OIV-MA-AS313-13A L-Ascorbic acid

Type IV method

1. Principle

The following methods enable the presence of L-ascorbic acid and dehydroascorbic acid in wines or musts to be determined.

Ascorbic acid is converted on activated carbon to dehydroascorbic acid. The latter forms a fluorescent compound on reaction with orthophenylenediamine (OPDA). A control prepared in the presence of boric acid enables spurious fluorescence to be determined (by the formation of a boric acid/dehydroascorbic acid complex). The sample and the control are analyzed fluorometrically and the concentration of dehydroascorbic acid calculated.

2. **Method (fluorimetric method)**

2.1. *Apparatus*

2.1.1. Fluorometer.

A spectrofluorometer equipped with a lamp giving a continuous spectrum and using it at minimum power.

The optimum excitation and emission wavelengths for the test are to be determined beforehand and depend on the equipment used. As a guide, the excitation wavelength will be approximately 350 nm and the emission wavelength approximately 430 nm. Cells of 1 cm path length.

2.1.2. Sintered glass filter of porosity 3.

2.1.3. Test tubes (diameter approximately 10 mm).

2.1.4. Stirring rods for test tubes.

2.2. Reagents

2.2.1. Orthophenylenediamine dihydrochloride solution ($C_6H_{10}Cl_2N_2$), 0.02 % (*m/v*), prepared just before use.

2.2.2. Sodium acetate trihydrate solution ($CH_3COONa.\ 3H_2O$), 500 g/L.

2.2.3. Mixed solution of boric acid and sodium acetate:

Dissolve 3 g of boric acid, (H_3BO_3) in 100 mL of a 500 g/L sodium acetate solution.

This solution must be prepared just before use.

2.2.4. Acetic acid solution (CH_3COOH) 56%: glacial acetic acid (ρ_{20}= 1.05 g/mL), diluted to 56% (*v/v*), pH approximately 1.2.

2.2.5. L-Ascorbic acid standard solution, 1 g/L.

Just before use, dissolve 50 mg of L-ascorbic acid previously dehydrated in a desiccator and protected against light, in 50 mL of acetic acid solution (2.2.4).

2.2.6. Very pure analytical grade activated carbon.

Place 100 g of activated carbon into a 2-liter conical flask and add 500 mL aqueous hydrochloric acid solution, 10% (*v/v*), (ρ_{20}= 1.19 g/mL). Bring to a boil, and filter through a sintered glass filter of porosity 3. Collect the carbon treated in this way in a 2-liter conical flask. Add 1 liter of water, shake and filter using a sintered glass filter of porosity 3. Repeat this operation two more times. Place the residue in an oven controlled to 115°C ± 5 °C for 12 hours (or overnight).

2.3. *Procedure*

2.3.1. Preparation of the sample of wine or must

Take a volume of the wine or must and dilute to 100 mL in a graduated flask with the acetic acid solution, 56% (2.2.4), in order to obtain a solution with an ascorbic acid concentration between 0 and 60 mg/L. Thoroughly mix the contents of the flask by shaking. Add 2 g of activated carbon and allow to stand for 15 minutes, shaking occasionally. Filter using ordinary filter paper, discarding the first few milliliters of filtrate.

Pipette 5 mL of the filtrate into two 100 mL graduated flasks. Add to the first 5 mL of the mixed solution of boric acid and sodium acetate solution (2.2.3) (sample blank) and to the second 5 mL of the sodium acetate solution (2.2.2) (sample). Allow to stand for 15 minutes, stirring occasionally. Make to 100 mL with distilled water. Pipette 2 mL from the contents of each flask into a test tube and add 5 mL of orthophenylenediamine solution. Stir with the stirring rod and allow the reaction proceed for 30 minutes in the dark and then make the spectrofluorometric measurements.

2.3.2. Preparation of the calibration curve.

Into three 100 mL graduated flasks pipette 2, 4, and 6 mL respectively of the standard ascorbic acid solution (2.2.5), make to 100 mL with acetic acid solution and thoroughly mix by stirring. The standard solutions prepared in this way contain 2, 4 and 6 mg per 100 mL of L-ascorbic acid respectively.

Add 2 g of activated carbon to each of the flasks and allow to stand for 15 minutes, stirring occasionally. Filter through ordinary filter paper, discarding the first few milliliters. Pipette 5 mL of each filtrate into three 100 mL graduated flasks (first series). Repeat the operation and obtain a second series of three graduated flasks. To each of the flasks in the first series (corresponding to the blank test) add 5 mL of the mixed solution of boric acid and sodium acetate (2.2.3), and to each of the flasks in the

second series add 5 mL of the sodium acetate solution (2.2.2). Let stand for 15 minutes, stirring

occasionally. Make up to 100 mL with distilled water. Take 2 mL of the contents of each flask; add 5 mL of orthophenylenediamine solution. Stir and allow the reaction to proceed for 30 minutes in the dark and then make the spectrofluorometric measurements.

2.3.3. Fluorometric determination

Set the zero on the scale of measurement using the corresponding control test sample for each solution. Measure the intensity of the fluorescence for each solution over the calibration range and for the solution to be determined. Plot the calibration curve, which should be a straight line passing through the origin. From the graph determine the concentration C of ascorbic acid and dehydroascorbic acid in the solution analyzed.

2.4. Expression of results

The concentration of L-ascorbic acid and the dehydroascorbic acid in the wine in milligrams per liter is given by:

$$C \times F$$

where F is the dilution factor.

Bibliography

- AFNOR standard, 76-107, ARNOR, Tour Europe, Paris.
- PROM T., *F.V., O.I.V.*, 1984, n° 788.