

Polarimetric Determination of Lactose Content in High Lactose Dairy Products

Relevant for: Food

The quality of milk and milk powders depends, among others, on the lactose content. The lactose needs to be extracted from dairy products having high lactose content (e.g. milk and milk powders) before using the polarimeter for concentration determination. Please note that the polarimetric approach is not suitable for lactose-free products.



Product	Anhydrous lactose [%]	Lactose monohydrate [%]
Raw milk	4.5	4.7
Lactose-reduced milk	0.27 - 0.84	0.32 – 0.95
Skimmed fresh milk	4.6	4.8
Condensed milk	7.6 – 11.9	8.0 – 12.5
Low-fat milk powder	49	51.5
Whole milk powder	36.1	38
Whey powder 4.4 % H ₂ O	69	72.8
Farmer's cheese	0.3 – 3.8	0.3 – 4.0

Table 1: Lactose content in cow's milk and processed products.

1 Sugar in milk

The characteristic carbohydrate of milk is lactose, commonly referred to as “milk sugar”. Lactose is present in the milks of mammals in various amounts, e.g. goat milk contains 4.0-4.9 %, cows' milk 4.7-5 %, and horse milk contains up to 6.3 % lactose. The average lactose content is around 4.8 % anhydrous lactose, while in human milk it varies from 4.5 % to 9.5 %.

In dairy products the amount of lactose differs depending on processing. In concentrated and dried products it increases proportional to the dry substance, whereas fermented products have a lower lactose concentration.

Table 1 gives an overview on examples of lactose monohydrate and anhydrous lactose content in cow's milk as well as in some processed products.

2 Lactose properties

In milk or milk products, lactose exists in two isomeric forms, called α - and β - lactose respectively. The molecular structures of α - and β - lactose differ in the orientation of a hydrogen- and a hydroxyl group on carbon atom no.1 in the glucose moiety. A solution of lactose at equilibrium has an optical rotation of 55.7 °. The distribution of anhydrous α and β -lactose is about 37.3 % anhydrous α -lactose and 62.7 % β -lactose.

2.1 Lactose in powder

The most common way to obtain lactose in solid form is crystallizing from solution. When crystallization is performed at temperatures below 93.5 °C, exclusively α -lactose monohydrate is obtained. α -Lactose has the specific peculiarity that in the crystalline state each lactose molecule is associated with 1 molecule of water. In other words, α -lactose crystallizes as monohydrate.

2.2 Lactose in liquids

In liquids α -lactose and β -lactose is present, both changing into one another continuously. This phenomenon is referred to as mutarotation. The velocity of mutarotation is determined by factors like temperature, concentration and pH (acidity) of the solution. Lactose solutions strive after a state of equilibrium between the α and β form. At room temperature the equilibrium results in a ratio of about 40 % α -lactose and 60 % β -lactose.

Therefore, the measured optical rotation and the resulting specific rotation (SR) changes over time until the steady state of the equilibrium is reached.

3 Apparatus

Depending on the needed accuracy and features, the MCP 5X00 and the MCP 4100 (no FillingCheck-camera) polarimeters from Anton Paar can be chosen for this application.



Figure 1: The MCP polarimeter from Anton Paar.

4 Methods

The polarimetric determination of the lactose content is based on the optical activity of lactose.

Fats and proteins in milk and milk powder, basically those components that cause the white or cloudy color, have to be removed before the measurement can be started. Consequently, the volume of precipitated proteins and fats is considered as a correction factor in some methods.

Two different clarification methods are recommended, the Carrez clarification and the AOAC method 896.01. These methods consist of three basic steps:

1. Mixing the milk or milk powder with a precipitation reagent.

2. Filtration in order to separate fats and proteins from the lactose.
3. Measuring the optical rotation in the polarimeter.

4.1 Lactose in milk (AOAC 896.01)

The „AOAC Official Method 896.01 Lactose in Milk“ (<http://www.aoac.org>) offers the possibility to determine the percentage of lactose in milk polarimetrically.

As a sample cell length of 200 mm is proposed, the MCP 4100/5X00 polarimeter from Anton Paar can be used.

Preparation of reagents

Keep in mind that mercury (Hg) compounds are toxic.

Acid-mercuric nitrate solution (acid-Hg(NO)₃):

Dissolve mercury (Hg) in twice its weight of nitric acid (HNO₃) and dilute with 5 volumes of water.

Alternatively: Dissolve 33.2 g potassium iodine (KI) and 13.5 g mercuric chloride (HgCl₂) in 200 ml Acetic acid (CH₃COOH) and 640 ml water (H₂O).

5 % phosphotungstic acid (PTA) solution: Dissolve 5 ml PTA in 95 ml water.

65.8 g milk is weighted into each of 2 volumetric flasks of 100 ml and 200 ml volume. 20 ml of acid-Hg(NO)₃ solution or 30 ml mercury diiodide (HgI₂) solution are added to each flask.

Fill the 100 ml flask with 5 % PTA solution up to the mark.

Both flasks are frequently shaken during 15 minutes. The solutions of the flasks are then filtered separately through a dry filter. The optical rotations of the filtrates are measured independently in the MCP using a 200 mm cell. Calculate the percent lactose content as follows:

$$\%lactose = \frac{b - (b - 2a) * 2}{2}$$

α is the polarimeter reading from the 200 ml flask

β is the polarimeter reading from the 100 ml flask

See also: AOAC Official Method 945.48: Lactose in Evaporated Milk (Unsweetened).

