

Reflectometric Determination of D- and L-Lactic Acid in Dairy Products after Enzymatic Reaction with Lactate Dehydrogenase (LDH)

Abstract

This protocol describes a method for the determination of lactic acid enantiomers in dairy products using the Reflectoquant® Lactic Acid Test. The technique uses an enzymatic reaction facilitated by lactate dehydrogenase to oxidize DL-lactic acid, leading to a detectable change in color. The protocol outlines the procedure for preparing samples of different dairy products, such as milk, yogurt, and cheese. It also offers instructions on how to obtain precise measurements using the RQflex® 20 Reflectometer. The dependability of the Reflectoquant® Lactic Acid Test in assessing DL-lactic acid levels in various dairy products is demonstrated by the results, which were confirmed against a photometric UV method according to DIN 10335:2010-09

Introduction

Lactic acid is one of the hydroxycarboxylic acids. It contains a carboxy group and a hydroxyl group. Salts and esters of lactic acid are called lactates. There are two isomeric forms of lactic acid, L-lactic acid and D-lactic acid. These differ in their optical activity. L-(+)-lactic acid is dextrorotatory and D-(-)-lactic acid is levorotatory. The racemate of both enantiomers is called DL-lactic acid.^{1,2}

The racemate is mainly found in sour milk and whey products, tomato juice and beer. L-lactic acid is found in the blood, muscles and organs of animals and humans. D-lactic acid is primarily produced during the microbial breakdown of glucose.^{1,2,3}

If milk products are left to stand for a long time, the lactic acid bacteria contained in them multiply. These convert the existing milk sugar (lactose) into lactic acid. The pH falls below pH 5 and the milk protein (casein) is denatured and flocculated.³

The main application of lactic acid is in the food industry. There, lactic acid fermentation is used to acidify and preserve many products. Other diverse areas of application are e.g., the production of biodegradable plastics, the use in soaps and liquid cleaners due to their antibacterial effect, as a limescale remover in tanneries, the textile industry, and the printing industry. Lactic acid is also used as an auxiliary agent in the pharmaceutical industry to convert water-insoluble active ingredients into lactates and thereby increase their solubility.^{2,3}

Experimental

This application note describes the reflectometric determination of lactic acid in dairy products.

Method

DL-Lactic acid (lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) under the catalytic effect of L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH) to a pyruvate. In the presence of diaphorase, the NADH formed in the process reduces a tetrazolium salt to a blue formazan that is determined reflectometrically.

Measuring range

3.0-60.0 mg/L DL-lactic acid

Sample material

Dairy products (e.g., milk products, sour milk products, yogurt, kefir, whey products, cheese)

Reagents, Instruments, and Auxiliaries

Reagents

- Reflectoquant® Lactic Acid Test (**1.16127**)
- L-(+)-Lactic acid monolithium salt - optional (**L2250**)

Instrument

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

Auxiliaries

- Volumetric flask, 100-mL
- Standard laboratory glassware (e.g. glass beakers)
- Analytical balance
- Homogenizer (optional)
- Magnetic stirrer (optional)

Analytical Approach

Sample preparation

Temper the sample to room temperature and homogenize. Crush solid samples before homogenization in an adequate way. For specific hints for sampling and homogenization see "DIN 10335:2010-09 Milk and milk products except milk powder - Determination of L- and D-lactic acid (L- and D-lactate) content - Enzymatic method".⁴

Then dilute the sample so that the estimated DL-lactic acid concentration in the diluted sample solution is between 5 mg/L and 60 mg/L DL-lactic acid. Milk can be used undiluted.

The following weights are recommended for dilution (add 100 mL distilled water):

- | | |
|--------------------|-------|
| • Milk powder | 15 g |
| • Whey powder | 0.5 g |
| • Yogurt | 0.5 g |
| • Creme Fraiche | 0.5 g |
| • Sour cream | 0.5 g |
| • Processed Cheese | 0.5 g |
| • Hard Cheese | 0.2 g |

Weigh the appropriate quantities to the nearest 1 mg and note the weight.

- Weigh **liquid samples** into a 100-mL-volumetric flask and make up to the mark with distilled water and mix.
- Weigh **pasty or solid, easily soluble samples** into a beaker, add about 60 mL of hot distilled water (about 55 °C) and mix well. Transfer the mixture quantitatively into a 100-mL-volumetric flask and make up to the mark with distilled water and mix.

