RESOLUTION OENO 12/2002

OENOLOGICAL TANNINS

THE GENERAL ASSEMBLY,

HAVING CONSIDERED Article 5 of the October 13, 1954 International Convention on Unification of the Methods of Analysis and Appraisal of Wines,

WITH THE PROPOSAL of the Sub Commission on Methods of Analysis and Appraisal of Wines,

DECIDES to replace the existing monograph by the following monograph in the aforementioned International Oenology Codex:

OENOLOGICAL TANNINS

N° SIN: 181

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Oenological tannins are extracted from nutgalls, or a wood rich in tannin: chestnut trees, oak, exotic wood, or grape seeds. Tannins are made up of a mixture of glucosides either from gallic acid (gallotannins), or from dilactone, ellagic acid (ellagitannins) (hydrolysable tannins) or from a mixture of proanthocyanidines (condensed tannins). Tannins are used to facilitate the clarification of wines and musts. Tannins must not change the olfactory properties and the colour of wine.

2. LABELLING

The nature of the extraction solvent (water or alcohol) , the botanical origin and an estimation of the total phenols contained must be clearly labelled.

3. CHARACTERISTICS

Oenological tannins range in colour from pale-yellow to reddish brown, with an astringent taste. Tannins are partially soluble in ethyl acetate, water-soluble, ethanol and methanol for condensed tannins and insoluble in most organic solvents, with the exception of ethanol and methanol for hydrolysable tannins.

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4. IDENTIFYING CHARACTERISTICS

4.1 – The aqueous solution of tannins produces, along with iron (III) salts, a blue/black precipitation between pH 3 and 5. This precipitation disappears with the addition of small quantities of strong acids.

4.2 – The aqueous solution of condensed tannin precipitate gelatine, egg whites, blood serum, etc. with a pH level between 3 and 6. Tannins precipitate alkaloids (quinine, strychnine) with a pH level between 4 to 6.

5. CHARACTERISATION

It is possible to characterise the botanical origin with the aid of the following criteria: ultraviolet absorption spectrum, flavanol content, proanthocyanidines, digallic acid, and scopoletine. (see appendix)

6. TEST TRIALS

6.1. Foreign matter

Tannin must be almost completely water-soluble and the content of insoluble substances should be under 2%, after shaking for 15 minutes 10 g of tannin in one litre of water.

6.2. Loss during drying

Determine the weight loss in an incubator at 100 – 105°C for 2 hours, of 2g of test solution. The weight must be constant and weight loss must be under 10%.

The limits below refer to the dry product.

6.3. Ashes

Incinerate progressively without going over 550 °C, the residue left over in the determination of loss during drying. The weight of the ashes should be under 4%.

6.4. Preparation of test solution

Take the ashes from 2 g of tannin by 1 ml of diluted hydrochloric acid (R) and one drop of concentrated nitric acid (R). Heat in 100°C water a little to dissolve. Pour this into a 50 ml volumetric flask. Rinse the capsule with distilled water and fill up the line on the flask.

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6.5. Arsenic

Take 0.25 g of tannin, and determine arsenic using the method described in Chapter II by atomic absorption spectrometer, after destroying organic matter by the wet method. (Arsenic content must be under 3 mg/kg).

6.6. Iron

Add 2 ml of 5% potassium thiocyanate solution (R) and 1 ml of concentrated hydrochloric acid (R) to 10 ml of test solution prepared according to article 6.4. The resulting colour should not be more intense than the control sample prepared with 2ml of iron (III) salt solution at 0.010 g of iron per litre (R), 8 ml of water and the same volumes of the same reagents. (Iron content must be less than 50 mg/kg). It is also possible to measure the iron with the atomic absorption spectrometer.

6.7. Lead

Measure the lead in the solution prepared according to article 6.4 and using the method outlined in the Compendium of International Methods of Analysis of Wine and Musts by atomic absorption spectro-photometer. Content must be less than 5 mg/kg.

