

Temperature and the rate of decay of milk

Investigate the effect of temperature on the rate of decay of fresh milk by measuring pH change.

For AQA GCSE Biology 8461 and Combined Science: Trilogy 8464. **Triple only** This practical is not in Combined Science. Teacher answers start on a new page.

Name

Date

Aim

To find how temperature affects the time taken for lipase to break down fat in milk, as a model of the rate of decay.

Background

Decay is the breakdown of dead biological material by microorganisms. Decomposers release enzymes that break the material down, and they respire as they grow. The rate of decay depends on the conditions, including **temperature**, water and the availability of oxygen.

Milk contains fat. The enzyme **lipase** breaks down fat into **fatty acids** and glycerol. The fatty acids lower the pH of the mixture. This practical uses lipase to model the way decomposers release enzymes that break down food. Another version leaves fresh milk at different temperatures and measures the fall in pH with a pH probe over several days, as bacteria in the milk produce acid.

Phenolphthalein is an **indicator** that is pink in alkaline conditions and colourless in acid. Sodium carbonate makes the milk alkaline at the start, so the mixture is pink. As the fatty acids are made, the pH falls and the pink colour disappears. The time taken for this to happen shows the rate of the reaction.

Rate is worked out as $1 \div \text{time}$, because a shorter time means a faster rate. Like all enzymes, lipase has an **optimum temperature**. Above this temperature the enzyme **denatures** and stops working.

Hypothesis

As the temperature increases, the time taken for the pink colour to disappear will decrease, up to an optimum temperature. This is because particles have more kinetic energy, so lipase and fat collide more often. Above the optimum, lipase will denature and the rate will fall.

Variables

Independent	Temperature of the water bath (°C)
Dependent	Time taken for the pink colour to disappear (s), used to calculate the rate ($1 \div \text{time}$)
Control	• Volume of milk (5 cm³) • Volume and concentration of sodium carbonate solution (7 cm³ of 0.05 mol/dm³) • Volume and concentration of lipase solution (1 cm³ of 5 % lipase) • Number of drops of phenolphthalein indicator (5 drops) • Type of milk (the same carton) • How the mixture is stirred or swirled

Equipment

- Fresh whole milk, about 5 cm³ for each test

- Sodium carbonate solution, 0.05 mol/dm³ (7 cm³ each test)
- Phenolphthalein indicator solution (5 drops each test)
- Lipase solution, 5 % (1 cm³ each test)
- Boiling tubes (two for each test) and test tube rack
- Syringes or measuring cylinders: 10 cm³ for milk and sodium carbonate, 1 cm³ for lipase
- Dropper for the indicator
- Water baths (or beakers of water with a kettle and ice) at 20, 30, 40, 50 and 60 °C
- Thermometer
- Stopwatch
- White tile or white paper, to see the colour change
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Phenolphthalein indicator (usually in ethanol)	Flammable, irritant to eyes and skin	Wear eye protection. Use small volumes, keep away from flames and wash off any splashes.
Sodium carbonate solution	Low hazard at 0.05 mol/dm ³ but can irritate eyes	Wear eye protection. Rinse splashes off skin and eyes with plenty of water.
Lipase	Enzymes can cause skin irritation or allergic reactions	Avoid skin contact and wash hands after use. Do not breathe in any dust if using solid lipase.
Hot water baths	Scalds and burns	Use a water bath at 60 °C or below, carry hot water carefully and place tubes in a rack. Mop up spills.
Milk	Allergy risk, and milk left at warm temperatures grows microorganisms	Do not taste or drink anything in the lab. Dispose of the mixtures down the sink with water and wash hands.

Method

1. Set the water baths to 20, 30, 40, 50 and 60 °C. Check each temperature with a thermometer.
2. Label two boiling tubes for the first temperature. Into tube 1, measure 5 cm³ of milk, then add 7 cm³ of sodium carbonate solution and 5 drops of phenolphthalein. The mixture should be pink.
3. Into tube 2, measure 1 cm³ of lipase solution.
4. Place both tubes in the water bath at the first temperature (20 °C) for 5 minutes, so that the contents reach this temperature.
5. Pour the lipase from tube 2 into tube 1, swirl gently and start the stopwatch straight away.
6. Keep the milk mixture in the water bath, standing in front of a white tile. Swirl it gently every 10 seconds.
7. Stop the stopwatch when the pink colour disappears. Record the time in s.
8. If there is no colour change after 10 minutes (600 s), stop the stopwatch and record 'No change in 600 s'.
9. Repeat steps 2-7 twice more at the same temperature, using clean tubes.
10. Repeat steps 2-9 at 30, 40, 50 and 60 °C.
11. Calculate the mean time for each temperature.

