RESOLUTION OIV-OENO 574-2017

Monograph on tannins – UPDATE OF the method for determination of polyphenols

THE GENERAL ASSEMBLY,

In view of Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

At the proposal of the "Specifications of Oenological Products" Expert Group,

DECIDES to replace the point 6.9 "Estimation of total phenols" of the sheet COEI-1-TANINS of the *International Oenological Codex* with the following point:

6.9 Estimation of richness in total phenols

Total phenol richness is estimated according the method described in Annex.

For total phenols the results must be greater than 65%.

METHOD FOR THE ESTIMATION OF THE TOTAL POLYPHENOLS CONTENT

1. PRINCIPLE

This method will measure the polyphenol concentration of preparations of oenological tannins and is based on gravimetric analysis using solid-phase extraction (SPE). Tannins in aqueous solution are adsorbed onto a polymer in a SPE column – polyvinylpolypyrrolidone, in this case – able to retain the polyphenols. The substances not retained by the PVPP are non-phenolic compounds that were present in the original sample.

The complete diagram of the method is shown below:

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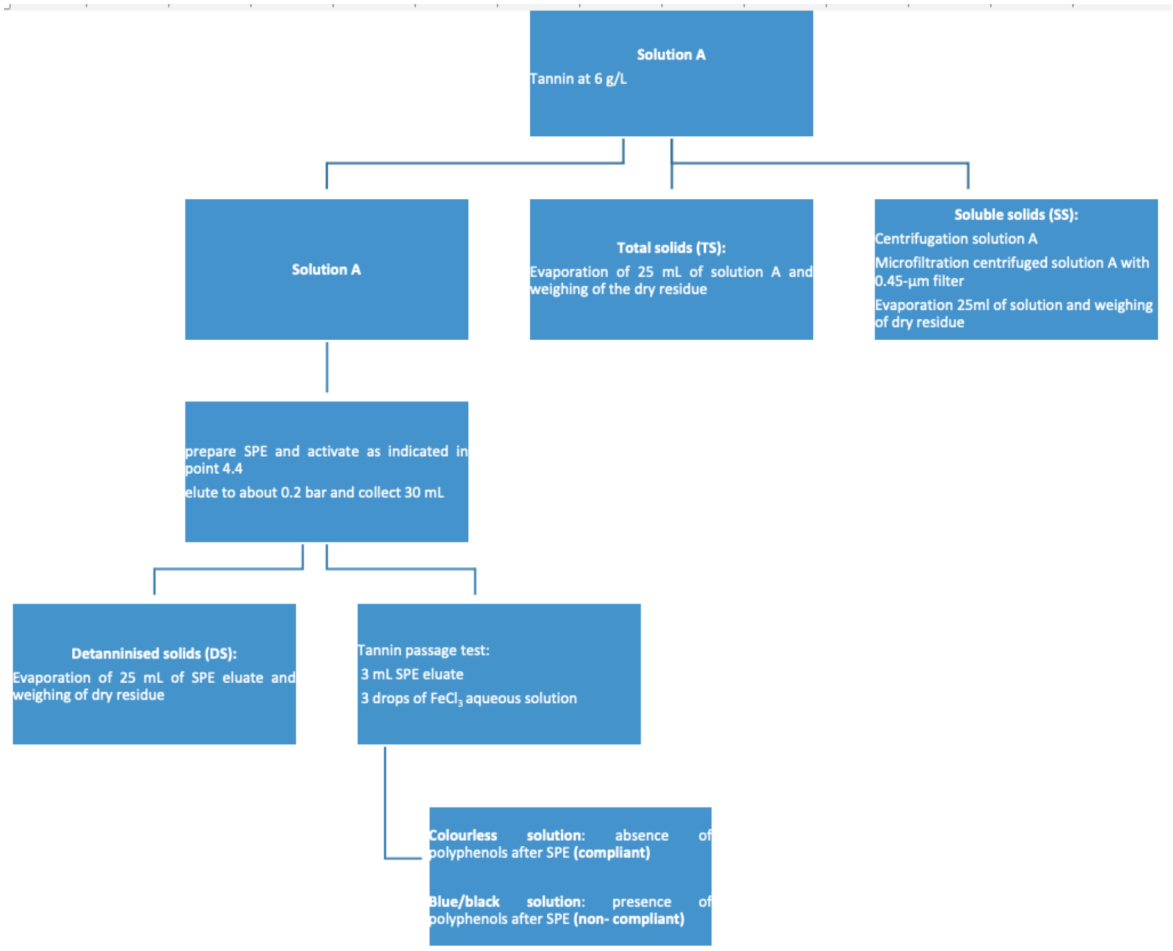

