OENOLOGICAL TANNINS

Specific monograph for oenological tannins containing procyanidins/prodelphinidins

(OIV-OENO 675A-2022)

Procyanidins/prodelphinidins are a sub-class of condensed (or proanthocyanic) tannins. Tannins from *Vitis vinifera* grapes, grape skins and grape seeds are included in this sub-class.

1. Method for the determination of sub-class affiliations

Characterisation by high-performance liquid chromatography (HPLC)

1. Principle

This method is designed to verify the presence of characteristic components of condensed tannins of the procyanidin/prodelphinidin sub-class and measure their total concentration. It is applicable to preparations of oenological tannins that are declared as pure and thus as not containing tannins with small masses from different families or sub-classes.

2. Reagents, material and apparatus

1. Reagents

(+)-catechin, CAS No.: 154-23-4
Ultrafiltered water (resistivity: 18.3 MΩ·cm)
Water (HPLC quality)
Methanol (HPLC quality)
Formic acid (HPLC quality)

2. Materials

100-mL borosilicate-glass flask
Cellulose filters with 0.45 µm pore size diameter
Plastic 1-mL syringe

3. Apparatus

Analytical balance with precision of 0.01 g
Analytical balance with precision of 0.1 mg
Class-A volumetric glassware
Mass chromatographic system with spectrometry detection composed of:
- Gradient pump for binary or quaternary mix
- Injector with a loop of 10 µL
- Spectrophotometric detector at 280 nm fixe wavelength
- Column Eclipse Plus C-18 (for example): 2.1 x 100 mm, 1.8 µm particle size
- ESI-SIM (Selected Ion Monitoring mode via Electro Spray Ionisation) ionisation source
- Mass spectrometer detector: quadrupole time of flight (Q-TOF)

3. Preparation of samples and standards

Samples: weigh approximately 0.5 g of oenological tannins on the analytical balance and make a note of the weight. Dissolve the oenological tannins in 100 mL of ultrafiltered water in a 100-mL borosilicate-glass flask and mix well.

Preparation of standard solutions: put 10 mg of (+)-catechin in solution into 50 mL of ultrafiltered water, corresponding to a 200 mg/L concentration. Then carry out dilutions in ultrafiltered water to obtain 5, 10, 20, 40, 60, 80 and 100 mg/L concentrations.

Solvent A: HPLC-quality water containing 0.1% of formic acid.

Solvent B: methanol containing 0.1% of formic acid.

4. Procedure

The sample solution and standard solutions are filtered on 0.45 µm (pore size diameter) filters and analyse by chromatography under the following conditions:

Injected volume: 10 µL of sample solution or standard solution of (+)-catechin

Detection at 280 nm

Composition of elution gradient: (time, % of solvent A)

0 min, 99.0%; 0.5 min, 94.0%; 20 min, 50.0%; 25 min, 0.0%; 32 min, 94.0% and 10 min for equilibrium.

Flow rate: 0.3 mL/min

Quantification and detection of the characteristic components of condensed tannins

of the procyanidin/prodelphinidin sub-group (sub-class) ((+)-catechin, (-)-epicatechin, (+)gallocatechin, epigallocatechin, (-)-epicatechin-3-*O*-gallate, (-)-epigallocatechin-3-*O*-gallate, dimers B1, B2, B3 and B4, B2-3-*O*-gallate, trimers) according to the ESI-SIM scan and Q-ToF detection, for example.

Table 1: Example chemical formulas and masses of the different procyanidins/prodelphinidins

Compound	Chemical formulas	m/z
(+)-catechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin-3-O-gallate	$C_{22}H_{18}O_{10}$	442.1
Dimer B1	$C_{50}H_{26}O_{12}$	578.1
Dimer B2	$C_{50}H_{26}O_{12}$	578.1
Dimer B3	$C_{50}H_{26}O_{12}$	578.1
Dimer B4	$C_{50}H_{26}O_{12}$	578.1
B2-3-O-gallate	$C_{37}H_{30}O_{16}$	730.1
Trimers	$C_{45}H_{38}O_{18}$	866.2