L2 . Calculate the rate for each temperature using $\text{rate} = 1 \div \text{mean time}$.

L3 . Plot a graph of rate (y-axis) against temperature (x-axis).

Results

Time taken for the pink colour to disappear at different temperatures					
Temperature (°C)	Time 1 (s)	Time 2 (s)	Time 3 (s)	Mean time (s)	Rate = $1 \div \text{mean time (s}^{-1})$
20					
30					
40					
50					
60					

Graph

Line graph of rate of reaction ($1 \div \text{time}$, in s^{-1}) on the y-axis against temperature in °C on the x-axis. Plot each point with a cross and draw a smooth curve (not straight lines point to point). The curve should rise to a peak at the optimum temperature and then fall. Use more than half of the grid and equal scale steps.



Exam questions 10 questions · 27 marks

1 State the dependent variable in this investigation.

[1 mark]

- 2** The milk and the lipase were each placed in the water bath for 5 minutes before they were mixed. Explain why. **[2 marks]**

- 3** Explain why the pink colour disappears during the investigation. **[2 marks]**

- 4** At 40 °C the times taken were 80 s, 78 s and 82 s. Calculate the rate of the reaction at 40 °C, using $\text{rate} = 1 \div \text{time}$. Give your answer to 2 significant figures. **[3 marks]**

Answer =

- 5** Use the sample results to explain the change in rate from 20 °C to 40 °C. **[3 marks]**

Mean time and rate		
Temperature (°C)	Mean time (s)	Rate (s ⁻¹)
20	320	0.0031
30	150	0.0067
40	80	0.013

6 At 60 °C there was no change in colour after 10 minutes. Explain why. **[3 marks]**

7 The student repeated each temperature three times. Give two reasons for repeating the experiment. **[2 marks]**

8 The student wants to find the optimum temperature more precisely. Suggest how the student should change the investigation. **[2 marks]**

9 Milk goes off more slowly in a refrigerator at 4 °C than on a kitchen table at 22 °C. Use your knowledge of decay to explain why. **[3 marks]**

10 Describe how you would investigate the effect of temperature on the rate of decay of milk [6 marks]
using lipase, sodium carbonate solution and phenolphthalein. Include how you would
make the results valid and repeatable.

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Teacher answers

Required practical 10: Temperature and the rate of decay of milk. The example results are practice data, not real measurements.

Example results

Example results (practice data)					
Temperature (°C)	Time 1 (s)	Time 2 (s)	Time 3 (s)	Mean time (s)	Rate = $1 \div \text{mean time}$ (s ⁻¹)
20	310	330	320	320	0.0031
30	145	155	150	150	0.0067
40	80	78	82	80	0.013
50	112	108	110	110	0.0091
60	No change in 600 s	No change in 600 s	No change in 600 s	> 600	< 0.0017

Conclusion

From 20 °C to 40 °C the time taken fell from 320 s to 80 s, so the rate of the reaction **increased** from 0.0031 s⁻¹ to 0.013 s⁻¹. At higher temperatures, particles move faster and collide more often, and with more energy, so lipase breaks down fat into fatty acids faster. This lowers the pH sooner. The rate was fastest at 40 °C, the optimum temperature in this experiment. At 50 °C the rate fell, and at 60 °C there was no colour change, because the lipase was **denatured**: its active site changed shape, so fat could no longer fit. In nature, decomposers break down dead material in the same way, so decay is fastest in warm conditions up to an optimum, and slow in cold conditions.

