Student's Guide

Microbes and food

Microbes and food spoilage

Although some microbes are deliberately used to make foods such as yoghurt and cheese, other microbes spoil food. Various methods are used to prevent foods from 'going off', such as preservatives, heat treatment, and drying and freezing. You are going to investigate what happens to microbes on frozen peas after the peas have been defrosted and stored.

Learning objectives

To show:

- ▷ the rapid increase in microbial numbers during storage of defrosted food
- ▷ the role of microbes in food spoilage
- ▷ the dangers of re-freezing thawed food

Techniques required

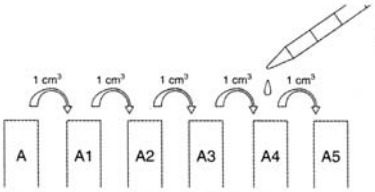
See *Basic Practical Microbiology*

- ▷ using a pipette (p. 10)

Procedure

1. Use the 10 cm^3 syringe or a pipette to add 5 cm^3 distilled water to test tube A containing freshly defrosted peas. Use a glass rod to gently crush the peas in the tube and mix them with the water as thoroughly as possible. Allow the mixture to settle.

2. Label five test tubes A1, A2, A3, A4, A5. Use the 10 cm^3 syringe or pipette to place 9 cm^3 distilled water in each one.



3. Using a 1 cm^3 syringe or pipette, mix the contents of tube A thoroughly by filling and emptying the syringe/pipette several times. Then transfer 1 cm^3 water from tube A to A1. Now transfer 1 cm^3 from A1 to A2. Mix thoroughly after each transfer. Continue to prepare the 'dilution series' as shown.

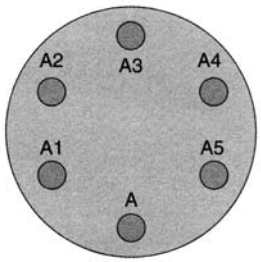
4. Mark an agar plate on the bottom as shown below. Label it with your name, the date and FP. Use the calibrated dropping pipette to draw up a small amount of sample from tube A5. Lift the lid of the Petri dish and release one drop on to the agar at position A5. The drop should be released as closely to the surface of the agar as possible to avoid splashing. Replace the lid. Return any excess sample from the dropping pipette to tube A5. Using the same pipette, repeat with the contents of tubes A4, A3, A2, A1 and A in that order. Discard the pipette into disinfectant. Allow the drops to soak into the agar. Tape the agar plate and invert it.

5. Label the other agar plate with '1DP', your name and the date. Repeat steps 1–4, using the 'day old' peas and clean test tubes and syringes/pipettes. Tape up and invert the plate as before. Both plates will be incubated until the next lesson.

Safety! *Do not open the plates.*

Next lesson...

6. Examine your agar plates and count the number of colonies visible at A, A1, A2, A3, A4, A5. Record your results in a table in two columns headed 'Fresh peas' and '1-day old peas'. Answer the questions.



Teacher's Guide

Microbes and food

Microbes and food spoilage

Almost any kind of food is a good medium for microbial growth. The process of 'going off' occurs when microbes grow on or in the food, altering its consistency, taste and smell. The processes used to preserve foods (see p. 30) are those that delay or prevent microbial growth. This investigation demonstrates the rate of bacterial growth in food, using a routine method of estimating microbial numbers.

Learning objectives

To show:

- the rapid increase in microbial numbers during storage of defrosted food
- the role of microbes in food spoilage
- the dangers of re-freezing thawed food

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Materials (each group)

- test tube A containing 3 freshly defrosted peas
- test tube B containing 3 peas defrosted and left at room temperature for 24 hours
- 2 nutrient agar plates
- 2 clean glass rods
- 2 × sterile 1 cm^3 syringes/pipettes and fillers
- 2 × sterile 10 cm^3 syringes/pipettes and fillers
- 150 cm^3 sterile distilled water
- adhesive tape
- marker pen
- 10 test tubes
- 2 calibrated dropping pipettes
- beaker of disinfectant

Recommendations

1. Ideally the apparatus should be sterile for best results; it should certainly be very clean.
2. The peas are in tubes to avoid contamination by the students.
3. The agar plates should be poured prior to the investigation to ensure that their surfaces are dry. After inoculation, they should not be disturbed until the drops have dried.
4. The plates should be incubated at 20–25 °C or at room temperature for 2–3 days and refrigerated until examined.
5. A Pasteur pipette can be calibrated by drawing into it a known volume, e.g. 1 cm^3 water, and counting the number of drops formed when this volume is discharged. Alternatively, plastic disposable Pasteur pipettes are available from school science suppliers. The drop size from these is ca 0·02 cm^3.

Notes

1. It is safe to use uncooked vegetables because of the types of microbe present, unlike raw meat which may contain pathogens and must not be used.
2. This is known as the Miles and Misra technique for estimating viable cell counts. Drops that contain large numbers of viable cells give rise to circular areas of confluent growth. Any drop containing less than about 15 viable cells will produce a small, countable number of colonies.

Questions

Session 1

1. Describe some types of food spoilage.
2. Which peas do you expect to have most microbes on them?
3. What is the purpose of making the two dilution series?
4. Why are the samples used in the order suggested?
5. What do you expect your agar plates to look like next lesson?

Session 2

6. Why do some of the six spots on each plate show more growth than others?
7. Do all the colonies look the same? If not, what does this suggest?
8. If one microbe gives rise to each colony, work out how many microbes were in each of the original pea suspensions.
9. Has storage for 24 hours after defrosting made any difference to the numbers of microbes?
10. Why is it unwise to refreeze frozen food that has thawed and been kept at room temperature?
11. Suggest the main sources of error in this investigation. How would you improve it to obtain reliable estimates of microbial numbers?

