OIV-MA-AS313-09 Citric acid

Type II method

1. Principle

Citric acid is converted into oxaloacetate and acetate in a reaction catalyzed by citratelyase (CL):

$$Citrate \overset{CL}{\leftrightarrow} oxaloacetate + acetate$$

In the presence of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), the oxaloacetate and its decarboxylation derivative, pyruvate, are reduced to L□malate and L□lactate by reduced nicotinamide adenine dinucelotide (NADH):

$$oxaloacetate + NADH + H^{+} \overset{MDH}{\longleftrightarrow} L\ malate + NAD^{+}$$

$$pyruvate + NADH + H^{+} \overset{LDH}{\longleftrightarrow} L - lactate + NAD^{+}$$

The amount of NADH oxidized to NAD+ in these reactions is proportional to the amount of citrate present. The oxidation of NADH is measured by the resultant decrease in absorbance at a wavelength of 340 nm.

2. Apparatus

2.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorbance of NADH is a maximum.

Alternatively, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 nm or 365 nm, may be used.

Since absolute absorbance measurements are involved (i.e. calibration curves are not used but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbance of the apparatus must be checked.

2.2. Glass cells with optical path lengths of 1 cm or single□use cells.

2.3. Micropipettes for pipetting volumes in the range 0.02 to 2 mL.

3. Reagents

1. Buffer solution pH 7.8 (glycylglycine, 0.51 M; pH 7.8; Zn^{+}(0.6 x 10^{-3} M):

dissolve 7.13 g of glycylglycine in approximately 70 mL of double distilled water. Adjust the pH to 7.8 with approximately 13 mL sodium hydroxide solution, 5 M, add 10 mL of zinc chloride, $ZnCl_8$, (80 mg in 100 mL double distilled water) solution and make up to 100 mL with double distilled water.

3.2. Reduced nicotinamide adenine dinucleotide, NADH, solution (approximately 6 x $10^{\square 3}$M): dissolve 30 mg NADH and 60 mg $NaHCO_3$ in 6 mL of double distilled water.

3.3. Malate dehydrogenase/lactate dehydrogenase solution (MDH/LDH) (0.5 mg MDH/mL; 2.5 mg LDH/mL): mix together 0.1 mL MDH (5 mg MDH/mL), 0.4 mL ammonium sulfate solution, 3.2 M, and 0.5 mL LDH (5 mg/mL).

This suspension remains stable for at least a year at 4°C.

3.4. Citrate□lyase (CL, 5 mg protein/mL): dissolve 168 mg lyophilisate in 1 mL ice□cold water. This solution remains stable for at least a week at 4°C and for at least four weeks if frozen.

It is recommended that, prior to the determination, the enzyme activity should be checked.

3.5. Polyvinylpolypyrrolidone (PVPP).

Note: All the reagents above are available commercially.

4. Preparation of the sample

Citrate determination is normally carried out directly on wine, without preliminary removal of pigmentation (coloration) and without dilution, provided that the citric acid content is less than 400 mg/L. If not, dilute the wine until the citrate concentration lies between 20 and 400 mg/L (i.e. between 5 and 80 µg of citrate in the test sample).

With red wines that are rich in phenolic compounds, preliminary treatment with PVPP is recommended:

Form a suspension of about 0.2 g of PVPP in water and allow to stand for 15 min. Filter using a fluted filter.

Place 10 mL of wine in a 50 mL conical flask, add the moist PVPP removed from the filter with a spatula. Shake for two to three minutes. Filter.

5. Procedure

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbance using the 1 cm cells, with air as the zero absorbance (reference) standard (no cell in the optical path). Place the following in the 1 cm cells:

	Reference cell	Sample cell
Solution 3.1	1.00	1.00
Solution 3.2	0.10	0.10
Sample to be measured	-	0.20
Double distilled water	2.00	1.80
Solution 3.3	0.02	0.02

Mix, and after about five min read the absorbance of the solutions in the reference and sample cells (A_1).

Solution 3.4	0.02 mL	0.02 mL

Mix; wait until the reaction is completed (about five min) and read the absorbance of the solutions in the reference and sample cells (A_2).

Calculate the absorbance difference ($A_1 - A_2$) for the reference and sample cells, ΔA_S and ΔA_R.

Finally, calculate the difference between those differences:

$$\Delta A = \Delta A_S - \Delta A_R$$

Note: The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

6. Expression of results

Citric acid concentration is given in milligrams per liter to the nearest whole number.

6.1. Method of calculation

The general formula for calculating the concentration in mg/L is:

$$C = \frac{V \times M}{\varepsilon \times d \times v} \Delta A$$

where:

V = volume of test solution in mL (3.14 mL)

v = volume of the sample in mL (0.2 mL)

M = molecular mass of the substance to be determined (for anhydrous citric acid, M = 192.1)

d = optical path in the cell in cm (1 cm)

□ = absorption coefficient of NADH, (at 340 nm, □ = 6.3 mmol-1 x l x cm-1).

so that:

$$C = 479 \times \Delta A$$

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

□ At 334 nm: C = 488 x ΔA (□= 6.3 mmol□1 x l x cm□1).

□ At 365 nm: C = 887 x ΔA (□= 3.4 mmol□1 x l x cm□1).

6.2. *Repeatability (r)*

Citric acid concentration less than 400 mg/L: *r* = 14 mg/L.

Citric acid concentration greater than 400 mg/L: *r* = 28 mg/L.

6.3. *Reproducibility (R)*

Citric acid concentration less than 400 mg/L: *R* = 39 mg/L.

Citric acid concentration greater than 400 mg/L: *R* = 65 mg/L.

Bibliography

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