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Explore enzymes and the science of lactose intolerance using lactase tablets

Extension activity

Why lactase tablets should not be taken with hot drinks or food

Introduction: Instructions on the packaging of lactase tablets typically warn against exposing the drugs to heat. In this activity, we scrutinize this disclaimer experimentally. Previous experiments have shown that lactase enzyme is substrate specific and that its activity is highly dependent on environmental pH. This experiment explores the influence of heat on enzyme activity. Lactose and maltose solutions, as well as whole and oat milk, are treated with lactase solution that has been preheated by boiling, followed by sugar detection using Fearon's reagent to monitor lactase activity.

This activity takes about 20 minutes.

Safety notes

- Lactase tablets, whole milk, and oat milk used in experiments are no longer suitable for consumption.
- Danger of scalding when using a water bath (65°C).
- Eye protection should be worn when using strongly alkaline Fearon's reagent.
- Contact of chemicals with the skin and eyes should be avoided in general. In case of contact, affected areas should be rinsed thoroughly with water.

Materials

Equipment per group:

- 5 test tubes
- 1 test tube stand
- 2 large beakers (e.g., 600 ml) to serve as water baths
- 2 thermometer (up to 100°C)
- 1 heating plate if necessary
- 5 drip pipettes
- Waterproof pencil
- 1 stopwatch
- 1 mortar and pestle
- Aliquots of liquid chemicals
- Student worksheet (see below)
- Bunsen burner
- Test tube holder

Chemicals:

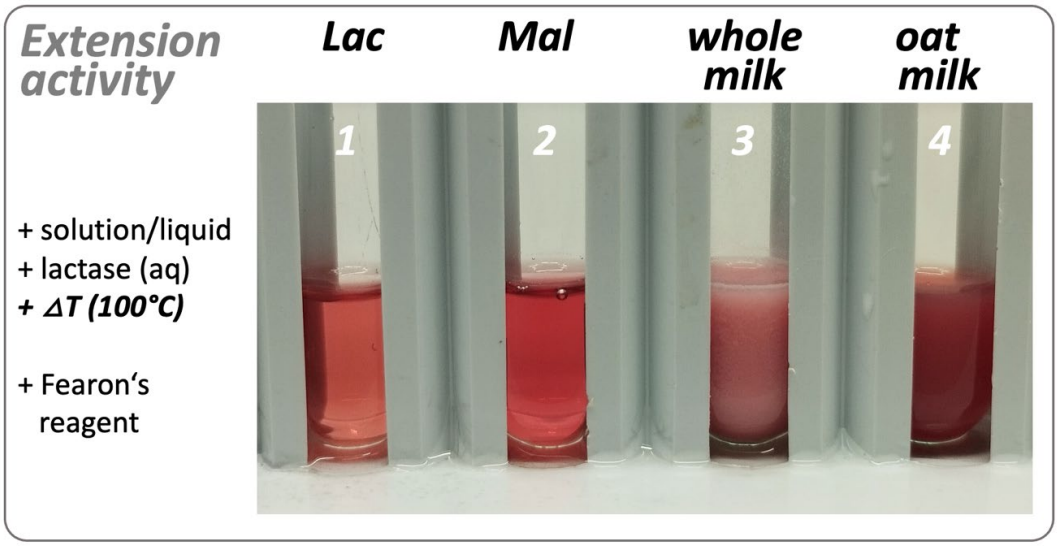
- Lactose solution (4% w/w)
- Maltose solution (4% w/w)
- Oat milk
- Whole milk
- Fearon's reagent (see the extension activity)
- Lactase express tablets (12 000 FCC ALU (Food Chemical Codex[1]), effective from the first minute)

Note: It is important to use tap water and not distilled water to prepare enzyme solutions, as it contains certain ion species, for example, calcium, which can act as an enzyme cofactor.

