OENOLOGICAL TANNINS

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1. GENERAL STATEMENTS

Oenological tannins contain polyphenols. Their complex structure is generally made up of a great variety of monomer units that are covalently bound to each other.

These are derived from different parts of plant species and extracted using solvents permitted under current regulations. They are classified into two classes according to the nature of the monomeric units that characterise them: hydrolysable tannins and condensed tannins *or proanthocyanic tannins*. Besides the two classes of tannins, each monomer unit itself (e.g. flavan-3-ol monomers, gallic acid, ellagic acid) can also occur in tannin preparations.

Hydrolysable tannins form a heterogeneous class that is divided into two sub-classes:

- gallotannins or gallic tannins consist of a glucose or quinic acid unit that is esterified with one or more molecules gallic acid and/or its depsides.
- ellagitannins or ellagic tannins consist of glucose unit(s) that is/are esterified with one or more molecules of ellagic acid and related molecules. In addition, the glycosidic unit(s) may be esterified with gallic acid and/or its/their depsides.

The structure of hydrolysable tannins varies according to the degree of esterification and polymerization (1)

Condensed or proanthocyanic tannins (of the procyanidin and prodelphinidin sub-classes and the profisetinidin and prorobinetidin sub-classes) and consist of polymers of flavan-3-ols. The flavan-3-ol units may differ stereochemically as well as in the degree of hydroxylation and may be present as gallic acid esters. The large variety of monomeric units, different interflavan bonds and the degree of polymerization result in a large number of possible structures of proanthocyanidins. Depending on the

molecular structure, the reactivity of proanthocyanidins can vary greatly.

Oenological tannins come exclusively in powder, granule or flake form, from light beige or beige and brick red to dark brown in colour.

They can be dissolved in a portion of must or wine for incorporation into the total amount of must or wine.

2. LABELLING

The labelling of oenological tannins must specify the following:

- their botanical origin (e.g. oak, quebracho, etc.),
- the classes to which they belong (hydrolysable or condensed),
- the sub-classes (e.g. gallotannins, prodelphinidins, etc.),
- the batch number and the expiry date,
- the minimum content of total polyphenols according to the method in Annex 1,
- the technological functions,
- when tannin preparations are a mixture of several tannin classes or tannin sub-classes the different tannin classes or sub-classes and associated technological functions,
- the recommended dosage and conditions of use,
- the storage conditions to guarantee their stability,
- the possible presence of potentially allergenic residues,
- where applicable, the indication that the oenological tannins were obtained from genetically-modified plants.

3. PERMITTED OENOLOGICAL TANNINS

The oenological tannins that are described in Chapter 1, that have demonstrable and measurable properties as well as a technological interest duly proven in practice, and that completely fulfil the conditions and criteria mentioned below, are permitted in compliance with the files of the Code of Oenological Practices.

The oenological tannins used should not:

- liberate substances in concentrations that could lead to potential health risks,
- cause fraud resulting from the addition of aromas or coloring,

- be harmful to the quality of the products made,
- result in the modification of the well-known organoleptic profile of wine (flavouring).

4. REACTIVITY PROPERTIES AND FUNCTIONS OF OENOLOGICAL TANNINS

4.1 Reactivity

Tannins are likely to be involved in numerous reaction pathways in wine. The reactivity of oenological tannins is directly related to the specific characteristics of their chemical structures; it also depends on the technical operations of production (techniques of extraction, concentration, fractionation, etc.), which influence the polyphenol content (thus the degree of purity) and the proportion of free functional groups. The nature of the plant determines the tannin class(es) or sub-class(es) to which they belong.

4.2 Properties and functions of tannins

The functions of oenological tannins are directly linked to their properties. The methods of analysis used to determine the functions of tannins should correspond to the state of the art and, if possible, be validated according to the appropriate international standards.

The following table lists properties as well as oenological applications, some of which are recognised and some of which remain to be demonstrated.

Theoretical properties	**Possible oenological applications**
Reactivity with proteins	Clarification aid
Reactivity with oxygen	Antioxidant
Iron chelation	Reduction of iron content
Polymerisation	Colour stabilisation
Formation of complexes	Colour stabilisation
Anti-laccase activity	Inhibition of laccase-activity
Microbiological action	

Antibacterial effect	Microbiological stability, reduction in SO_2 use

The properties and resulting oenological applications will be the object of specific monographs by classes of tannins and/or sub-classes, in which the methods of measurement shall be specified.

4.3 Estimation of the total polyphenol content

The estimation of the total polyphenol content of preparations of oenological tannins is measured by the method described in Annex 1.

