RESOLUTION OENO 6/2008

DIFFERENTIATION METHOD FOR OENOLOGICAL TANNINS - AMENDMENT TO THE MONOGRAPH

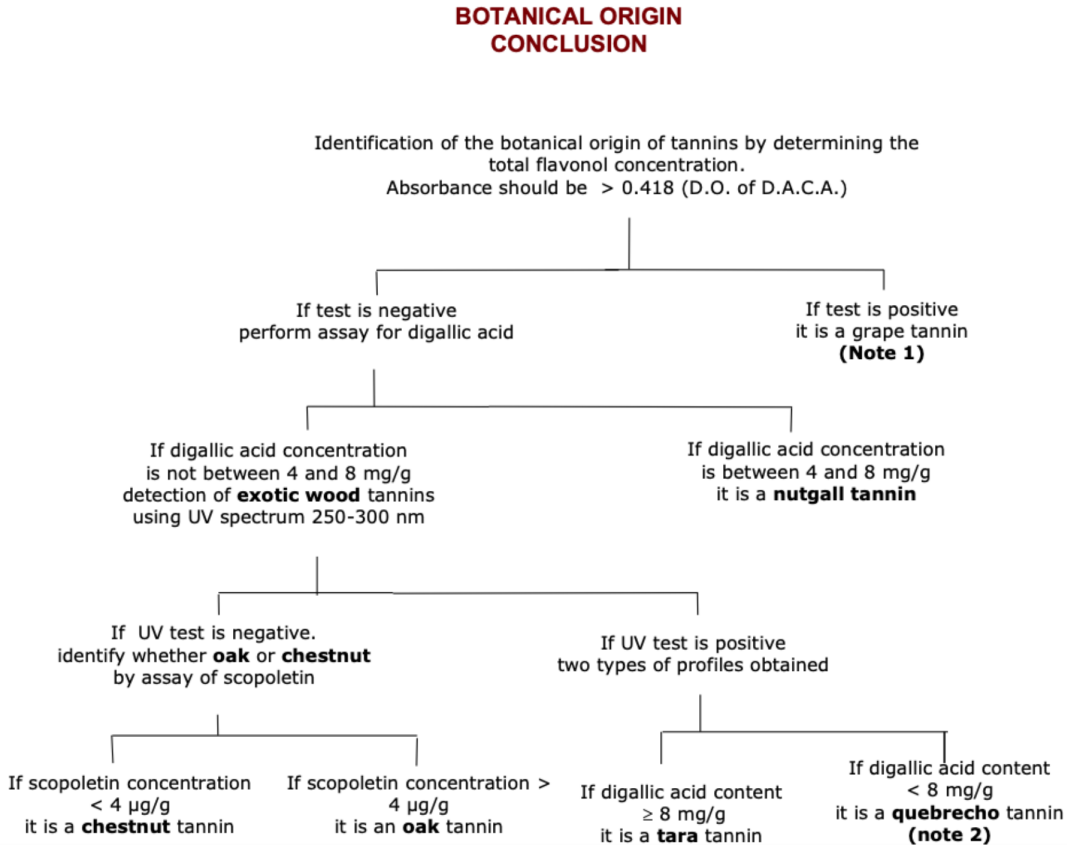
THE GENERAL ASSEMBLY

IN VIEW of Article 2 paragraph 2 iii of the Agreement establishing the International Organisation of Vine and Wine,

Having studied the research by the "Methods of Analysis" Sub-commission,

CONSIDERING resolution Oeno 12/2002 concerning the oenological tannins monograph,

DECIDES, upon the proposal of Commission II "Oenology" to amend and supplement the last page of the annex to resolution Oeno 12/2002 with the following text:



Certified in conformity Verona, 20th June 2008
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2008

Note 1

Grape tannins are formed from 3-flavonol units, which can be released by thiolytic cleavage of the flavonol intermonomer linkages in proanthocyanidols under heat in an acid medium. The monomers thus released are then separated and assayed using HPLC. This means that the procyanidols and prodelphinidols can be quantified separately. This method is used to identify tannins from grape skins, stems and seeds. Under these conditions, Quebracho tannin does not produce a peak (see method and diagram below).

