

Determination of D-malic acid in fruit juice samples

Introduction

The addition of malic acid to fruit juice, to increase the acid content for example, is strictly illegal, although the EC permits its addition to wine. The naturally occurring form of malic acid in fruits is the L-enantiomer. Synthetically produced malic acid consists of the racemic D/L mixture. The much higher cost of the L-enantiomer, which is currently 20 times that of the D/L mixture, discourages its use as an adulterant. Therefore, the measurement of D-malic acid alone is sufficient to detect the illegal addition of synthetic malic acid. A sample of orange juice sampled during 1991 and analysed also by the Leatherhead Food RA contained 103 ppm of D-malic acid (authentic orange juice should not contain any).

D-malic acid can be measured spectrophotometrically using the enzymatic method, recently developed by Beutler and Wurst, available also as a test kit from Boehringer Mannheim GmbH.

A survey carried out by the Ministry of Agriculture Fisheries and Food in the U.K. in January 1991, compared methods for detection of added substances in leading brands of orange juice, of 17 samples analysed, 10 contained D-malic acid, as a consequence of adulteration.

Principle

D-malic acid is oxidised by nicotinamide-adenine dinucleotide (NAD) to oxaloacetate in the presence of D-malate dehydrogenase. Oxaloacetate is decomposed by the same enzyme to pyruvate and carbon dioxide. The amount of NADH formed from NAD as a consequence is proportional to the amount of D-malic acid and is measured by the increase in absorbance at 340nm.



Method

The analysis can be rapidly carried out directly on orange juice. It is recommended that the suspended solids are first removed by centrifugation for 0.5 mins or via a syringe filter. The above reaction can be carried out in a UV grade cuvette of 1cm pathlength, with a stopper. Alternatively disposable methacrylate cells UV grade can be used. In this case the volumes given below should be halved. Solutions are conveniently dispensed using an automatic pipette.

Add 1.00ml HEPES buffer pH 9.0

0.1ml NAD aqueous solution containing 52mg

1.7ml distilled water

0.1ml sample.

Mix by inversion and read absorbance after 6mins (A1).

Libra S21/S22 operation

- Select Applications (key 2) from the main menu
- Select Reaction rate
- Enter wavelength 340 and press OK (F3)
- Select time in mins
- Enter delay time of 6 and press OK (F3)
- Enter duration time of 20 and press OK (F3)
- Enter factor 0.628 to convert readings directly to concentration units and press OK (F3) [Analyses at Biochrom showed that concentration of D-malic acid g/l = 0.628 x absorbance value]
 - To go back and change the parameters press Method (F1)
- A blank determination is necessary. The preparation is identical apart from omission of the sample and addition of an equivalent volume of water.
- Insert blank and press green run key. This reference value is used for subsequent samples until changed.
- Insert samples as required and press the green run key.
- After reading the sample at 6 mins (abs A1), add 0.10ml aqueous enzyme solution containing 1.3 units of activity.
- Mix and stand for 20 minutes at ambient temperature, recommended range 20-25 C.
- Place the sample in the instrument as the instrument count down timer approaches 20 mins and read absorbance (A2)

The absorbance difference, calculated as A2-A1, is shown as delta A on the results screen.

Other information is displayed which, although not required for this calculation, is a check on the chemical stability of the assay. The assay is shown graphically as it proceeds and the results show slope (abs/min) and the line quality (a coefficient of determination of > 95 % is expected if the assay was carried out over a linear section).

Print outs may be obtained by setting up the printer options in System Utilities and Preferences (3) prints results. This is automatic with autoprint.

Additionally press . to print result if auto-print is off, or to re-print result if auto-print is on

The above procedure can be easily used with other instruments in the Libra range by using the concentration mode and the 0.628 factor.

Ordering Details

Libra S5	80-2115-00
Libra S11	80-2115-15
Libra S12	80-2115-10
Libra S21	80-2115-25
Libra S22	80-2115-20
Libra S32	80-2115-30

The reaction can be accelerated for increased sensitivity if warmed. For this purpose the Libra S21/S22 have the following accessories:

- 8 position water heated cell changer (80-2109-70) used with an external heating bath
- 6 position Peltier heated cell changer (80-2106-04) and Temperature Control Unit (80-2112-49)
- Single position water heated cell holder (80-2106-08) used with an external heating bath
- Single position electrical cell holder (80-2106-12), temperatures selectable from 25, 30 and 37°C
- Single position Peltier cell holder, temperatures selectable over the whole range from 20-49°C (80-2106-13).

The Sipper (80-2112-25) enables some automation of the analyses, and can be used together with a heated (not water heated) or non-heated single cell holder.

Reference

Beutler H.O and Wurst B. Deutsche Lebensmittel-Rundschau 86, 341-344 and 386-389 (1990).