The total polyphenol content should be greater than or equal to 65% and a maximum concentration is not required

5. PHYSICAL PROPERTIES

5.1 Insoluble materials

Place 5 g tannin in a solution of 100 mL double-distilled water at room temperature and stir for 15 minutes. After that filter this solution over a 0.8 µm membrane previously weighed. Evaporate and dry the membrane at 100-105 °C. Weight the membrane. The insoluble matter content should not be greater than 5% w/w.

The procedure described later in the total polyphenol content method (Annex 1, point 4.3) could be used as an alternative.

5.2 Loss on drying

Determined up to constant weight, for a 2 g sample, the weight loss in an oven at 100-105 °C for 2 hours should be less than 10%.

6. MAXIMUM CONTAMINANTS LEVELS

Oenological tannins should be produced in accordance with good manufacturing practices. According to the origin of the plant used, some maximum contaminant levels – such as those for heavy metals – may be different (e.g. the iron content of chestnut-derived tannins).

All of the limits set below relate to dry products.

6.1 Pentachlorophenol

Proceed with determination according to the method described in the OIV Compendium of the International methods of musts and wines. Content should be less than 1 µg/kg.

6.2 Total polycyclic aromatic hydrocarbons (PAH)

Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoroanthene and chrysene

Proceed with determination according to the method described in Chapter II of the International Oenological Codex. Content should be less than 30 µg/kg.

6.3 Benzo(a)pyrene

Proceed with determination according to the method described in Chapter II of the International Oenological Codex. Content should be less than 5 µg/kg.

6.4 Ashes

Without going above 550 °C, progressively incinerate the residue left by the determination of the loss on drying. The weight of the ashes should be less than 5%. A Higher concentration could mean that the extraction was done with non-authorized solvents.

6.5 Preparation of the test solution

Dissolve the ashes obtained from 2 g tannin into 1 mL diluted hydrochloric acid (R) and a drop of concentrated nitric acid (R). Heat in a water bath at 100 °C for a short time to ensure dissolution is complete. Transfer to a 50-mL calibrated flask, rinsing the capsule with distilled water, and make up to the mark. The test solution is ready to dose following elements.

6.6 Arsenic

For 0.25 g tannin, detect arsenic using the atomic-absorption-spectrophotometry method described in Chapter II of the International Oenological Codex or using ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis, after destruction of the organic matter using the wet method.The arsenic content should be less than 3 mg/kg.

6.7 Iron

On the basis of 10 mL of test solution prepared according to paragraph 6.5, determine the iron using atomic-absorption-spectrometry according to the method in Chapter II of the International Oenological Codex or using the ICP/MS method described in the Compendium of International Methods of Wine and Must Analysis.

The iron content should be less than 50 mg/kg, with the exception of chestnut-derived tannins, whose iron content should be less than or equal to 200 mg/kg .

6.8 Copper

Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis.Content should be less than 5 mg/kg.

6.9 Lead

Proceed with determination according to the method described in Chapter II of the

International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis.□Content should be less than 5 mg/kg.

6.10 Mercury

Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis.□Content should be less than 0.5 mg/kg.

6.11 Cadmium

Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis.□Content should be less than 0.5 mg/kg.

6.12 Salmonella

Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Absence should be checked on a 25 g sample of dry matter.

6.13 Total coliforms

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.□Content should be less than 30 CFU/g of dry matter.

6.14 Escherichia coli□

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.□Absence should be checked on a 25 g sample of dry matter.

6.15 Moulds

Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Content should be less than 100 CFU/g of dry matter.