Errors and improvements

Error	Effect on the results	Improvement
Judging the end point (when the pink colour disappears) is subjective (random error).	Different people stop the stopwatch at different times, giving variation in the time.	Use the same person each time, put a white tile behind the tube, compare against a colourless tube, or use a pH meter or colorimeter.
The mixture changes temperature after it is taken out of the water bath or while the enzyme and milk are being mixed.	The temperature during the reaction is different from the temperature recorded, so the rate is wrong.	Keep the tube in the water bath all the time, pre-warm both tubes for 5 minutes and check the temperature with a thermometer.
Water bath temperature varies during the practical.	The temperature is not constant, so the results are not valid.	Use a thermostatically controlled water bath, or add hot or cold water as needed, and check the temperature at the start and end.
Measuring small volumes of lipase and indicator with limited accuracy.	Differences in volume change the amount of enzyme or indicator, which changes the time.	Use a 1 cm ³ syringe or a pipette for lipase and a dropper that gives drops of equal size.
Only five temperatures were used, 10 °C apart.	The optimum temperature cannot be found precisely.	Repeat at temperatures closer together around the fastest rate, for example 35, 40 and 45 °C.

Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

1. [1]

- time taken for the pink colour to disappear / time for the indicator to change colour (1) allow the rate of reaction

2. [2]

- so both reach the temperature of the water bath (1)
- so the reaction takes place at the chosen temperature from the start (1)

3. [2]

- lipase breaks down fat in the milk to make fatty acids (1)
- which lower the pH so that the indicator changes from pink (alkaline) to colourless (acid / neutral) (1)

4. [3]

- mean time = $(80 + 78 + 82) \div 3 = 80 \text{ s}$ (1)
- $1 \div 80$ (1)
- $= 0.013 \text{ s}^{-1}$ (1) 0.0125 (not to 2 significant figures) scores 2 marks

5. [3]

- as temperature increases, the rate of reaction increases / the time taken decreases (1)
- particles have more energy / move faster (1)
- so lipase and fat collide more often / with more energy, so more successful collisions per second (1)

6. [3]

- the lipase is denatured (1)
- the shape of the active site changes (1)
- so the fat no longer fits / no fatty acids are made, so the pH does not fall (1)

7. [2]

- to calculate a mean (1)
- to identify anomalous results / to check the results are repeatable (1)

8. [2]

- test more temperatures (1)
- closer together / at smaller intervals, between 30 °C and 50 °C, for example 35, 40 and 45 °C (1)

9. [3]

- decay is caused by microorganisms (decomposers) (1)
- at the lower temperature enzymes / respiration of microorganisms work more slowly (1)
- so they grow and break down the milk more slowly / the rate of decay is lower (1)

10. [6]

Level 3 5-6	A clear, logical method which includes: milk with sodium carbonate and indicator, lipase added after both are brought to the same temperature in a water bath, a stopwatch started at mixing and stopped at the colour change, a range of at least four temperatures, repeats and a mean, and the calculation of rate as $1 \div \text{time}$. At least three variables are controlled. The method could be followed by another person.
Level 2 3-4	A mostly clear method with the main steps in order. Some details are missing, such as pre-warming, the rate calculation or repeats.
Level 1 1-2	A few simple points, for example 'mix milk and lipase and time it', with little detail or no control of variables.

Indicative content

- put a set volume of milk, sodium carbonate and a set number of drops of phenolphthalein in a tube (pink)
- put lipase in a separate tube; place both in a water bath and leave until they reach the temperature
- mix and time how long the pink colour takes to disappear
- repeat at 20, 30, 40, 50 and 60 °C, and repeat each temperature to calculate a mean
- rate = $1 \div \text{time}$; plot a graph of rate against temperature
- control volumes, concentrations, number of drops, type of milk

Exam tips

- Rate is $1 \div \text{time}$. A shorter time means a faster rate, so do not say 'the rate decreased' when the time decreases.
- Above the optimum, say the enzyme is denatured and the shape of the active site has changed. Do not say the enzyme is 'killed', because enzymes are not alive.
- Name what the pH change shows: fatty acids are made when lipase breaks down fat, so the pH falls and the indicator changes colour.
- In evaluation questions, point out that the end point of a colour change is subjective, and suggest a pH probe or colorimeter as an improvement.
- Link the practical to decay: warmer conditions (up to an optimum) speed up decay because the microorganisms' enzymes and respiration work faster.