OIV-MA-AS322-05B Iron

Type IV method

1. Principle

After digestion in hydrogen peroxide, 30%, the total iron, present as Fe (III) state, is reduced to the Fe (II) and quantified by the formation of a colored *ortho*-phenanthroline complex.

2. Method

2.1. Apparatus

2.1.1. Kjeldahl flask, 100 mL.

2.1.2. Spectrophotometer enabling measurements to be made at a wavelength of 508 nm.

2.2. Reagents

2.2.1. Hydrogen peroxide, H_2O_2, 30% (*m/v*), solution, iron free.

2.2.2. Hydrochloric acid, 1 M, iron free.

2.2.3. Ammonium hydroxide (ρ_{20} = 0.92 g/mL).

2.2.4. Pumice stone grains, pretreated with boiling hydrochloric acid_diluted 1/2 and washed with distilled water.

2.2.5. Hydroquinone solution, $C_6H_6O_2$, 2.5%, acidified with 1 mL concentrated sulfuric acid (ρ_{20} = 1.84 g/mL) per 100 mL of solution. This solution must be kept in an amber bottle in the refrigerator and discarded at the slightest sign of darkening.

2.2.6. Sodium sulfite solution, Na_2SO_3, 20%, prepared from neutral anhydrous sodium sulfite.

2.2.7. *ortho*-phenanthroline solution, $C_{12}H_8N_2$, 0.5%, in alcohol, 96% vol.

2.2.8. Ammonium acetate solution, CH_3COONH_4, 20% (*m/v*).

2.2.9. Fe (III) solution containing 1 g of iron per liter. Use of a commercial solution is preferred. Alternatively, a 1000 mg/L Fe (III) solution can be prepared by dissolving 8.6341 g of ferric ammonium sulfate, $FeNH_4(SO_4)_2.12H_2O$, in 100 mL of hydrochloric acid, 1 M, and making up the volume to one liter with the hydrochloric acid, 1 M.

2.2.10. Dilute standard iron solution containing 100 milligrams of iron per liter.

2.3. Procedure

2.3.1. Digestion

2.3.1.1. For wines with sugar content below 50 g/L

Combine 25 mL of the wine, 10 mL of the hydrogen peroxide solution and a few grains of pumice into the 100 mL Kjeldah1 flask. Concentrate the mixture to a volume of 2 to 3 mL by heating. Allow to cool and add sufficient ammonium hydroxide to make the residue alkaline thus precipitating hydroxides while taking care not to wet the walls of the flask.

After cooling, carefully add hydrochloric acid, to the alkaline liquid to dissolve the precipitated hydroxides and transfer the resulting solution to a 100 mL volumetric flask. Rinse the Kjeldahl flask with hydrochloric acid, and combined the solutions in the volumetric flask and make up to 100 mL.

2.3.1.2. For musts and wines with sugar content above 50 g/L

- If the sugar content is between 50 and 200 g/L, the 25 mL wine sample is treated with 20 mL of hydrogen peroxide solution. Continue as in 2.3.1.1.
- If the sugar content is greater than 200 g/L, the samples of wine or must should be diluted 1/2 or possibly 1/4 before being treated with 20 mL of hydrogen peroxide solution. Continue as in 2.3.1.1.

2. Blank experiment

Carry out a blank trial with distilled water using the same volume of hydrogen peroxide solution as the amount used for the mineralization, following the experimental protocol described in 2.3.1.1.

2.3.3. Determination

Introduce 20 mL of the hydrochloric acid wine digest solution and 20 mL, of the hydrochloric acid solution obtained from the 'blank experiment' into two separate 50 mL volumetric flasks. Add 2 mL of hydroquinone solution, 2 mL of sulfite solution and 1 mL of *ortho*-phenanthroline. Allow to stand for 15 minutes, during which time Fe (III) is reduced to Fe (II). Then add 10 mL of ammonium acetate solution, make each up to 50 mL with distilled water and shake the two volumetric flasks. Use the solution originating from the blank experiment to zero the absorbance scale at 508 nm and measure the absorbance of the wine solution at the same wavelength.

2.3.4. Calibration

Place 0.5, 1, 1.5 and 2 mL of the 100 mg of iron per liter solution into each of four 50

mL volumetric flasks, and add 20 mL of distilled water to each. Carry out the procedure described in 2.3.3 to measure the absorbance of each of these standard solutions, which contain 50, 100, 150 and 200 micrograms of iron respectively.

2.4. Expression of results

2.4.1. Method of calculation

Plot a graph giving the variation in absorbance as a function of the iron concentration in the standard solutions. Record the absorbance of the test solution and read off the iron concentration C in the hydrochloric acid digestion solution, i.e. in 5 mL of the wine being analyzed.

The iron concentration in milligrams per liter of the wine to one decimal place is given by: 200 □ C

If the wine (or must) has been diluted, the iron concentration in milligrams per liter of the wine to one decimal place is given by: 200 □ F □ C

where F is the dilution factor.