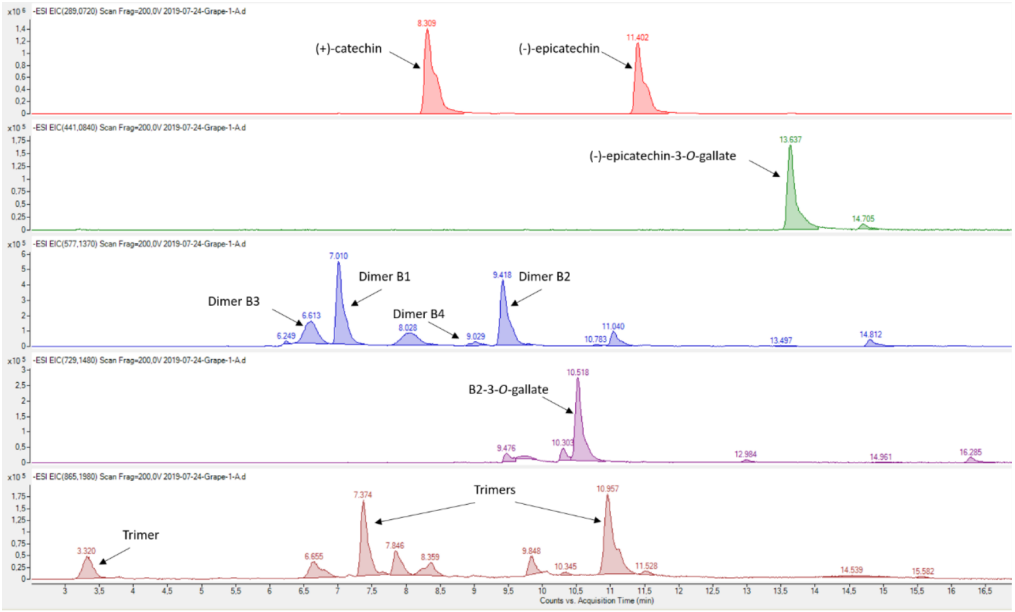

Figure 1: Example of ESI-SIM scan of procyanidins/prodelphinidins

2. Conclusion

An oenological tannin is recognised as a procyanidin/prodelphinidin when:

- its total polyphenol content is higher than 65% (gravimetric method in Annex 1 of the general monograph OIV-OENO 624-2022),
- its procyanidin/prodelphinidin content as characterised by the HPLC method is higher than 120 mg equivalent of (+)-catechin per gram of oenological tannins.
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2. Methods of measurement of properties and functionalities

The following compliance methods and criteria are only applicable when the property/functionality is claimed on the preparation of tannins.

1. Antioxidant ability

1. Principle

Determination of procyanidins/prodelphinidins' ability to contribute to the protection of must and wine from oxidation.

2. Products

1. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl): MM = 394.32
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid): MM = 250.29
Methanol at 99.9% volume
96-well microplate reader (FLUOstar Omega - BMG Labtech, for example)

2. Direct oxygen consumption (OCR)

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Iron (III) chloride hexahydrate: MM = 270.30, CAS No. 7705-08-0
Copper (II) sulfate pentahydrate: MM = 249.68, CAS No. 7758-98-7
Clear glass bottles with inserted pills of 0.75-cL capacity
NomaSens oximeter, for example

3. Protocols

1. Antioxidant capacity (DPPH assay)

0.15 g/L oenological tannin solution: dissolve 37.5 mg of oenological tannins in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5). Dilution of oenological tannins solution could be needed if the measurement absorbance is higher than 1 unit (in this case the dilution should be included in the calculation).
1mM Trolox solution: dissolve 125 mg of Trolox in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).
Calibration curve: dissolve in 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mL of 1mM Trolox solution into 0, 0.2, 0.4, 0.6, 0.8 and 0.9 mL of model wine solution. These quantities correspond to 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM final concentration of Trolox respectively.

6.10^{-5} M DPPH solution: dissolve 2.36 mg of DPPH in 100 mL of methanol. The solution should be freshly prepared.

2. Direct oxygen consumption (OCR)

1 g/L oenological tannin solution: dissolve 0.75 g of oenological tannins in 750 mL of model wine solution.

Model wine solution: dissolve 4 g of tartaric acid, 2.25 mg of Iron (III) chloride hexahydrate and 0.225 mg of Copper (II) sulfate pentahydrate in 90 mL of ethanol and 660 mL of distilled water. The pH should be adjusted at 3.5.