2. REAGENTS, MATERIALS, EQUIPMENT

2.1. Reagents

2.1.1. PVPP (polyvinylpolypyrrolidone [CAS No. 9003-39-8]

2.1.2. $FeCl_3$ aqueous solution (1 g/L)

2.1.3. Double-distilled water

2.1.4. Ethanol (20% v/v)

2.2. Materials

2.2.1. Aluminium dishes (70 mL)

2.2.2. Disposable tubes with caps (50 mL)

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2.2.3. SPE columns (70-mL reservoir, 150*29,75 mm)
2.2.4. SPE column frits (27-mm diameter – 20 µm PE)
2.2.5. 1000-mL Pyrex flask
2.2.6. Class A 50-mL cylinders
2.2.7. Cellulose acetate membrane filter 0.45 µm;; Ø 47 mm
2.2.8. Plastic syringe; 50 mL
2.2.9. Graduated glass pipettes (2 marks); 25 mL; Class A

2.3. Equipment

2.3.1. Bath thermostated to 20 °C
2.3.2. Technical balances with 0.01 g scale
2.3.3. Analytical balances with 0.1 mg scale
2.3.4. Oven thermostated to 105 °C
2.3.5. Oven thermostated to 80 °C or alternatively a thermostatic water bath
2.3.6. Centrifuge
2.3.7. Vacuum manifold
2.3.8. Q Class A volumetric glassware
2.3.9. Desiccator

3. PREPARATION OF SAMPLES

The solution (referred to as solution A) is used for measuring total solids (TS), soluble solids (SS) and detanninised solids (DS).

Weigh about 6 g of tannin on the analytical balance and record the weight. Dissolve the tannin in about 950 mL of warm (60–70 °C) double-distilled water in a litre Pyrex flask and shake well. Leave the flask to stand at room temperature for 30 minutes. Cool the solution in a bath thermostated to 20–22 °C, top up the volume with double-distilled water and mix well.

4. OPERATING MODE

4.1. Measuring total solids (TS):

- Collect and transfer 25 mL of solution A to an aluminium dish (see 2.2.1),
- evaporate in an oven thermostated to 80 °C until dry,

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- move to an oven thermostated to 105 °C to dry until constant weight and weigh the residue (cool the dishes in the desiccator before weighing).

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

$$\%TS = \frac{TS\_dry\_residue\ (g)}{weight\_of\_tanning\ (g)} \cdot \frac{1000}{(mL)solA}.100$$

4.2. Measuring soluble solids (SS):

- Centrifuge solution A at 10 000 g during 5 minutes,
- microfilter centrifuged solution A through the membrane filter in order to obtain a clear solution, then evaporate 25 mL of solution in an oven thermostated to 80 °C until dry,
- move to an oven thermostated to 105 °C to dry until constant weight and weigh the residue (cool the dishes in the desiccator before weighing).

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

$$\%TS = \frac{SS\_dry\_residue\ (g)}{weight\_of\_tanning\ (g)} \cdot \frac{1000}{(mL)solA}.100$$

4.3. Measuring insoluble solids (IS):

Calculate the difference between the total solids and the soluble solids as follows:

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

4.4. Measuring detanninised solids (DS):

- Prepare the SPE columns: introduce the first frit, 7.0 ± 0.1 g of PVPP previously rehydrated with a 20% hydroalcoholic solution for 15 minutes, and the second

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frit, then pack the stationary phase well,

