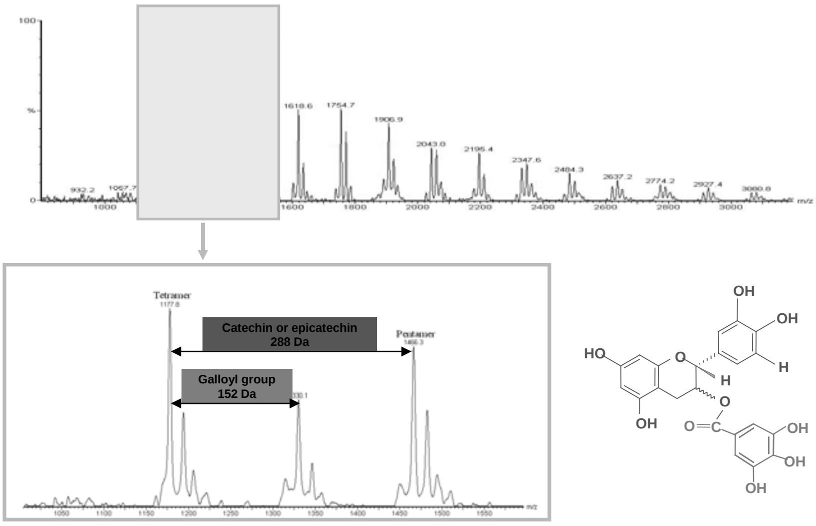

Figure 1: MALDI-TOF mass spectrum of grape seed tannin extract

Grape seed PA presents a profile characteristic of the procyanidin-based PA*c* tannins (Figure 1). A repeating series of mass peaks with a mass difference of 288 Da (288 atomic mass units) between them (see Figure 1, enlarged inset, upper horizontal arrow) corresponds to catechin and epicatechin units joined by C-C linkages in the polymer. A second repeating series of mass peaks with a mass difference of 152 Da between them (see Figure 1, enlarged inset, lower horizontal arrow) corresponds to the galloyl group. In all samples, the PA*c* tannins showed a degree of polymerisation from dimeric (two flavan-3-ol units) to octameric (eight flavan-3-ol units), and showed partial galloylation (0-4 galloyl units) in the polymer.

Grape skin PA presents a PA*c* profile as shown in Figure 1. It also shows a prodelphinidin-based PA*d* profile, characterized by peaks with a mass difference of 304 Da, arising from the presence of an additional –OH group in the flavan-3-ol units. Such an increase of mass difference from an additional oxygen atom can be seen in Figure 2, in which the fisetinidol and epifisetinidol units (with a mass difference of 272 Da between mass peaks) can be seen together with the robinetinidol and epirobinetinidol units (with a mass difference of 288 Da between mass peaks).

