firmer jam and finer fruit

Pectin esterase (or pectin methyl esterase) is one of the first 'pure' pectinase preparations available from commercial enzyme suppliers. It strips methyl ($-CH_3$) groups from methoxyl pectin (see Figure 6, page 9). This allows divalent ions (such as Ca^{2+}) to form cross-links between the pectin molecules, causing them to form a gel. The process can be used to thicken fruit purées without boiling them, and to stiffen fruit pieces used in yoghurts and desserts, reducing wastage and improving the quality and 'mouthfeel' of the product.

Aim

To investigate the effect of pectin esterase on pectin.

Preparation

A pectin solution can be prepared in advance (see box, below) or purchased from a supermarket.

Timing

This activity takes about 50 minutes.

Materials and equipment

Needed by each person or group

- A solution of apple pectin, 50 cm^3 (the type sold for jam-making)
  OR
  A solution of lemon pectin extracted as described below (see box). *These solutions must be made up with hard tap water or 20 mM calcium chloride solution*
- Pectin esterase, *e.g.*, Gist-brocades *Rapidase™ FP Super* or Novozymes *NovoShape™*, 0.5 cm^3
- 1 cm^3 syringe (for dispensing the enzyme)
- 10 cm^3 syringe (for dispensing the pectin solution)
- Small beakers, *e.g.*, *100 cm^3*, 4
- 25 cm^3 syringe barrels, 2 (for assaying flow rate)
- Retort stand with two bosses and clamps
- Stopclock

Procedure

1. Fix both syringes in the clamps and attach them to the stand so that each syringe is about 10 cm above the bench surface.
2. Add 25 cm^3 of pectin solution to each of two beakers.
3. To the first beaker add 0.5 cm^3 of distilled water and mix it into the pectin solution. *Start the clock*.
4. To the second beaker add 0.5 cm^3 of enzyme and mix it well into the pectin solution.
5. Carefully but smartly pour the two solutions into the vertically clamped syringes. Ideally, both solutions should be added at the same time. If you do this, it is wise to place a finger over each syringe nozzle until both are full (a friend will come in handy here!).
6. Record the time taken for the solutions to run through the two syringes.
7. Repeat steps 5 and 6 until three similar readings are obtained.
8. Calculate the relative pectin esterase activity (see the instructions opposite).

Safety

Do not consume materials treated with enzyme

If fruit purée or pieces are used (see *Further activities*), they should not be eaten. The enzyme used in this protocol is of food grade, but it has not been used in aseptic conditions. Therefore materials produced using such enzymes must *not* be consumed. *Please refer to the general enzyme safety guidelines on page 11.*

Further activities

1. Investigate the effect of different sources of calcium ions on the gelling of pectin treated with pectin esterase (see sample graphs opposite).
2. Investigate the effect of pectin esterase on purées made from different types of fruit. Fruit purée can be prepared by blending fruit with water in a liquidiser. The purée must be 'thin' enough to pass through a syringe barrel. It should be stored, frozen, until required. Remember that calcium ions are required for gelling, so you may need to add an appropriate salt (*e.g.*, $CaCl_2$). If you live in a hard water area, tap water may suffice.
3. Devise a method for testing the strength of fruit pieces. Use this method to measure the effect of treating the fruit pieces with pectin esterase.
4. Pectin esterase is inhibited by high concentrations of sucrose. Devise an investigation to study this effect.

resources

Keep fruit in good shape with *NovoShape™* (1999) *BioTimes* March, No. 1, p. 3. www.biotimes.com/biotimes9903/main.htm

prepare your own pectin

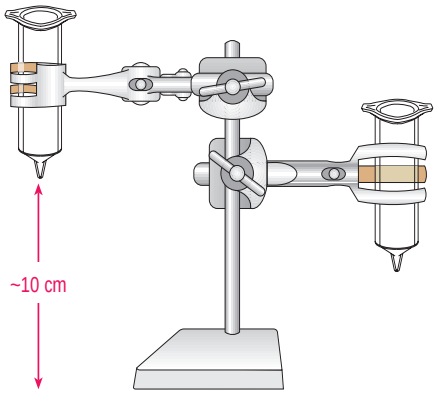
Extracting pectin from fruit

You can buy bottles of pectin solution for jam-making. Usually this product has been extracted from apple cores and peels and is suitable for use in this investigation. It contains a large proportion of high-methoxyl pectin (see Figure 6, page 9).

An alternative is to extract pectin from fruit yourself. This is simple to do, and a comparison of the type of pectin found in different fruits could form a worthwhile practical investigation.

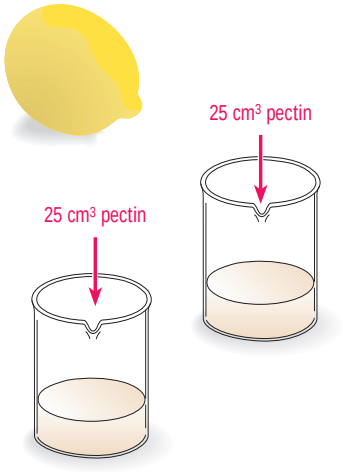
Pectin is found in the *albedo* or white layer immediately beneath the skin of citrus fruits. To obtain pectin from lemons, for example, slice a lemon, then carefully cut away a thin layer of the outer, yellow, peel so that the white layer is exposed (fine scissors are useful for this). Cover the slices with water and simmer gently until the solution thickens and the edges of any peel that remain start to become transparent (about 30 minutes). Strain the liquid from the fruit, then allow the solution to cool. Store the pectin solution in a 'fridge. One lemon should yield about 100 cm^3 of pectin solution.