Procedure

1) Test tubes should be labelled with numbers 1–4, or three-letter abbreviations for sugars (Lac, Mal) and "whole" or "oat" for milk products, using a waterproof pencil. Add 1 ml of the following solutions to the test tube using dropping pipettes: 1) lactose solution, 2) maltose solution, 3) whole milk, and 4) oat milk.
2) Using a mortar and pestle, pulverize a lactase express tablet and transferred to a test tube.
3) Add 10 mL of tap water is added using a dropping pipette, followed by mixing to dissolve the pulverized lactase tablet.
4) The lactase solution is briefly heated to boiling point using a Bunsen burner.
5) Add 1 mL of this lactase solution to each of the test tube using a dropping pipette and shake gently to mix.
6) Place the test tubes in a preheated water bath (38°C) for 5 minutes, simulating human body temperature.
7) Add 1 mL of Fearon's reagent to each test tube with a dropping pipette and thoroughly swirl to mix.
8) Place test tubes in a water bath preheated to 65°C.
9) Incubate the solutions for approximately 5 minutes.
10) Observe and document the results, for example, on the worksheet (see below).

Observation: Unlike in Activity 2, with preheating of the lactase solution, the formation of a red dye is observed in all four assays (see figure). This result is identical to those of the Fearon test on lactose, maltose, whole milk, and oat milk without prior lactase digestion.



Heat inactivates lactase so the lactose is not digested
Image courtesy of the author

Evaluation: Enzyme activity is highly dependent on protein integrity or structure. Preboiling the enzyme solution simulates heat exposure of lactase tablets. The persistence of red-dye formation using Fearon's reagent and the absence of yellow-dye formation – indicating glucose formation as a result of lactose breakdown (as observed during a previous experiment without acid pre-treatment, figure S1) – demonstrate the loss of lactase activity after heat treatment. This can be explained by changes to the 3D structure of the enzyme through heat denaturation at boiling temperature, well above human body temperature.

Discussion

Three fundamental aspects of enzyme activity are addressed with the activities presented here: 1) substrate specificity, 2) pH dependence, and 3) temperature dependence. The experiments are centred around a topical, medical, and motivating context (lactose intolerance) and make use of everyday food products (milk, oat milk). In contrast to more widely used urea/urease experiments that typically involve malodorous and sticky chemicals in a somewhat abstract context, which is not easily accessible to lower-grade students, this series of sugar/lactase-centred experiments provides an alternative, more relatable, and olfactorily pleasant access to the subject area of enzymology.

Worksheet 4 for extension activity

1) Perform the experiment 'Why lactase tablets should not be taken with hot drinks or food'.
2) Record your observations by completing Table 1, column 2.
3) Complete Table 1, column 3. (Tip: remember what colours the Fearon test gives with monosaccharides and disaccharides.)
4) Summarize your results in a short text. What effect does temperature have on the enzyme lactase?

Table 1: Results

Sugar/milk	**Colour detected by using Fearon's reagent and lactase**	**Are monosaccharides or 1,4-linked-disaccharides contained in the sample?**
Lactose		
Maltose		
Whole milk		
Oat milk		

Worksheet 4: Model answers

1) Perform the experiment 'Why lactase tablets should not be taken with hot drinks or food'.
2) Record your observations by completing Table 1, column 2.
3) Complete Table 1, column 3. (Tip: remember what colours the Fearon test gives with monosaccharides and disaccharides.)
4) Summarize your results in a short text. What effect does temperature have on the enzyme lactase?

Table 2: Results

Sugar/milk	**Colour detected by using Fearon's reagent and lactase**	**Are monosaccharides or 1,4-linked-disaccharides contained in the sample?**
Lactose	red	1,4-linked disaccharide
Maltose	red	1,4-linked disaccharide
Whole milk	red	1,4-linked disaccharide
Oat milk	red	1,4-linked disaccharide

Summary: The red dye shows us that, despite the addition of the enzyme lactase, 1,4-linked disaccharides can still be detected in all solutions.
The enzyme is therefore dependent on temperature, and temperatures that are too high lead to the enzyme becoming ineffective.