4. Tests

1. Antioxidant capacity

First a blank containing solely the DPPH reagent (RB) is measured at 515 nm by placing 190 µL of DPPH solution (1.3.1) in all the wells of the plate. Then, add 10 µL of oenological tannin solution (samples), distilled water (blank) or Trolox curve solution (standards) into the wells and measure (MS) at 515 nm after 30 min.

See Figure 2 for an example of how to fill the plate.

The formula to be applied for the calculation of the antioxidant capacity is as follows:

1. $$RB - MS = x$$

2. $$antioxidant\ capacity\ (mg\ eq.\ Trolox\ per\ g\ of\ tannins) = \frac{250.29\ (mg)}{0.15\ (g)}\ x\ \frac{x-b}{a}$$

where “a” and “b” correspond respectively to the slope and the constant of the Trolox calibration curve: Absorbance = f ([Trolox]) □ Absorbance = ax + b

In all cases, procyanidins/prodelphinidins should demonstrate antioxidant capacity, and more specifically they should have more than 500 ± 50 mg equivalent Trolox per gram of tannins (commercial extract).

	1	2	3	4	5	6	7	8	9	10	11	12
A	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
B	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
C	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
D	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
E	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
F	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
G	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
H	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11

T = Trolox OT = Oenological Tannins

Figure 2: example 96-well plate

2. Direct oxygen consumption (OCR)

First the model wine solution is saturated with oxygen at 8 mg/L by bubbling with air for 10 min at 20-25 °C. Then, add the oenological tannins to the model wine solution in the bottles filled to 0.75 cL. Seal the bottles hermetically and shake to fully homogenise.

1. Measure the oxygen consumed every two days starting 1h after the filled of the bottles.

2. To determine the oxygen consumption rate, follow the pathway as shown in **Figure 2**:

   - represent the oxygen consumption versus the time,
   - then represent the inverse of the oxygen consumed versus the inverse of the time,
   - the oxygen consumption rate corresponds to the inverse of the slope coefficient:
     - OCR t mg of O_2 consumed per day and per g of tannins = 1/A, A being the slope coefficient

In all cases, procyanidins/prodelphinidins should demonstrate an ability to consume the oxygen directly, and more specifically they should be able to consume at least 0.10 ± 0.05 mg of O_2 per litre, per day and per gram of tannins (commercial extract).

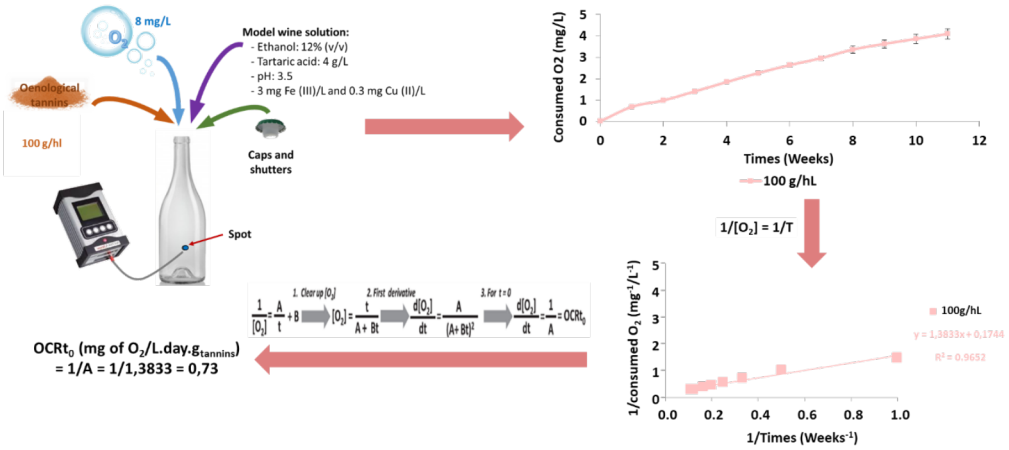

Figure 3: Pathway to determine oxygen consumption rate

3. Antioxidasic ability

1. Principle

Determination of procyanidins/prodelphinidins' antioxidasic ability to contribute to antioxidasic protection in terms of the laccase activity of compounds in must and wine.