DIFFERENTIATION METHOD FOR PROANTHOCYANIDIN TANNINS BY HPLC

Definition

Identification of Quebracho, grape skin and grape seed tannins

Apparatus and methods

Apparatus and test conditions

- 1 ml straight-sided pipette with 0.05 ml calibrations
- 10 ml volumetric flask
- HPLC system

Must be equipped with: a pump with the capacity for extremely precise constant or programmmed flow-rate or , and a 20 μl sample loop.
A C18 type reversed-phase column, with a particle diameter of for example 10 µm.
Length: 250 mm; internal diameter: 4.6 mm.
A UV/visible detector.

- Oven
- 10 ml teflon-stoppered hydrolysis tubes
- Cellulose ester filters, pore diameter 0.45 μm
- Vacuum filtration system

Certified in conformity Verona, 20th June 2008
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2008

- 1000 µl automatic pipette

Analytical balance to 1 mg

Reagents and calibration solutions

- HPLC grade methanol
- Distilled water
- Toluene-α-thiol (CAS 100-53-8) 99%
- Hydrochloric acid (12M) 37%
- Phosphoric acid 84%

Preparation of reagents

- Preparation of solvents for HPLC:

Solvent A: into a 1l volumetric flask, introduce 1ml phosphoric acid and bring up to volume with distilled water which has been previously filtered in a vacuum filtration system.
Solvent B: into a 1l volumetric flask, introduce 1ml phosphoric acid and bring up to volume with methanol that has been previously filtered in a vacuum filtration system.

- Methanol containing 1.7% HCl: into 10 ml methanol, introduce 140 µl hydrochloric acid, using a 1000 µl automatic pipette.
- Thioacidolysis reagent = 5% toluene-□-thiol solution: into 10 ml of the solution, introduce 470 µl toluene-α-thiol using a 1000 µl automatic pipette.
- Oenological tannins (commercial preparations)
- Tannin solutions at 1 g/l: 10 mg tannins are introduced into 10 ml methanol.

Procedure

0.5 ml of tannin solution and 0.5 ml of the thioacidolysis reagent (5% toluene-α-thiol solution) are introduced into a hydrolysis tube. The mixture is stirred and heated at

Certified in conformity Verona, 20th June 2008
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2008

60°C for 10 min. The tube is then cooled and 0.5 ml distilled water added.

The sample is analysed using HPLC on a C18 reversed-phase column. The eluents used are solvents A and B. The elution sequence is as follows: from 70% (for 5 min.) of solvent B to 10% in 40 min., then from 10 to 70% (for 5 min.) in 10 min. (return to initial conditions). The flow-rate of 1ml/min is constant for the whole sequence and the wavelength used is 280 nm.

The peaks are identified and respectively quantified according to the data provided by Vivas et al. (2004)*.

Tannins from seeds, skins and Quebracho have different profiles. Grape seed tannins are composed exclusively of procyanidols, and are identified by a high galloylation level, a high epicatechin content and a low mean degree of polymerisation (MDP). Skin tannins are identified by a combination of procyanidols and prodelphinidols, with a predominance of procyanidols, a low level of galloylation, a significant quantity of epicatechin and a variable MDP. Quebracho tannins do not produce any 3-flavonols. It is therefore possible to determine their composition in terms of proanthocyanidol tannins.

* N. VIVAS, M.F. NONIER, N. VIVAS de GAULEJAC, C. ABSALON, A. BERTRAND, M. MIRABEL, "Differentiation of proanthocyanidin tannins from seeds, skins and stems of grapes (Vitis Vinifera) and heartwood of Quebracho (Schinopsis balansae) by MALDI-TOF/MS and thioacidolysis/LC/methods", Analytica Chimica Acta, 2004, 513, Issue 1, 247-256.