6.8. Mercury

Measure the mercury using the method outlined in Chapter II by atomic absorption spectrometer. Content must be less than 1 mg/kg.

6.9. Estimation of total phenols

On an aqueous tannin solution at 1 g/l diluted to 1/100th, measure the absorbency at 280 nm on an optical path of 1 mm. Total phenol content is given in gallic acid equivalents/g and transformed in p. 100 of tannin powder. For total phenols the results must be greater than 65%.

6.10. Nature of tannins

6.10.1 - Proanthocyanidic tannins are estimated by the DMACH method: mix 5 ml of reagent (100 mg of dimethylaminocinnamaldehyde + 10 ml of 12 M HCl solution; after bring to 100 ml with methanol) to 1 ml of aqueous tannin solution (1g/l). Wait 10 minutes; take a reading of the absorbency at 640 nm on 1 mm optical path. The results are given in equivalent catechin. The result for condensed tannins must be greater than 10 mg/g.

6.10.2 – The nitrous acid method is used to estimate ellagitannins. Mix 1 ml of

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aqueous tannin solution (1 g/l), 1 ml of methanol and 160 µl of 6% acetic acid (m/v). Displace the oxygen by nitrogen sparging for 10 minutes, add 160 µl of 6% sodium nitrite (m/v) followed by a brief nitrogen sparging (1 mn), the tube is vacuum sealed and its reaction takes in 60 mn in water bath at 30°C. The intensity of the colour is measured by absorbency at 600 nm. The results are estimated in mg/g in equivalents of castalagine ($\varepsilon_{600\ nm}$: 983 g-1). For hydrolysable tannins and ellagic type, the result must be greater than 20 mg/g.

6.10.3 – Gallic like hydrolysable tannins correspond to other categories of products, and test negatively to 6.10.1 and 6.10.2.

6.11. Extraction process

6.11.1. IS solubility indicator

It is expressed in % of solubility for 5 g of tannin in 100 ml of diethylether/ethanol (9/1, v/v) mixture. For tannins extracted from water, the indicator must be less than 5.

6.11.2. Iex extractability indicator:

$$IEx = (D.0._{370\ nm} \times 2) - (D.O._{350nm} + D.0._{420\ nm}$$

When lEx is greater than 0.05, the products come solely from extraction by water.

7. STORAGE CONDITIONS

Oenological tannins must be kept in sealed closed packages.

Declaration of Danemark

"When differences in specifications of purity, definitions and analytical methods exist between OIV and other competent intergovernmental organizations, such as Codex Alimentarius and European Union, Denmark believes that every possible effort must be done to identify why these differences exist and to reconcile them as far as possible, in order to avoid the existence of different international regulations on the same subject."

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APPENDIX IDENTIFICATION OF THE BOTANICAL ORIGINS OF OENOLOGICAL TANNINS

MATERIALS AND METHODS

Principle

The recognition of the botanical origin of oenological tannins requires the formulating of the following observations in order:

1. The presence of condensed tannins taken from grapes,
2. The presence of tannins from nutgalls,
3. The presence of tannins from exotic wood,
4. Differentiating the tannin from oak and the tannin for chestnut wood.

Tannins from grapes is characterized by high content of flavanols, as expressed in (+) catechin.
Nutgall tannins have a high content of digallic acid.
The ultraviolet spectrum for tannins from exotic wood has a specific peak.
Tannins from oak trees are richer in coumarines, in particular scopoletine, than chestnut tannins.

Equipment and analytical conditions

- Laboratory glassware.
- Magnetic mixer.
- UV/visible absorption spectrophotometer double beam.
- 1 cm optical pathway glass cuvette
- 1 cm optical pathway quartz cuvette,
- 100° C water bath (optional)
- Heated rotating evaporator
- Composed chromatographic system (as an example):

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- pressure gradient pump for binary mixtures
- an injector equipped with a 20-µl loop
- a spectrophotometer detector with wave length 280 nm
- a fluorimetric detector

An reversed phase column (C18) diameter of particles 5µm,
dimensions of the column: 20 cm X 4.6 mm to measure the gallic acid and the scopoletine.