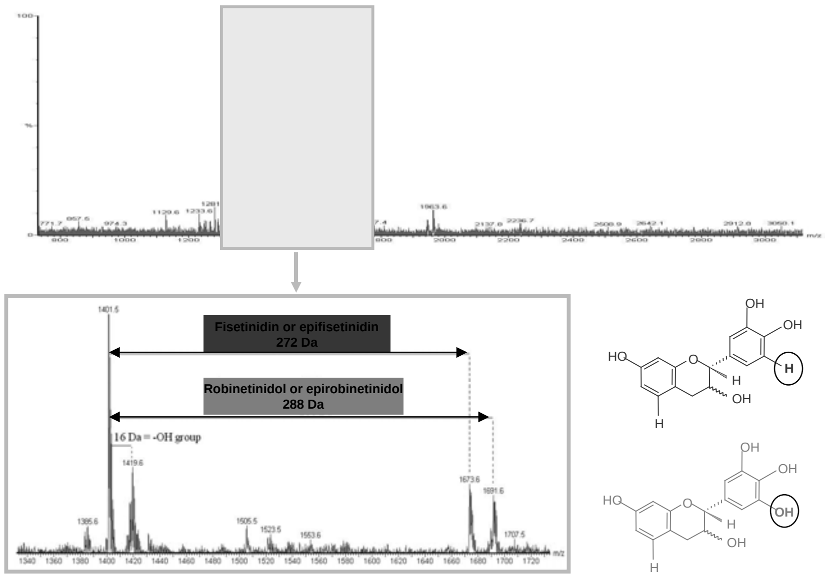

Figure 2: MALDI-TOF mass spectrum of quebracho tannin extract

Quebracho PA presents both a profisetinidin-based PA*f* profile and a prorobinetinidin-based Pa*r* profile. The PA*f* profile shows a mass difference of 272 Da between repeating mass peaks (Figure 2, enlarged inset, upper horizontal arrow) corresponding to the masses of the fisetinidol and the epifisetinidol units that are joined by C-C linkages in the polymer. The PAr profile shows a mass difference of 288 Da, corresponding to the masses of robinetinidol and the epirobinetinidol units joined by C-C linkages in the polymer. Like the skins, a 16 Da increment indicates the occurrence of flavan-3-ols that are trihydroxylated in ring B, as in the lower chemical structure in Figure 2.

Finally, the main products released by thioacidolysis were identified by electrospray ionisation (ESI) mass spectrometry. The compounds identified from the spectra are shown in Figure 3. In agreement with the chemical reaction of thioacidolysis, two classes of compounds were characterised.

- Terminal units of flavan-3-ols. These are catechin and epicatechin and epicatechin-3-*O*-gallate for PA*c*, and gallocatechin and epigallocatechin for PA*d* ;
- Extension units, with C4-C6 or C4-C8 links. These provided catechin benzylthioether I, and II (isomeric forms), epicatechin benzylthioether, epicatechin-3-*O*-gallate benzylthioether for PA*c* and epigallocatechin benzylthioether for PA*d*.

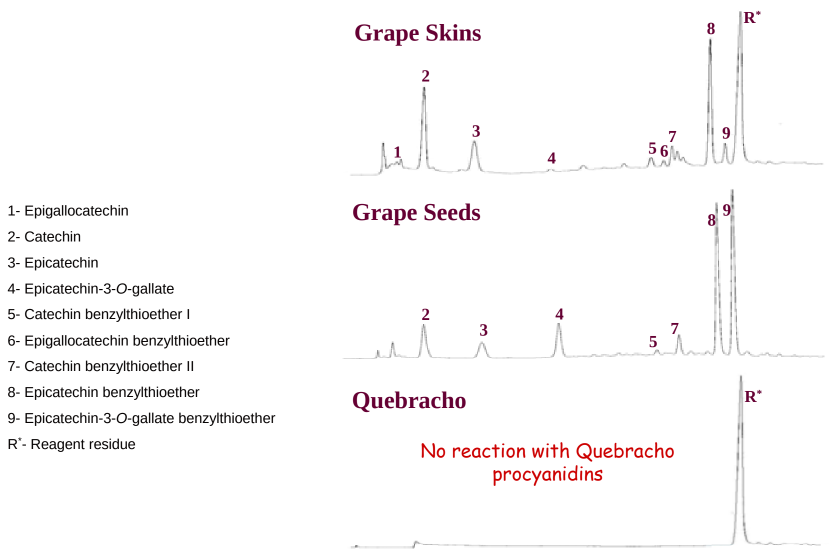

Figure 3 : HPLC chromatograms of grape skin, seed, and Quebracho proanthocyanidins, after thioacidolysis

It was found that seed PAs contain only procyanidins, with the majority in the form of oligomeric tannins. Skin PAs are a mixture of procyanidins and prodelphinidins, but with procyanidins predominant. Quebracho PAs have no flavan-3-ols because they have phenolic units with a resorcinol ring A (lacking a hydroxyl group in the C5 position), not the phloroglucinol ring A that is found with the grape seed and skin compounds. This difference could explain the absence of reaction between Quebracho PA and wine PA.