1 Clamp two empty 25 cm^3 syringe barrels onto a retort stand, about 10 cm above the bench top.

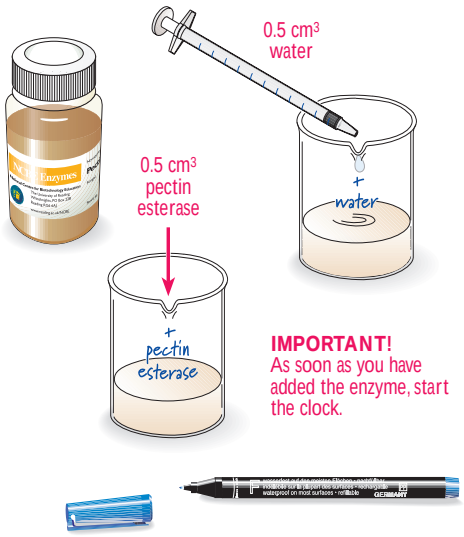


2 Prepare two identical beakers of pectin solution.

You can easily extract pectin from fruit such as lemons (see box on page 18).



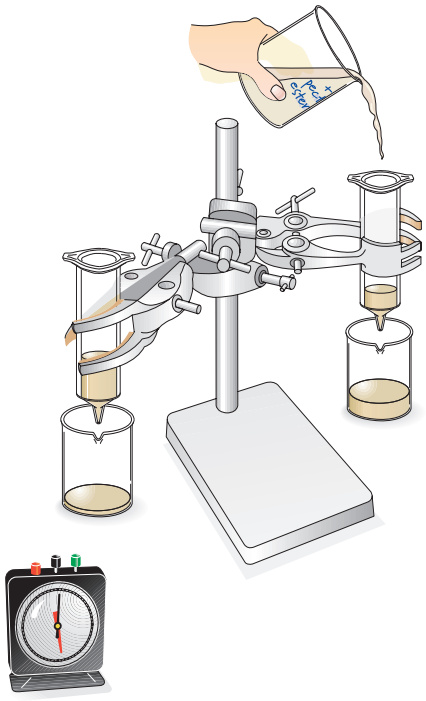
3 4 Label the beakers appropriately. Add pectin esterase to one beaker and distilled water to the other (a 'control').



IMPORTANT!
As soon as you have added the enzyme, start the clock.

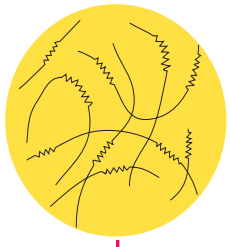
5 6 Pour the two solutions into the syringes.

Record the time taken for each solution to run through its syringe.



7 Repeat steps 6 and 7 until three similar readings are obtained (in other words, keep going until the enzyme activity appears to have stopped).

What's going on?



The black lines represent chains of pectin molecules.

Regions where methyl ($-CH_3$) groups have been stripped off by the pectin esterase are shown as zig-zagged lines.

Ca^{2+}

Calcium ions (shown as small dots) form 'bridges' between the non-methylated regions, linking the pectin molecules.

This causes the pectin solution to form a semi-solid gel.

Note:
This is not the process by which pectin gels in normal jam or marmalade. There, the acidic conditions plus sugar provide the conditions necessary for pectin chains to be cross-linked.

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ANALYSIS

The decrease in flow rate may be calculated as follows:

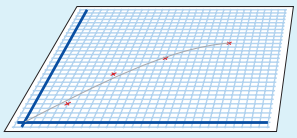
$$\% \text{ decrease} = \frac{F - F_t}{F - F_W} \times 100$$

F = flow rate of untreated pectin
F_t = flow rate of the pectin solution after incubation time, t
F_W = flow rate of pectin with distilled water

This can be plotted against time of incubation, and the time required for a 50% decrease in flow rate (t50) read off the x axis of the graph.

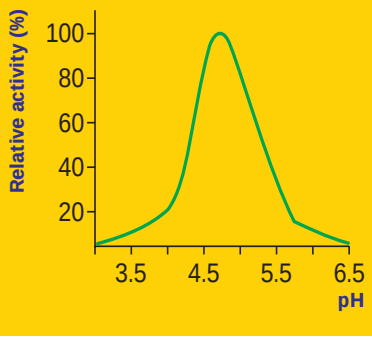
This can be used to calculate the relative pectin esterase activity:

$$\text{Relative pectinase activity} = \frac{1}{t50} \times 100$$

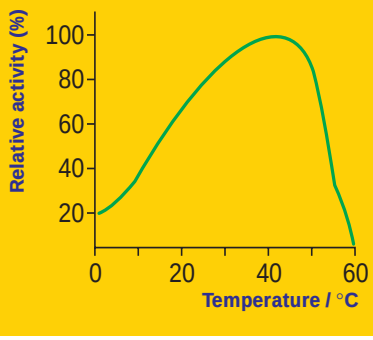


Pectin esterase

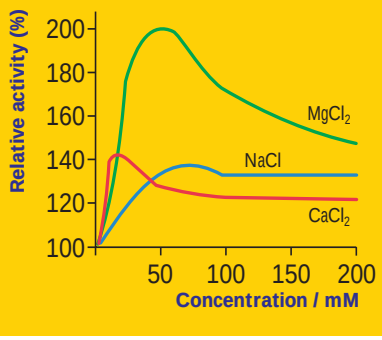
Pectin esterase activity at 20 °C



Pectin esterase activity at pH 4.8



Pectin esterase activity at pH 4.8, 20 °C



Data from Novozymes A/S.

Dean Madden