4.2 Lactose in milk powders

Especially for products with high lactose-content this method can be used to determine the ratio of

crystalline α -lactose monohydrate and thus the percentage of amorphous lactose in powders. Among others, raw lactose powder, whey powder, milk powder, but also concentrated permeates can be analyzed.

Note:

- In dissolved powders the mutarotation of both, α - and β -lactose should be in equilibrium (stable OR, Fig. 2).
- The specific rotation of α -lactose-hydrate and β -lactose is 52.5 and the specific rotation of water-free α -lactose and β -lactose is 55.4.
- A correction factor for the volume of the protein precipitation has to be considered for the calculation of the lactose content.

Carrez clarification – procedure

10 g of milk powder are mixed with a small amount of water to make a slurry. In order to determine the ratio of α - and β -lactose, the time has to be measured until the equilibrium between α - and β -lactose is established (Fig. 2).

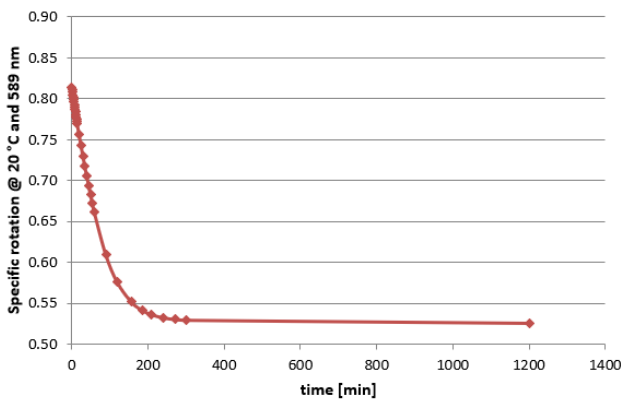


Figure 2: Mutarotation of lactose monohydrate powder. As soon as the α -lactose (specific rotation +89.4 ml · °/(dm·g)) comes into contact with water, the mutarotation of α - and β -lactose (+35 ml · °/(dm·g)) starts, leading to a decrease of the determined specific rotation. At the end, the equilibrium between α and β -lactose, with a specific rotation of 52.7 +89.4 ml · °/(dm·g), is reached.

The time at the moment the powder comes into contact with the water should be counted as zero (t = 0). The concentrate is diluted with water to 50 ml and transferred to a 250 ml volumetric flask. Now 20 ml of 105.6 g/l potassium hexacyanoferrate(II) trihydrate (K₄[Fe(CN)₆] · 3H₂O), 2 drops of 1-Octanol, 15 ml of 237.4 g/l zinc acetate dihydrate

(Zn(CH₃COO)₂ · 2 H₂O) and 30 ml concentrated acetic acid (CH₃COOH) are added and carefully mixed.

The 250 ml volumetric flask is filled up to the mark with H₂O and shaken well. Now the sample is filtered and the optical rotation of the filtrate is measured with the MCP 10 minutes after the time measurement has been started. Its optical rotation is recorded in intervals of every minute for 20 minutes. The solution is stored for 24 hours at 20 °C before the final optical rotation value α_{∞} is measured.

The content of α -lactose (a) and β -lactose (b) of the powder can be calculated according to the following formulas:

$$a = \left(\frac{\alpha_0}{\alpha_{\infty} - 0.622} \right) * 99.1$$

$$b = \left(\frac{1.631 - \alpha_0}{\alpha_{\infty}} \right) * 99.1$$

The amount of α -lactose hydrate can be calculated from this data according to the following formula:

$$c = \left(\frac{a - b}{R} \right) * 100$$

R represents the equilibrium between β -lactose and α -lactose in amorphous lactose and is usually set to 1.25.

Limitations

The quantification of polarimetric measurements is limited to samples which are free of other optically active compounds which cannot be precipitated with Carrez solution.

4.3 How to calculate the overall lactose content

Independent from the method and after the equilibrium of α - and β -lactose has reached, the final measured optical rotation is used to calculate the lactose content.

The concentration of the lactose $c_{lactose}$ [g/100 ml] depends on the optical rotation measurement, on the optical path length [dm] and on the concentration:

$$\alpha = \frac{[\alpha]_D^{20} * l * c_{lactose}}{100}$$

$$c_{lactose} = \frac{\alpha}{[\alpha]_D^{20} * l} * 100$$

The specific rotation $[\alpha]_D^{20}$ of powders containing α -lactose monohydrate is 52.3 (mL·°)/(dm·g).

The equation shows the specific rotation of lactose powder over time. At equilibrium the specific rotation is 52.6 (mL·°)/(dm·g). The mutarotation can be accelerated by adding a few drops of ammonia solution (10 %).

4.4 Safety precautions

These methods do not contain any safety instructions. It is the responsibility of the user of this method to establish appropriate health and safety practices and to determine the applicability of regulatory limitations prior to use.

5 Literature

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Fundamentals of Dairy Chemistry, Springer Verlag, Berlin, ISBN-10: 0-8342-1360-5, ISBN-13: 978-0834213609.

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