2. Products

Ethanol at 96% volume, CAS No. 64-17-5

Tartaric acid: MM = 150.09, CAS No. 87-69-4

Sodium acetate: MM = 82.03, CAS No. 6131-90-4

Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine): MM = 360.36, CAS No. 14414-32-5

Polyvinylpolypyrrolidone (PVPP): CAS No. 25249-54-1

Must botrytised with laccase activity

Distilled water (HPLC quality)

3. Protocols

2 g/L oenological tannin solution: dissolve 200 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Buffer solution (8.2 g/L): dissolve 410 mg of sodium acetate in 50 mL of distilled water.

Syringaldazine solution (0.06 g/L): dissolve 30 mg of syringaldazine in 500 mL of ethanol.

4. Tests

1. Add 4 mL of botrytised must to 1 mL of oenological tannin solution in one tube, which will correspond to sample modality.
2. Add 4 mL of botrytised must to 1 mL of model wine solution in one tube, which will correspond to control modality.
3. After 4 minutes (precisely), add 0.8 g of PVPP to both tubes (sample and control modalities), stir and centrifuge for 10 minutes at 8,500 rpm.
4. Recover 1 mL of the supernatant (for both sample and control modalities), into 1.4 mL of buffer solution and 0.6 mL of syringaldazine solution. Put the mixture into a plastic spectrophotometer cuvette (10 mm path length).
5. Measure the absorbance at 530 nm every minute for 5 minutes (including time measurements at 0 minutes).
6. Then determine the laccase activity and the residual laccase activity by using the following equations and **Figure 3**:
7.

□□□□□□□ □□□□□□□□ = 46.15 □ Δ□ μ□□□. □$^{-1}$. □□□$^{-1}$ = 46.15 □ Δ□ □□

% □□ □□□□□□□□ □□□□□□□□ = (□□□□□□□ □□□□□□□□$_{□□□□□}$ / □□□□□□□ □□□□□□□□$_{□□□□□□}$) □ 100

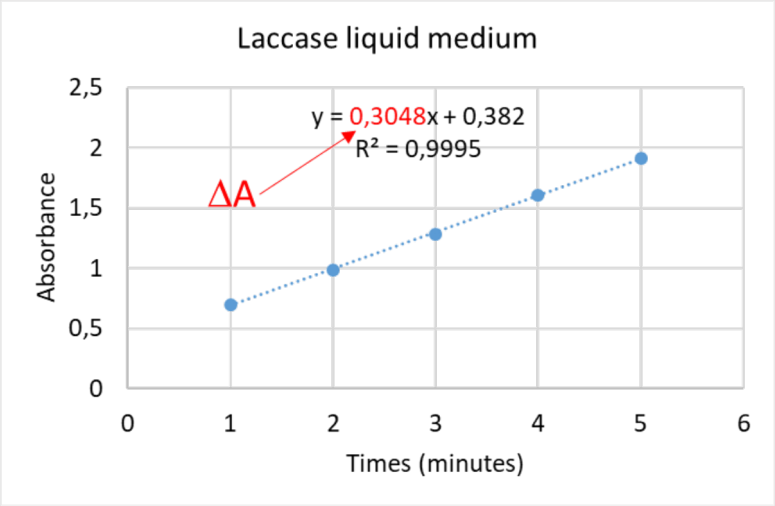

Figure 4: Example determination of □□.

In all cases, procyanidins/prodelphinidins should demonstrate an antioxidasic ability, and more specifically they should be able to reduce the residual laccase activity by at least 50%. This value is valuable for must and wine containing less than 5 UL (units of laccases).

5. Colour stabilisation

1. Principle

Determination of procyanidin/prodelphinidin colour stabilisation properties to promote the expression, stabilisation and preservation of colour in red must and wine.

2. Products

Ethanol at 96% volume, CAS No. 64-17-5

Tartaric acid: MM = 150.09, CAS No. 87-69-4

Malvidin-3-*O*-glucoside: MM = 528.87, CAS No. 18470-06-9

3. Protocols

0.8 g/L oenological tannin solution: dissolve 80 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

0.1 g/L malvidin-3-O-glucoside solution: dissolve 10 mg of malvidin-3-*O*-glucoside in

100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

4. Tests

1. Place 0.75 mL of oenological tannin solution and 0.75 mL of model wine solution in one 2-mL stoppered conical tube – hereinafter a "tube" – and keep it in the dark at room temperature. This tube will be called "T".