The structural diversity among oenological tannins from grape seeds.

A study of grape seed tannins (Kahn and Bertrand 2005) demonstrated the presence of three groups of tannins.

Group 2 is characterised by a low degree of polymerization, a high tannin fraction, and an excellent antiradical activity. Group 3 is characterized by the highest degree of polymerization and galloylation. Finally, group 1 has intermediary properties between group 2 and 3. Groups 1 and 2 have a high total polyphenolic and PA content (the purest tannins), whereas group 3 has the lowest content and is thus less pure.

The mean degree of polymerisation (mDP) does not indicate the distribution of molecular weights, but this was assessed by examining the polyphenolic profile of the tannin samples, as obtained by normal phase HPLC with UV detection at 280 nm. This method can rapidly assess the mass distribution of proanthocyanidins in commercial tannin preparations. Three very characteristic profiles were obtained (Figure 4). The chromatograms of the three tannin groups demonstrate that the polyphenolic profile was directly related to the molecular weight: the larger the proanthocyanidin molecules, the more their HPLC profile was shifted towards the right. This profile allows assessment of both the polyphenolic content and the mDP.

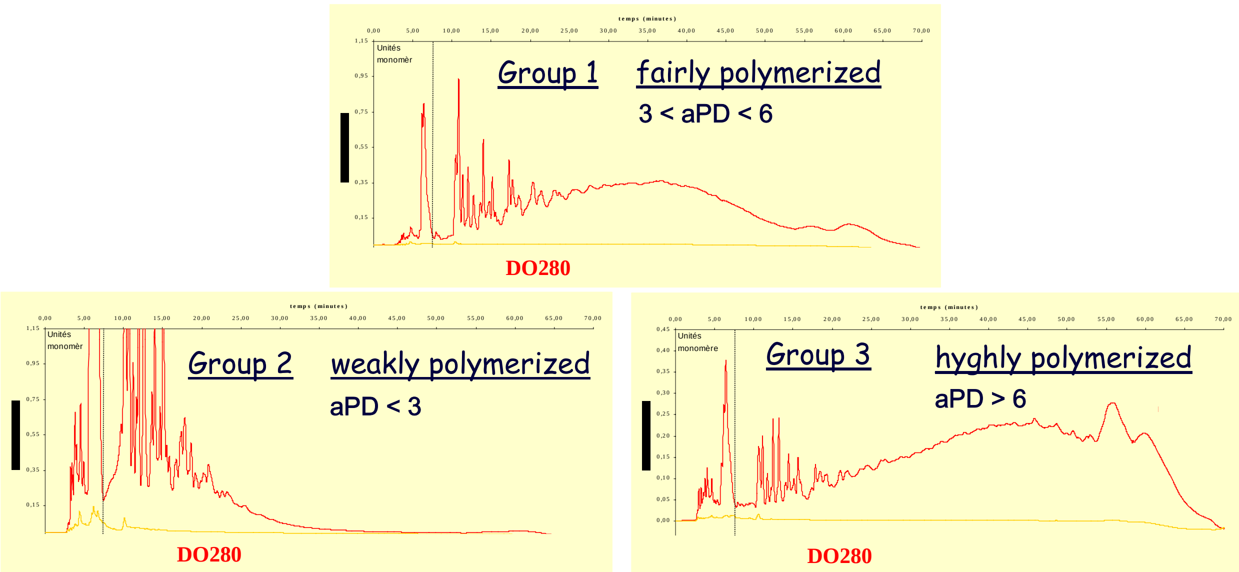

Figure 4: Seed tannin chromatogram

This study has demonstrated the structural diversity of grape seed tannin components. This diversity leads to very different properties in commercial seed tannin preparations, particularly with regards to their astringency, colour stabilization, cross-reactivity with proteins, antiradical activity, and other aspects.