- Weigh **solid cheese samples** into a beaker and add about 30 mL of hot distilled water (about 55 °C). Homogenize the mixture with a homogenizer (e.g., Ultra-Turrax) for about 1 min. Completely transfer the adhering sample residues to the beaker with about 20 mL of distilled water. Shake this mixture for 15 min or stir with a magnetic stirrer. Then transfer the mixture quantitatively into a 100-mL-volumetric flask and make up to the mark with distilled water and mix.

Measurement

Use the RQflex® manual and the test strip's instruction. for use for a detailed description.

The following applies to the Lactic Acid Test:

- Measurement procedure A
- Stored reaction time: 300 sec

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 measurement sample (15 - 25 °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, read off the lactic acid concentration from the display. The result is automatically stored.

Notes on the measurement:

- The two reaction zones change color differently.
- If the measurement value exceeds the measuring range (HI is shown on the display), repeat the measurement using fresh, diluted samples until a value of less than 60.0 mg/L lactic acid is obtained.
- **Protect the reaction zones from light during the reaction time.**
- If the test strip is inserted into the adapter after the reaction time has expired, renewed depression of the START button may produce a false result.
- Reclose the tube containing the test strips immediately after removing the needed number of strips.
- **Do not touch the reaction zones with the fingers since hand perspiration contains lactic acid.**
- At the end of each workday, cleanse the strip adapter thoroughly with distilled water or ethanol.

Result calculation

The mass fraction (w) of DL-lactic acid in g per 100 g of the sample is calculated using the following equation:

$$w = \frac{\beta \times V \times 100}{m}$$

Where:

- w the content of DL-lactic acid in grams per 100 grams of the sample
- β the mass concentration of DL-lactic acid in milligrams per liter of the prepared sample solution
- V the volume of the sample solution in the volumetric flask, in liters
- m the weight of the sample, in milligrams
- 100 the conversion factor from grams to 100 grams

Method control

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

- Dissolve 1.066 g of L-(+)-lactic acid monolithium salt in distilled water, make up to 1000 mL with distilled water, and mix.
- Lactic acid content: 1000 mg/L.
- Dilute this standard solution with distilled water to 25.0 mg/L lactic acid and analyze as described in section "Measurement".

Free lactic acid is not suited for preparing the standard solution.

Results

The results were verified by performing a reference analysis with the photometric UV method according to "DIN 10335:2010-09 Milk and milk products except milk powder - Determination of L- and D-lactic acid (L- and D-lactate) content - Enzymatic method".⁴

The results are shown in the following table.

Sample	Reflectoquant® Lactic acid Test, Conc. DL-lactic acid (g/100 g)	Photometric UV-method, DIN 10335:2010-09 Conc. DL-lactic acid (g/100 g)
Yogurt 1	1.01	1.00
Yogurt 2	0.84	0.77
Greek yogurt	0.65	0.69
Goat milk yogurt	0.83	0.91
Kefir	0.65	0.70
Sour cream 1	0.60	0.66
Sour cream 2	0.55	0.58
Sour cream 3	0.62	0.64
Sour cream 4	0.48	0.47
Crème fraîche 1	0.50	0.50
Crème fraîche 2	0.44	0.48
Yogurt powder	4.70	4.63
Whey powder	0.66	0.64
Cheese 1, freeze-dried	2.25	2.14
Cheese 2, freeze-dried	1.57	1.58
Processed cheese	0.59	0.61
Milk powder 1	0.024	0.030
Milk powder 2	0.007	0.006

Conclusion

The Reflectoquant® Lactic Acid Test is a quick and easy way to analyze the DL-lactic acid content of dairy products.

For more information

Reflectoquant® Test strips see [SigmaAldrich.com/test-strips](https://sigmaaldrich.com/test-strips)

Applications see [SigmaAldrich.com/wfa-applications](https://sigmaaldrich.com/wfa-applications)

Technical Service see [SigmaAldrich.com/techservice](https://sigmaaldrich.com/techservice)

References

1. PubChem. Lactic Acid. Nih.gov. [accessed 2024 Aug 12]. <https://pubchem.ncbi.nlm.nih.gov/compound/Lactic-Acid>
2. Milchsäure, URL: <https://roempp.thieme.de/lexicon/RD-13-02408?searchterm=milchsäure&context=searche> (2022-09-22)
3. DL-Milchsäure, C₃H₆O₃, URL: https://seilnacht.com/Chemie/ch_milch.htm (2022-09-22)
4. DIN 10335:2010-09 Milk and milk products except milk powder - Determination of L- and D-lactic acid (L- and D-lactat) content - Enzymatic method. <https://www.din.de/en/getting-involved/standards-committees/nal/publications/wdc-beuth:din21:127761787>

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