2. Place 0.75 mL of malvidin-3-*O*-glucoside solution and 0.75 mL of model wine solution in one tube and keep it in the dark at room temperature. This tube will be called "M".

3. Place 0.75 mL of oenological tannin solution and 0.75 mL of malvidin solution in one tube and keep it in the dark at room temperature. This tube will be called "T_M".

4. After 7 days, measure the absorbance at 450, 520, 570 and 630 nm of the three tubes (T_M, T and M).

5. Subtract the absorbance values of T to T_M to obtain the absorbance, taking into account the absorbances of the oenological tannins used.

$A(T_M) - A(T) = A(T)$

6. Then, determine the CIELAB coordinates (L*, a* and b*) corresponding to the tannin solution with malvidin-3-*O*-glucoside (T) and malvidin-3-*O*-glucoside solution (M) with the free MSCV software (https://www.unirioja.es/color/descargas.shtml) or equivalent.

The formulas to be applied for the calculation of the copigmentation index are as follows:

1) $\Delta E_{ab}.TS = \sqrt{(L^*_T - L^*_W) + (a^*_T - a^*_W) + (b^*_T - b^*_W)}$

2) $\Delta E_{ab}.CS = \sqrt{(L^*_M - L^*_W) + (a^*_M - a^*_W) + (b^*_M - b^*_W)}$

3) Copigmentation Index (%) = $100 \times \frac{\Delta E_{ab}.TS - \Delta E_{ab}.CS}{\Delta E_{ab}.CS}$

ΔE_{ab}.TS: total colour difference between the solution of malvidin-3-*O*-glucoside containing commercial tannins (T) and a pure white colour solution (W).

ΔE_{ab}.CS: total colour difference between the solution of malvidin-3-*O*-glucoside (M) and a pure white colour solution (W).

The CIELAB coordinates of a pure white colour solution are L* = 100.00, a* = 0.00 and b* = 0.00.

In all cases, procyanidins/prodelphinidins should demonstrate an ability to stabilise the colour, and more specifically their copigmentation index should read as higher than 7.0 ± 2.0% after 7 days.

Note: Alternative methods of determination can be used in place of any of the methods described, on the condition that these have been internally validated.

3. Bibliography

- Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Australian Journal of Grape Wine Research **2006**, *12*, 39–49.
- Vignault, A.; González-Centeno, M.R.; Pascual, O.; Gombau, J.; Jourdes, M.; Moine, V.; Iturmendi, N.; Canals, J.M.; Zamora, F.; Teissedre, P.-L. Chemical characterization, antioxidant properties and oxygen consumption rate of 36 commercial oenological tannins in a model wine solution. *Food Chemistry* **2018**, *268*, 210–219.
- Vignault, A.; Pascual, O.; Jourdes, M.; Moine, V.; Fermaud, M.; Roudet, J.; Canals, J.M.; Teissedre, P.-L.; Zamora, F. Impact of enological tannins on laccase activity. *OENO One* **2019**, *53*, 27–38.
- Vignault, A.; Pascual, O.; Gombau, J.; Jourdes, M.; Moine, V.; Canals, J.M.; Teissedre, P.-L.; Zamora, F. Recent advances of the OIV working group on oenological tannins in the study of the functionalities of oenological. *BIO Web of Conferences* **2019**, *15*, 02015.
- Vignault, A.; Gombau, J.; Pascual, O.; Jourdes, M.; Moine, V.; Canals,

J.M.; Zamora, F.; Teissedre, P.-L. Copigmentation of Malvidin-3-O-Monoglucoside by Oenological Tannins: Incidence on Wine Model Color in Function of Botanical Origin, pH and Ethanol Content. *Molecules* **2019**, *24*, 1–15.

- Vignault, A.; Gombau, J.; Jourdes, M.; Moine, V.; Canals, J.M.; Fermaud, M.; Roudet, J.; Zamora, F.; Teissedre, P.-L. Oenological tannins to prevent Botrytis cinerea damage in grapes and musts: kinetics and electrophoresis characterization of laccase. *Food Chemistry* **2020,** *316*, 126334.
- Vignault, A. Tanins œnologiques : caractéristiques, propriétés et fonctionnalités. Impact sur la qualité des vins. Thèse de doctorat, Université de Bordeaux et Universitat Rovira i Virgili, **2019**.