

Determination of Malic Acid in Coconut Water

Reflectometric determination after enzymatic reaction with malate dehydrogenase

Singapore, 2024

Introduction

Coconut water has gained popularity as a natural beverage for health and sports as it contains various minerals, amino acids, organic acids, vitamins, and sugars.

The Malic acid content in coconut water varies with its maturity and can also be affected by processing. Malic acid is a crucial factor that can affect the taste of coconut water.¹ Therefore, manufacturing plants for coconut water will need to assess the malic acid content.

The RQflex® 20 instrument offers an easy way to measure malic acid in coconut water with the Reflectoquant® Malic Acid test strips (**1.16128**) from a range of 0.25 – 3.0 g/L malic acid (considering the dilution factor).

Experimental

Method

Malic acid (malate) is oxidized to oxalacetate by NAD under the catalytic effect of malate dehydrogenase. In the presence of diaphorase the NADH formed in the process reduces a tetrazolium salt to a red formazan that is determined reflectometrically.

Measuring Range

Test kit: 5 – 60 mg/L malic acid

Method: 0.25 – 3.00 g/L malic acid (considering the dilution factor of 50)

Applicable Sample

Coconut Water

Reagents, Instruments and Materials

Test/Reagents Kit

Reflectoquant® Malic Acid Test (**1.16128**)

Instrument(s) & Devices

RQflex® 20 Reflectoquant® (**1.17246**)

Other Reagents and Accessories

- Distilled water (e.g., from a suitable Milli-Q® system) or water for analysis (**1.16754**)
- Pipette for the volume of 1 mL
- 50 mL volumetric flask

Products for AQA

- RQcheck set for RQflex® 20 Reflectometer (**1.17247**)
- Recalibration Set for RQflex® 20 Reflectometer (**1.16954**)
- L-(-)-Malic acid (**8.00384**)
- Buffer solution pH 7.00 Certipur® (**1.09439**)

Analytical Procedure

Sample Preparation

- Mix coconut water sample well.
- Accurately pipette 1.00 mL of sample into a 50 mL volumetric flask.
- Top up to mark with water for analysis and mix well.

Measurement

- Press the START button of the reflectometer and **-this is imperative - at the same time** immerse **both reaction zones** of the test strip in the pretreated sample (15 - 30 °C) for 2 sec.
- **Carefully** allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel.
- **Immediately** insert the strip all the way into the strip adapter with the reaction zones facing the display.
- After the end of the reaction time (300 sec), read off the result from the display in mg/L malic acid. The result is automatically stored.

Calculation

Malic acid content $\left[\frac{\text{g}}{\text{L}}\right] = \text{Measured value} \left[\frac{\text{mg}}{\text{L}}\right] \times 0.05$

Analytical quality assurance

Analytical quality assurance (AQA) is recommended before each measurement series. Check the instrument using the RQcheck. If RQcheck failed, perform a recalibration using the recalibration set and repeat the RQcheck. For more details see RQflex® 20 manual.

To check test strips, test reagent, measurement device, and handling (recommended before each measurement series):

Dissolve 0.250 g of L-(-)-malic acid in buffer solution pH 7.00, make up to 100 mL with this buffer solution, and mix. Malic acid content: 2500 mg/L.

Dilute this standard solution with distilled water to 25.0 mg/L malic acid and analyze as described in section 7.

Results and Discussion

Here in this study, four different brands of coconut water were used as test samples for measurement and comparison.

The results were verified using ion chromatography as orthogonal method to the Reflectoquant® Test kit. The specific experimental ion chromatography conditions can be found in **Table 1**.

Table 1: Experimental Conditions with Metrohm Eco IC – modified from Metrohm Application Note²

Experimental Conditions			
Column:	Metrosep Organic Acids, 250 x 7.8 mm	Injection volume:	100 µL (Manual)
Detection:	Conductivity	Flow rate:	0.5 mL/min
Eluent:	0.5 mM Sulfuric Acid: Acetone (4:1)	Temperature:	25 °C
Suppressor:	MSM (200 mM Lithium Chloride)	Pressure drop:	~5.4 MPa (780 psi)

The comparison of the results obtained by reflectometric and ion chromatographic (IC) determination is shown in **Table 2**.