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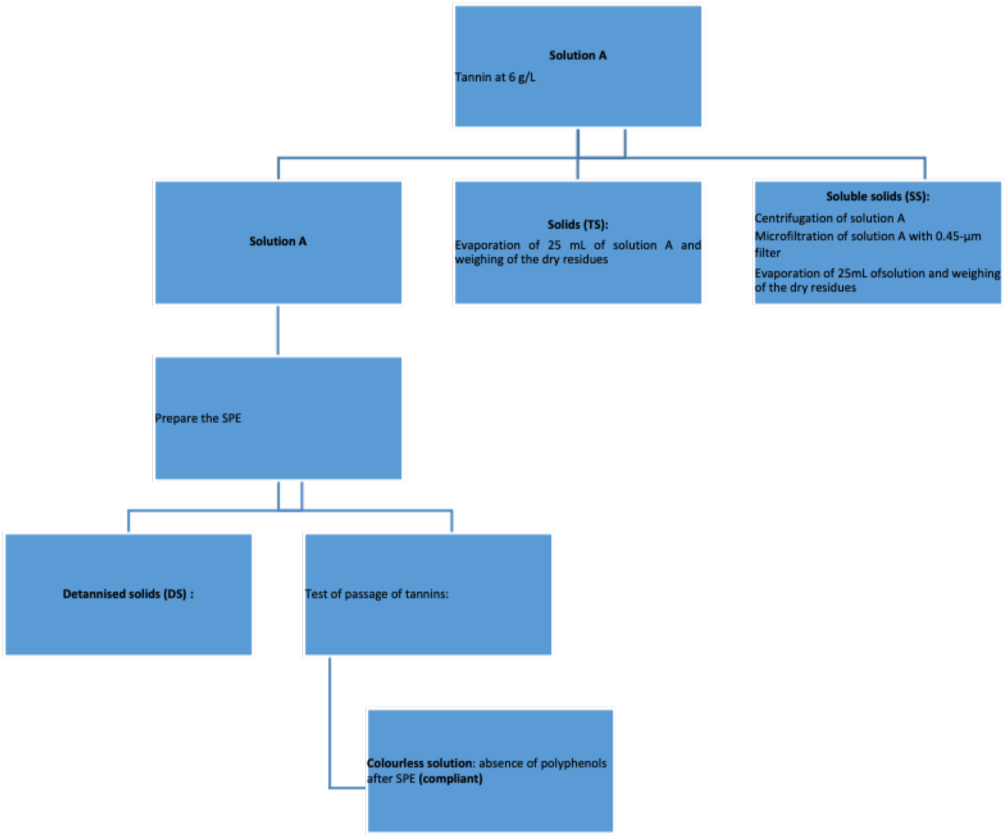
Annex 1 METHOD FOR THE ESTIMATION OF THE TOTAL POLYPHENOL CONTENT

1. PRINCIPLE

This method is designed to measure the concentration in polyphenols of preparations of oenological tannins and is based on gravimetric analysis using solid-phase

extraction (SPE). In an SPE column, tannins in aqueous solution are adsorbed onto a polymer (in this case polyvinylpolypyrrolidone) capable of retaining polyphenols. The substances not retained by the PVPP correspond to non-phenolic compounds that were present in the original sample.

The full diagram of the method is presented below:



2. REAGENTS, MATERIALS AND APPARATUS

2.1 Reagents

1. PVPP (approx. 100 µm polyvinylpolypyrrolidone, CAS No. 9003-39-8)
2. Aqueous solution of $FeCl_3$ (1 g/L)
3. Double-distilled water
4. Ethanol (20 % v/v)

2.2 Materials

1. Aluminium dishes (70 mL)
2. Disposable tubes with a conical base and stoppers (50 mL)
3. SPE columns (70 mL reservoir, 150 x 29,75 mm)
4. Frits for SPE column (diameter 27 mm, 20 µm PE)
5. 1 000-mL Pyrex flask
6. 50-mL Class-A test tubes
7. 0.45 µm Cellulose acetate membrane filter, Ø 47 mm
8. Plastic 50-mL syringe
9. 25-mL Class-A graduated glass pipettes (2 marks)

2.3 Apparatus

1. Thermostatic bath at 20 °C
2. Technical balance with precision of 0.01 g
3. Analytical balance with precision of 0.1 mg
4. Thermostatic oven at 105 °C
5. Thermostatic oven at 80 °C or, failing this, a thermostatic bath
6. Centrifuge
7. Vacuum manifold

2.3.8 Class-A volumetric glassware

2.3.9 Desiccator

3. PREPARATION OF SAMPLES

The solution (referred to below as solution A) is used to measure the total solids (TS), soluble solids (SS) and detannised solids (DS).

Weigh approximately 6 g tannins on the analytical balance and make a note of the weight. Dissolve the tannins in around 950 mL hot double-distilled water (60□70 °C) in a 1-L borosilicate-glass flask and mix well. Leave the flask to rest at room temperature for 30 minutes. Cool the solution at 20□22 °C in a thermostatic bath, make up to volume with double-distilled water and mix well.