- pH meter.

Reagents and reference solutions

- para-dimethylaminocinnamaldehyde
- concentrated hydrochloric acid solution(R)
- (+) catechin
- digallic acid
- absolute ethanol
- ethyl acetate
- concentrated sodium hydroxide solution(R)
- methanol
- ethyl ether
- acetonitrile
- acetic acid
- scopoletine
- umbelliferone
- distilled water or demineralised or ultra filtered water.

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Preparation of reagents

p-dimethylaminocinnamaldehyde (p-DACA) solution

100 mg of p-DACA are put into a solution of 10 ml 12 M hydrochloric acid and 90 ml of methanol.

Elution solvents for digallic acid

- solvent A: pure methanol
- solvent B: perchloric acid solution in water at pH 2,5

Elution solvents for scopoletine

- solvent A: distilled water containing 3% acetic acid
- solvent B: acetonitrile containing 3% acetic acid

Preparation of reference solutions

(+) catechin solution

Dissolve 10 mg of (+) catechin in 1 l of distilled water

Digallic acid solution at 100 mg / litre of distilled water

Scopoletine solution at 20 µg / litre of distilled water.

Operating methods

There are 2 methods for identifying the presence of grapes tannins:

Measuring total flavanols.

5 ml of p-DACA reagent are added to 1 ml of aqueous solution at 200mg / l of tannin.

After 10 mn measure the absorption of the mixture at 640 nm in a glass cuvette with an optical path of 10 mm.

The absorbance values are then read from the calibration curve obtained from an increasing concentration range in (+) catechin analysed under the same conditions.

Measuring proanthocyanic tannins.

Add 2 ml of distilled water and 6 ml of 12 M hydrochloric acid to 4 ml of solution of 200 mg/l of tannin in a hydrolysis tube. This tube is heated to 100 °C for 30 mn and

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cooled in a cold bath.

A second tube containing the same mixture stays at room temperature for the same amount of time.

Then, 1 ml of ethanol is placed in both tubes and the absorbance values are measured at 550 nm.

The difference between the 2 absorbance values is multiplied by 380 to give the Proanthocyanic tannin content.

Identification of tannins from nutgall

20 ml of aqueous tannin solution at 50 mg/l is brought to pH 7 with the aid of a concentrated sodium hydroxide solution (R).

An initial series of extractions carried out 3 times 20 ml of ethyl acetate to eliminate neutral substances.

Secondly, the aqueous state is brought to pH 2 by the addition of concentrated hydrochloric acid solution (R). and then followed by a new series of 3 extractions with ethyl acetate.

After the evaporation of the ethyl acetate, the residue is taken by 20 ml of methanol then analysed by chromatograph under the following conditions: (as an example): injected volume: 20 µl of extract or standard digallic acid solution

Detection at 280 nm

Composition of an elution gradient:

- from 10 to 20% of solvent A in 35 mn
- from 20 to 40% of solvent A in 15 mn
- from 40 to 98% of solvent A in 20 mn

Mobile phase flow: 0.8 ml / mn.

Identification of tannins from exotic wood

Prepare an aqueous solution of tannin so that when placed in a 1 cm optical pathway quartz cuvette. The solution has an absorbency measured at 280 nm between 1 and 1.5.

Carry out a continuous absorbency readings between 250 and 300 nm.

Note the presence or the absence of a maximum absorption peak.

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Identification of tannins from oak or chestnut

Scopoletine contained in the 20 ml aqueous solution of tannin at 5 g/l is extracted 3 times with 20 ml of ethylic ether.