Table 2: Method results comparison across three coconut water brands

Sample	Malic Acid content [g/L]		% Difference IC vs. RQflex® 20
	IC	RQflex® 20	
1	1.72	1.78	3.7
2	1.54	1.61	4.4
3	1.91	1.92	0.4
4	1.86	1.78	-4.1
5	1.76	1.72	-2.6
6	1.94	1.95	0.4
7	1.92	1.77	-8.6

Conclusion

- Rapid testing of malic acid content in coconut water can be performed with Reflectoquant® Malic Acid Test with RQflex® 20 reflectometer.
- Orthogonal method using ion chromatography has provided evidence that experimental results from both methods have differences within ± 10 %.

References

1. J.J. Lal et al. Coconut Palm. In: Caballero B, Trugo LC, Finglas PM. Encyclopedia of Food Sciences and Nutrition, 2nd Edition, Academic Press;2003. P. 1464-1475.
2. Metrohm IC Application Note No.: AN-O-009. Determination of organic acids and phosphate using ion-exclusion chromatography with direct conductivity detection. (https://www.metrohm.com/en_us/applications/application-notes/aa-o-001-100/an-o-009.html)

Featured Products

Product	Cat. No.
Reflectoquant® instruments and strips	
Malic Acid Test, reflectometric, 5.0-60.0 mg/L (malic acid), REFLECTOQUANT®	1.16128
Reflectometer RQflex® 20, pkg of 1 unit, Reflectoquant®	1.17246
Products for AQA	
Recalibration Set for RQflex® 20 Reflectometer, 1 unit	1.16954
RQcheck Set for RQflex® 20 Reflectometer, 1 set	1.17247
L-(-)-Malic acid, for resolution of racemates for synthesis	8.00384
Buffer solution, (di-sodium hydrogen phosphate/potassium dihydrogen phosphate), traceable to SRM from NIST and PTB pH 7.00 (20 °C) Certipur®	1.09439
Solvents and consumables	
Water for analysis EMSURE®	1.16754

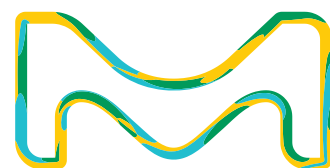
Related Products

Product	Cat. No.
Products for ion chromatography	
Sulfuric Acid Concentrate, 0.1 M H ₂ SO ₄ in water (0.2N), eluent concentrate for IC	68279
Lithium chloride monohydrate, Suprapur®	1.05677
Acetone for Liquid Chromatography LiChrosolv®	1.00020
Milli-Q® IQ 7000	ZIQ7000T0C
Millex® hydrophilic PTFE syringe filter, pore size 0.45 µm, diam. 33 mm, non-sterile	SLCR033NS
PTFE Membrane Filter, 0.2 µm Pore Size, 0.2 µm OmniporeTM PTFE filter membrane	JGWP04700
All-Glass Filter Holder Assembly with funnel, Omnipore, filter diam. 47 mm, hydrophilic	XX1504700
Greiner centrifuge tubes, 15 mL, 17x120 mm, conical (V) bottom, w/ graduation, I.D. field	T1943-1000EA
L-(-)-Malic acid, certified reference material, TraceCERT®	09172-100MG

For more information on

- Reflectoquant® Test strips see [SigmaAldrich.com/test-strips](https://www.sigmaaldrich.com/test-strips)
- Further Application Notes see [SigmaAldrich.com/wfa-applications](https://www.sigmaaldrich.com/wfa-applications)
- Order/Customer Service see [SigmaAldrich.com/order](https://www.sigmaaldrich.com/order)
- Technical Service see [SigmaAldrich.com/techservice](https://www.sigmaaldrich.com/techservice)

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