4. PROCEDURE

4.1 Measurement of total solids (TS)

- Take a sample and transfer 25 mL of solution A to an aluminium dish (2.2.1),
- evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of the total solids (TS) is as follows:

$$\%TS = \frac{TS_dry_residues\ (g)}{tannins_weight\ (g)} \cdot \frac{1\,000}{solA\ (mL)} \cdot 100$$

4.2 Measurement of soluble solids (SS)

- Centrifuge solution A at 10 000 g during 5 minutes,
- microfilter solution A centrifuged through a membrane filter in order to obtain a clear solution, then leave 25 mL of solution to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of soluble solids (SS) is as follows:

$$\%SS = \frac{SS_dry_residues\ (g)}{tannins_weight\ (g)} \cdot \frac{1\,000}{solA\ (mL)} \cdot 100$$

4.3 Measurement of insoluble solids (IS)

Calculate the difference between the total solids and soluble solids:

$$\%IS = \%TS - \%SS$$

4.4 Measurement of detannised solids (DS)

- Prepare the SPE columns: introduce the first frit, 7.0 g ± 0.1 g PVPP rehydrated beforehand with a hydro-alcoholic solution at 20% for 15 minutes, as well as the second frit, then pack the stationary phase in well,
- place the SPE column in a vacuum manifold (example in Figure 1),
- pack the column with three rinses (do not dry out the PVPP and apply a vacuum of approx. 0.2 bar in order to avoid compacting of the polymer): a first rinse with 50 mL ethanol (20 % v/v), a second rinse with 50 mL double-distilled water, and a third rinse with 20 mL solution A in order to eliminate the water residues of the PVPP,
- add 30 mL of solution A to the column and collect 30 mL eluate (DS, detannised solids) in a 50-mL Falcon tube with a conical base, interrupting the elution when the liquid reaches the level of the upper frit,
- take a sample of 25 mL eluate and transfer this into an aluminium dish,
- leave to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of detannised solids (DS) is as follows:

$$\%DS = \frac{DS_dry_residues\ (g)\ -\ BC\ (g)}{tannins_weight\ (g)} \cdot \frac{1\,000}{solA\ (mL)} \cdot 100$$

where BC is the value of the blank measured after SPE (see 4.5).

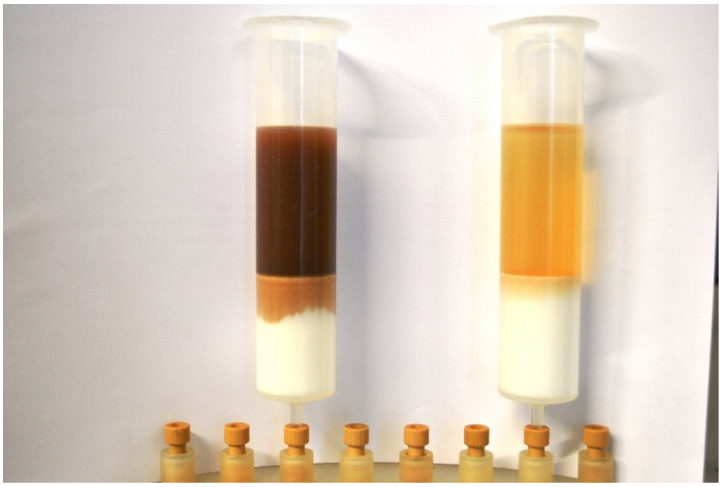


Figure 1 ▫ Example SPE extraction

In order to guarantee the absence of polyphenols in the eluate after passing through the column, add 3 drops of an aqueous solution of $FeCl_3$ to 3 mL detannised solids (DS) in solution. If the solution develops a blue-black hue, this means that the polyphenols have passed through the polymer; the analysis should then be repeated by reducing the quantity of initial product. If the solution remains colourless after this treatment, proceed with gravimetric analysis.

4.5 Measurement of the blank (BC)

A blank test should be carried out before conducting the SPE elution, in order to evaluate any interference caused by the analytical process. Proceed as follows:

- prepare the SPE columns: introduce the first frit, 7.0 g ± 0.1 g PVPP rehydrated beforehand with a hydro-alcoholic solution at 20% for 15 minutes, as well as the second frit, then pack in well,
- place the SPE column in a vacuum manifold (example in Figure 1),
- pack the column with two rinses (do not dry out the PVPP and apply a vacuum of approx. 0.2 bar in order to avoid compacting of the polymer): a first rinse with 50 mL ethanol (20 % v/v) and a second rinse with 70 mL double-distilled water,
- add 30 mL double-distilled water to the column and collect 30 mL eluate (blank for detannised solids) in a 50-mL Falcon tube with a conical base, interrupting the elution when the liquid reaches the level of the upper frit,
- take a sample of 25 mL eluate and transfer this into an aluminium dish, then leave to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residues (cool the dishes in the desiccator before weighing).

5. EXPRESSION OF RESULTS

Measurement of the percentage of total polyphenols (%polyphenols):

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

$$\%\text{polyphenols} = \frac{\%SS - \%DS}{\%TS} \cdot 100$$

- Determination of the suitability of the PVPP: refer to OENO 11-2002 - COEI-1-PVPP: 2007, § 6.