- place the SPE column on the vacuum manifold (as in Figure 1, for example),
- activate the column with three washes (do not dry the PVPP and apply a vacuum of about 0.2 bar to avoid compacting the polymer): first wash with 50 mL ethanol (20% v/v), second wash with 50 mL double-distilled water and third wash with 20 mL solution A to eliminate water residue from the PVPP,
- add 30 mL solution A to the top of the column and collect the 30 mL of eluate (DS, detanninised solids) in a 50-mL Falcon tube, then stop elution when the liquid reaches the level of the upper frit,
- take 25 mL of eluate and transfer to an aluminium dish,
- evaporate in an oven thermostated to 80 °C until dry,
- move to an oven thermostated to 105 °C to dry until constant weight and weigh the residue (cool the dishes in the desiccator before weighing).

The formula to apply for the calculation of detanninised solids (DS) is as follows:

$$\%DS = \frac{DS\_dry\_residue\ (g) - BK\ (g)}{weight\_of\_tanning\ (g)} \cdot \frac{1000}{(mL)solA}.100$$

Where *BK* is the blank value measured after SPE (see 4.5).

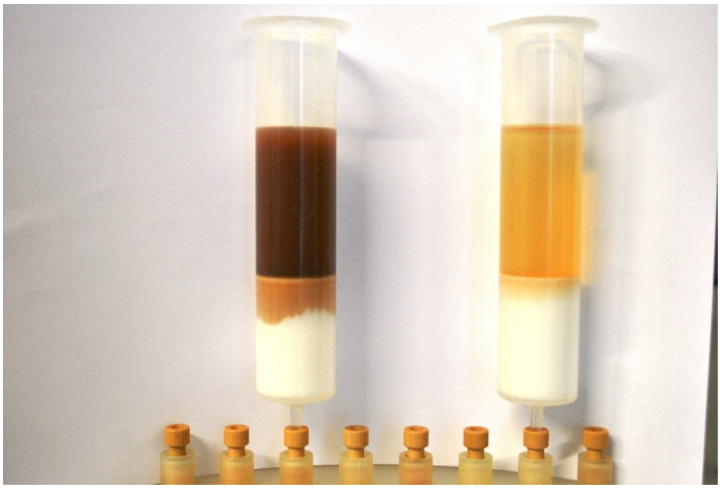

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Figure 1 – Example of SPE extraction

To ensure there are no polyphenols present in the eluate after passing through the column, add 3 drops of $FeCl_3$ aqueous solution to 3 mL of detanninised solids (DS) solution. If the solution develops a blueish-black hue, then polyphenols have passed through the polymer, so the analysis should be repeated reducing the initial product weight. If the solution remains colourless after this treatment, proceed with the gravimetric analysis.

4.5. Blank measurement (BK)

When performing SPE elution, a blank test is required before starting so as to assess any interference caused by the analytical process. Proceed as follows:

- prepare the SPE columns: introduce the first frit, 7.0 ± 0.1 g of PVPP previously rehydrated with a 20% hydroalcoholic solution for 15 minutes, and the second frit, then pack well,
- place the SPE column on the vacuum manifold (as in Figure 1, for example),
- activate the column with two washes (do not dry the PVPP and apply a vacuum of about 0.2 bar to avoid compacting the polymer): first wash with 50 mL ethanol (20% v/v), second wash with 70 mL double-distilled water,
- ~~add 30 mL double-distilled water to the top of the column and collect the 30 mL of eluate (blank for detanninised solids) in a 50 mL Falcon tube, then stop elution when the liquid reaches the level of the upper frit,~~
- take 25 mL of eluate and transfer to an aluminium dish, then evaporate in an oven thermostated to 80 °C until dry,
- move to an oven thermostated to 105 °C to dry until constant weight and weigh the residue (cool the dishes in the desiccator before weighing).

5. EXPRESSION OF RESULTS

Measuring the percentage of total polyphenols (%polyphenols):

The formula to apply for calculating the percentage of tannins is as follows:

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

- Measuring PVPP suitability: REFER TO OENO 11/2002 - COEI-1-PVPP: 2007, PARA. 6.

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