Certified in conformity Verona, 20th June 2008
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2008

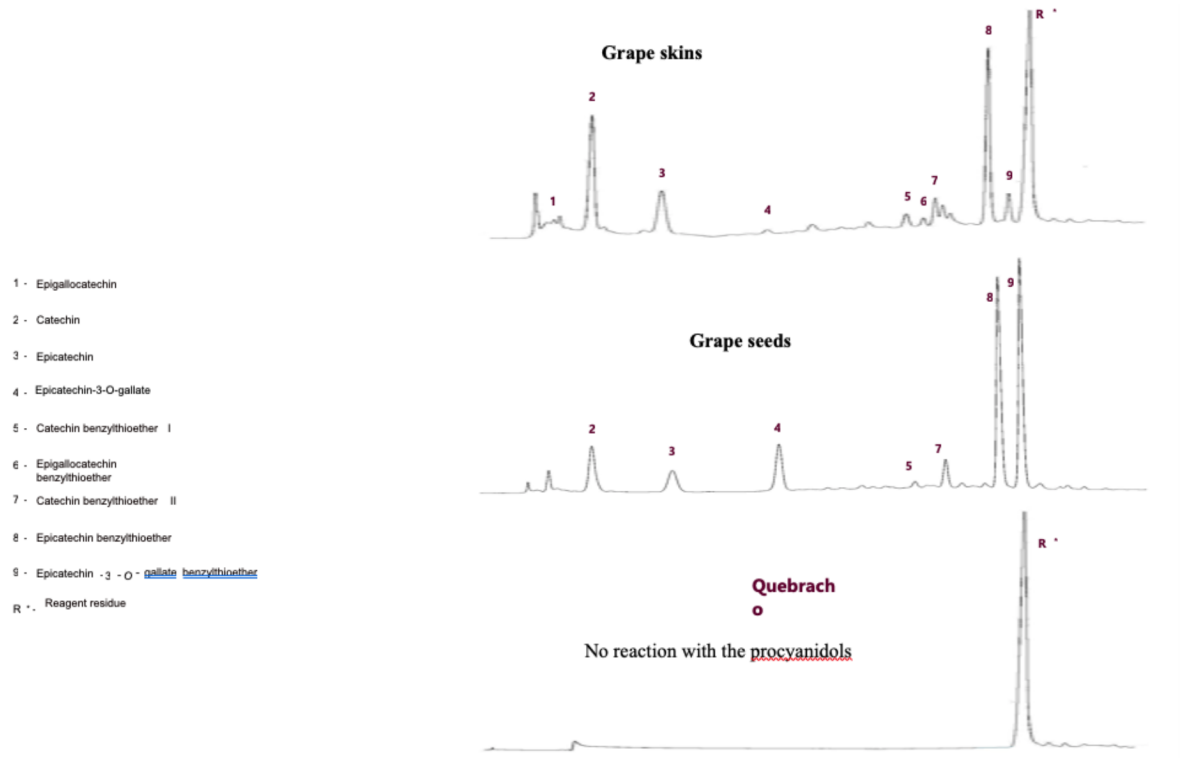


Fig .1 Chromatograms of proanthocyanidols from grape skins and seeds and Quebracho, obtained by HPLC after thiolysis.

Note 2

Identification of Quebracho as the botanical origin of a tannin is achieved by a process of elimination. Formal identification of the presence of Quebracho-derived tannin can be made using HPLC in combination with mass spectrometry (MALDI-TOF). The latter shows that the monomer constituents of this tannin are obtained from fisetinidol and robinetinidol, which have no hydroxyl in 5- position on the atomic nucleus (in other words grape-derived tannins are formed from monomers which have a trihydroxyl nucleus (phloroglucinol) whereas Quebracho-derived tannins are formed from monomers with a dihydroxyl nucleus (resorcinol).

Certified in conformity Verona, 20th June 2008
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2008