

Determination of lactase in milk

Introduction

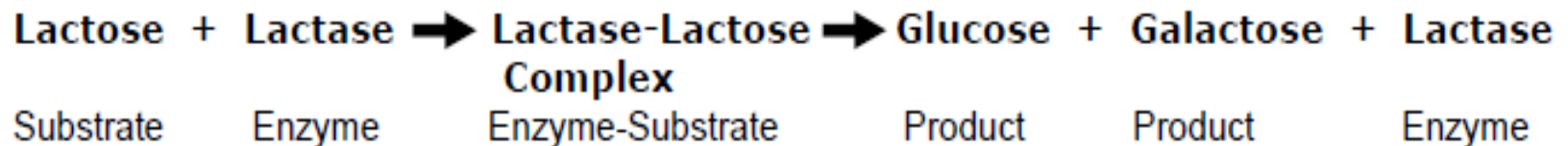


When some people eat dairy products (milk, ice cream, and cheese), they experience digestive discomforts such as flatulence (gas), bloating, cramping, and even diarrhea. These individuals do not produce enough of an enzyme called lactase, the enzyme that digests lactose, a carbohydrate found in milk.

When undigested lactose accumulates in the intestine of a person with lactose intolerance, bacteria in the intestine feed on the lactose and produce waste gases that cause flatulence and bloating. Large amounts of undigested lactose may also cause water to diffuse from the blood into the intestine resulting in diarrhea.

Lactase Activity

Lactase, is a protein enzyme that digests (breaks down) lactose (milk sugar) into glucose and galactose (smaller sugars). The chemical equation below illustrates what happens when the enzyme lactase digests lactose (milk sugar).



Test principle

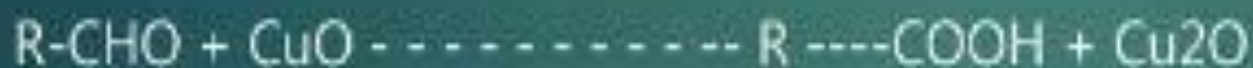
Lactose , is a disaccharide, sugar that is found in milk and is formed from galactose and glucose. Lactose makes up around 4.5~5% of milk (by weight).

The enzyme lactase is essential for digestive, hydrolysis of lactose in milk.

Deficiency of the enzyme causes lactose intolerance.

Principle

- ▶ Lactose are having a free ketonic or aldehyde group which react with alkaline copper salt reduced to cupric oxide.



- ▶ This reduction is using to calculate lactose % in milk sample.

▶ Reagent and instruments

- ▶ Fehling solution A (Copper sulphate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -33.6439 g in 1000 ml purified water)
- ▶ Fehling solution B (Potassium sodium tartrate 173 g and 50 g NaOH in 1000 ml purified water)
- ▶ Methylene blue solution
- ▶ True lactose solution (5 g anhydrous lactose in 1000 ml purified water)

▶ Procedure

▶ A) Standardization of Fehling solution (Calculation of factor)

- ▶ Take 5 ml each solution (A & B) in conical flask.
- ▶ Add 40 ml purified water
- ▶ Add 2 drops methylene blue indicator
- ▶ Using lactose solution as titer till get a clear solution.
- ▶ Heat conical flask and add methylene blue indicator
- ▶ Again titrate till clear faint blue color appeared.
- ▶ Calculate the factor by using below formula-

Factor = volume of solution (Burette reading) x mg/ml lactose

- ▶ B) preparation of sample solution
 - ▶ take 25 ml milk sample in a conical flask.
 - ▶ Add 200 ml purified water
 - ▶ Add 4 ml acetic acid (10%)
 - ▶ Make up the volume 250 ml.
 - ▶ Solution to be filtered.

▶ C) Titration

- ▶ Take 5 ml each solution (Fehling A & B) in a conical flask.
- ▶ Add 40 ml water
- ▶ Add methylene blue solution 2 drops
- ▶ Add 10 ml sample solution in conical flask, heat the flask followed by again Titration against sample solution

- ▶ Calculate the % lactose as below-

$$\text{▶ \% Lactose} = \frac{F \times V \times 100}{T \times W \times 1000}$$

- ▶ F = Factor
- ▶ V = volume (sample solution volume 250 ml)
- ▶ T = Burette Reading
- ▶ W = weight of sample
- ▶ 1000 stand for conversion of g to mg

Thank you