After the total recuperation and evaporation of the ether phase, the extract is taken from 50 ml of water and then analysed by chromatography under the following conditions : (as an example):

Injected volume: 20 µl of extract or scopoletine reference solution fluorimetric detection:

- excitation wavelength: 340 nm,
- emitting wavelength: 425 nm

Composition of an elution gradient:

- 94% of solvent A during 10 mn
- from 94 to 85% in 20 mn
- from 82 to 67% in 5 mn
- from 37 to 42% in 5 mn.
- Mobile phase flow: 1 ml/mn

CONCLUSION

Tannin is recognised as being from grapes when the total flavanol content, expressed as (+) catechin is over 50 mg/g or its proanthocyanic tannin content is over 0.5 mg/g.

Tannin is recognized as coming from nutgall when digallic acid content is between 4 and 8 mg/g.

Tannin is recognized as coming from exotic wood when its spectrum reveals an absorption peak between 270 and 280 nm.

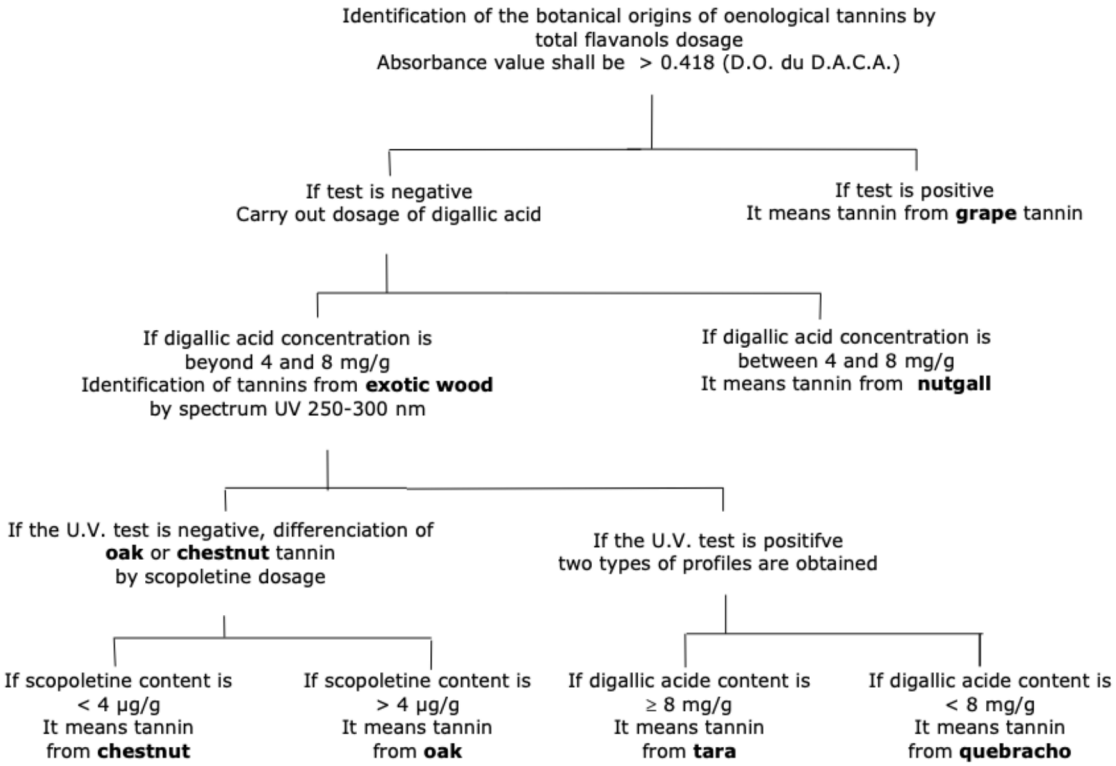
Tannin is recognized as coming from oak when scoploletine content is over 4 µg/g .

Tannin is recognized as coming from chestnut trees when its scopoletine content is equal to or less than 4 µg/g and if it is not identified as coming from another origin.

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BOTANICAL ORIGINS
CONCLUSION



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