	Group 1	Group 2	Group 3
Proportion of analysed tannins	majority	minority	minority
Mean degree of polymerization (mDP)	3 < mDP < 6 fairly polymerized	mDP < 3 weakly polymerized	mDP > 6 highly polymerized
Active tannin fraction	+ + +	+ + + +	+
Galloylation rate	+ + +	+	+ + + +
Antiradical activity	+ +	+ + +	+ +
Cross-reactivity with wine polyphenols	+ + +	+ + + +	+
Cross-reactivity with wine proteins	+ + +	+	+ + + +
Antioxidising potential	+ +	+ + +	+

These observations, of the diversity of properties within tannin fractions, could partly explain the contradictory views expressed in recent publications about the benefits and results of tannin additions. Thanks to this work, it is now possible to control the raw materials used in the production of enological tannins, and to offer a new approach to the use of tannins: a tailored use of tannins, in which the type of tannin used is a decision making tool with selection of the tannin based on scientific data and pertinent expertise, according to the wine type desired.

Different tannins for different wines: the tailored use of tannins.

Wine polyphenolic compounds can occur with various degrees of polymerisation and oxidation, and in quantities that vary with :

- grape variety;
- grape maturity at harvest;
- the sanitary condition of the fruit;
- the maceration management (pumping over, aerations); and
- the status of wine ageing.

Thus, it is important to add tannins carefully and according to the wine polyphenolic status, as detailed below.

1. Lack of fruit maturity

Observation	Explanation	Objective	Application[1]
Lack of colour, green tannins, bitter and astringent tannins.	Deficiency of anthocyanins, but a high level of reactive tannins with a high galloylation rate	Protect anthocyanins, and soften tannins.	During AF: use a tannin reactive with wine proteins, e.g. ellagitannin. Before MLF: use seed tannin that is highly reactive with wine tannin.
Lack of colour, lack of structure.	Deficiency of anthocyanins and tannins.	Protecting anthocyanins, and restoring the tannic potential.	During vinification: Compensate and structure with seed tannin During ageing: use seed tannin reactive with wine anthocyanins.

[1]AF: alcoholic fermentation, MLF: malolactic fermentation

2. Young wine

Observation	Explanation	Objective	Application
Astringency, stable purplish colour	Highly reactive tannins, free anthocyanins	Prioritising cross-reaction of tannins with anthocyanins before MLF, and initiating a soft polymerisation	Before MLF: use micro-oxygenation. Beginning of ageing: use ageing on lees + micro-oxygenation.
Suppleness, unstable purplish red colour	Few tannins, free anthocyanins	Stabilising colour before MLF. Restoring the tannic potential.	Before MLF: use highly reactive seed tannins with wine anthocyanins. During ageing: use reactive seed tannin, with wine anthocyanins, and structuring tannin.

3. Wines with a long maceration, green seeds

Observation	Explanation	Objective	Application
Harshness, dryness, bitterness, stable and deep colour	Large quantity of highly reactive tannins, stable anthocyanins	Eliminating harsh and bitter tannins, softening structure and volume	Beginning of ageing: fining End of ageing: oak tannin

4. Grapes and wines predisposed to oxidative characters

Observation	Explanation	Objective	Application
Spoiled harvest, orange-coloured juice.	Presence of quinones.	Reaction with oxygen and free radicals in order to limit the loss of wine polyphenols.	Before AF: gallotannin During AF: Chestnut tannin, then seed tannin reactive with dissolved oxygen in wine and effective over time. Structuring tannin
Grapes predisposed to oxidative characters.	Grapes with a low polyphenol content.	Reaction with oxygen, preserving and enhancing the ageing potential.	During AF: chestnut tannin. Before MLF: seed tannin reactive with polyphenols and dissolved oxygen in wine. Structuring tannin.

5. Wine at the end of ageing

Observation	Explanation	Objective	Application
Still high content of low polymerised tannins	Astringency, harshness, bitterness,		Ageing: seed tannin highly reactive with wine tannins.
Lack of polymerised tannins	Lack of structure, light colour		Ageing: seed tannin reactive with wine anthocyanins. Structuring tannin.

References

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