OIV-MA-AS313-07 Lactic acid

Type II method

1. Principle

Total lactic acid (L□lactate and D□lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) to pyruvate in a reaction catalyzed by L□lactate dehydrogenase (L□LDH) and D□lactate dehydrogenase (D□LDH).

The equilibrium of the reaction normally lies more strongly in favor of the lactate. Removal of the pyruvate from the reaction mixture displaces the equilibrium towards the formation of pyruvate.

In the presence of L□glutamate, the pyruvate is transformed into L□alanine in a reaction catalyzed by glutamate pyruvate transaminase (GPT):

(1) L-lactate + $NAD^+ \overset{L-LDH}{\underset{\rightleftarrows}{}}$ pyruvate +NADH+ H^+

(2) D-lactate + $NAD^+ \overset{L-LDH}{\underset{\rightleftarrows}{}}$ pyruvate +NADH+ H^+

(3) Pyruvate + L-glutamate $\overset{L-GPT}{\underset{\rightleftarrows}{}}$ L-alanine+ □-ketoglurarate

The amount of NADH formed, measured by the increase in absorbance at the wavelength of 340 nm, is proportional to the quantity of lactate originally present.

Note: L□lactic acid may be determined independently by using reactions (1) and (3), while D□lactic acid may be similarly determined by using reactions (2) and (3).

2. Apparatus

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

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

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

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

3. Reagents

Double□distilled water

3.1. Buffer solution, pH 10 (glycylglycine, 0.6 M; L□glutamate, 0.1 M):

Dissolve 4.75 g of glycylglycine and 0.88 g of L□glutamic acid in approximately 50 mL of double distilled water; adjust the pH to 10 with a few milliliters sodium hydroxide, 10 M, and make up to 60 mL with double distilled water.

This solution will remain stable for at least 12 weeks at 4°C.

3.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 40 x 10□3 M: dissolve 900 mg of NAD in 30 mL of double distilled water. This solution will remain stable for at least four weeks at 4°C.

3.3. Glutamate pyruvate transaminase (GPT) suspension, 20 mg/mL.

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

3.4. L□lactate dehydrogenase (L□LDH) suspension, 5 mg/mL.

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

3.5. D□lactate dehydrogenase (D□LDH) suspension, 5 mg/mL.

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

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

Note: All the reagents are available commercially.

4. Preparation of the sample

Lactate determination is normally carried out directly on the wine, without prior removal of pigmentation (coloration) and without dilution provided that the lactic acid concentration is less than 100 mg/L. However, if the lactic acid concentration lies between:

100 mg/L and 1 g/L, dilute 1/10 with double distilled water, 1 g/L and 2.5 g/L, dilute 1/25 with double distilled water, 2.5 g/L and 5 g/L, dilute 1/50 with double distilled water.

5. Procedure

Preliminary note:

No part of the glassware that comes into contact with the reaction mixture should be touched with the fingers, since this could introduce L□lactic acid and thus give erroneous results.

The buffer solution must be at a temperature between 20 and 25°C before proceeding to the measurement.

5.1. *Determination of total lactic acid*

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

Place the following in the 1 cm cells:

	Reference cell	Sample cell
	(mL)	(mL)
Solution 3.1.	1.00	1.00
Solution 3.2.	0.20	0.20
Double distilled water	1.00	0.80
Suspension 3.3.	0.02	0.02
Sample to be measured	-	0.20

Mix using a glass stirrer or a rod of synthetic material with a flattened end; after about five min, measure the absorbencies of the solutions in the reference and sample cells (A_1).

Add 0.02 mL of solution 3.4 and 0.05 mL of solution 3.5, homogenize, wait for the reaction to be completed (about 30 min) and measure the absorbencies of the solutions in the reference and sample cells (A_2).

Calculate the differences ($A_2 - A_1$) in the absorbencies of the solutions in the reference and sample cells, ΔA_S and ΔA_R.

Finally, calculate the difference between those differences:

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

5.2. *Determination of L□lactic acid and D□lactic acid*

Determination of the L□lactic acid or D□lactic acid can be carried out independently by applying the procedure for total lactic acid up to the determination of A_1 and then continuing as follows:

Add 0.02 mL of solution 3.4, homogenize, wait until the reaction is complete (about 20 min) and measure the absorbencies of the solutions in the reference and sample cells (

A_2).

Add 0.05 mL of solution 3.5, homogenize, wait until the reaction is complete (about 30 min) and measure the absorbencies of the solutions in the reference and sample cells (A_3).

Calculate the differences (A_2 – A_1) for L-lactic acid and (A_3 – A_2) for D□lactic acid between the absorbencies of the solutions in the reference and sample cells, ΔA_S and ΔA_R.

Finally, calculate the difference between those differences:

$$\Delta A = A_s - \Delta A_R$$

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. When determining the L□lactic acid alone, the incubation time may be reduced to 10 min.

6. Expression of results

Lactic acid concentration is given in grams per liter (g/L) to one decimal place.

6.1. Method of calculation

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

$$C = \frac{V \times M}{\varepsilon \times \delta \times v \times 1000} \times \Delta A$$

where

V= volume of test solution in mL (V = 2.24 mL for L-lactic acid, V = 2.29 mL for D□lactic acid and total lactic acid)

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

M = molecular mass of the substance to be determined (for DL□lactic acid, M = 90.08)

□ = 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).

6.1.1. Total lactic acid and D□lactic acid

$$C = 0.164 \times \Delta A$$

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

Note:

- Measurement at 334 nm: C = 0.167 x ΔA, (ε= 6.2 $mmol^{-1}$ x 1 x cm^{-1}).
- Measurement at 365 nm: C = 0.303 x ΔA, (ε = 3.4 $mmol^{-1}$ x 1 x cm^{-1}).

6.1.2. L□lactic acid

$$C = 0.160 \times \Delta A$$

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

Note:

- Measurement at 334 nm: C = 0.163 x ΔA, (ε = 6.2 $mmol^{-1}$ x 1 x cm^{-1}).

Measurement at 365 nm: C = 0.297 x ΔA, (□ = 3.4 $mmol^{□1}$ x 1 x $cm^{□1}$).

6.2. *Repeatability (r)*

r = 0.02 + 0.07 x_i

*x*i is the lactic acid concentration in the sample in g/L.

6.3. *Reproducibility (R)*

R = 0.05 + 0.125 x_i

*x*i is the lactic acid concentration in the sample in g/L.

Bibliography

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