OIV-MA-BS-19 Overall determination of phenolic compounds in spirituous beverages of viti-viniculture origin without added caramel

Type IV method

1. Definition

Folin-Ciocalteu assay measures the total quantities of phenolic compounds originating from wood present in barrel-aged spirits that haven't received any added caramel.

This assay is not specific to phenolic compounds (cf. principle). Caramel also reacts to the Folin-Ciocalteu reagent. However, in the case of wood-aged spirituous beverages, the vast majority of results are related to the presence of phenolic compounds derived from the oak wood (VIDAL and Al, 1991).

Folin-Ciocalteu phenolic compound content corresponds to the response to the result described below. This result is expressed in mg of gallic acid/l by calibration.

2. Principle

All the phenolic compounds are oxidized by the Folin-Ciocalteu reagent. This reagent consists of a mixture of phosphotungstic acid and phosphomolybdic acid which is reduced, during the oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides.

The blue colouring produced has a maximum absorption of about 750-760 nm. It is proportional to the quantity of oxidized phenolic compounds.

3. Apparatus

1. Standard laboratory apparatus, and in particular:

   - Temperature-controlled bath (70° C), spectrophotometer.

4. Reagents

4.1. Folin-Ciocalteu reagent

This reagent is available for purchase ready to use. It can be prepared in the following manner:

- 100 g of sodium tungstate (No. CAS : 13472-45-2),
- 25 g of sodium molybdate (No. CAS : 7631-95-0), are dissolved in 700 ml of distilled water (No. CAS : 7732-18-5).

Add:

- 50 ml of 85% phosphoric acid (No. CAS : 7664-38-2) (ρ20=1.71 g/mL),
- 100 ml of concentrated chlorhydric acid (No. CAS : 7647-01-0) (ρ20=1.19 g/mL).

Bring to a boil under reflux for 10 hours, then add:

- 150 G of lithium sulphate (No. CAS : 10377-48-7),
- a few bromine drops (No. CAS : 7726-95-6),
- and bring to a boil for another 15 minutes. Cool and add 1 litre of distilled water.

4.2. Anhydrous sodium carbonate

- Prepare a 4.25% solution (m/v) in distilled water.

4.3. Anhydrous gallic acid (No. CAS : 149-91-7),

5. Procedure

1. Calibration in gallic acid

Produce a hydroalcoolic gallic acid stock solution by weighed quantity, then some surrogate solutions by dilution (at least 2). The calibration range also includes a blank (hydroalcoolic solution). As an example, the range can include the following levels: 0.200 and 400 mg/L. Check the linearity of the calibration.

5.2. **Preparation of the samples**

The sample must be perfectly limpid and free of beeswing.

5.3. **Reaction**

In a test tube, introduce:

- 0.2 ml of sample (or of calibration solution)
- 1 ml of Folin-Ciocalteu reagent,

- 18.8 ml of sodium carbonate solution.

After stirring, bring to approximately 70°C for 20 minutes in the temperature-controlled bath, then cool under running cold water.

5.4. Absorptance measurement at 760 Nm

Absorbance at 760 nm is measured under a 1 cm optical path.

6. Expression of results

Express the result in mg of gallic acid/L (linear calibration), accounting for the possible dilution of the sample. If the absorptance is greater than 1, a new measurement is carried out after dilution of the sample, if linearity is guaranteed.

7. References

- BLOUIN J., LLORCA L., MONTREAU F.R., DUFOUR J.H., 1972. Etude des conditions optimales pour la détermination des composés phénoliques totaux par le réactif de Folin-Ciocalteu. Connaissance de la vigne et du vin, 6, 405-413.
- VIDAL J.-P., CANTAGREL R., FAURE A., BOULESTEIX J.M., 1991**.** Comparaison de trois méthodes de dosage des composés phénoliques totaux dans les spiritueux. FV OIV n°904.