clarifying fruit juice

The use of pectinase to extract juice from apple is a well-known school practical investigation. However, pectinase was first used industrially to *clarify* apple juice. Here we investigate the effect of pectinase and amylase on their own and combined. This work has the advantage that it can readily be extended to incorporate other enzymes *e.g., cellulase, amyloglucosidase (AMG)* or be tried using other cloudy fruit juices.

Aim

To produce clear apple juice it is absolutely necessary to remove pectin from it. This is achieved using enzymes. The purpose of this investigation is to investigate the effect of different enzyme preparations on cloudy apple juice.

Preparation

To ensure a reasonable reaction rate, if the juice has been stored in a 'fridge, it should be allowed to warm to room temperature before use.

Timing

This activity takes about 50 minutes. Further clearing may be observed if the enzyme is allowed to act overnight.

Materials and equipment

Needed by each person or group

- Cloudy pure apple juice, 40 cm^3
- Pectinase, *e.g.,* Novozymes *Pectinex*™, 1.5 cm^3
- Amylase, *e.g.,* Novozymes *Termamyl*®, 1.5 cm^3
- 1 cm^3 syringes for measuring out enzymes, 2
- 10 cm^3 syringe for measuring out apple juice
- Waterproof marker pen with which to label test tubes
- Test tubes, 4
- Test tube rack suitable for use in a water bath
- Water bath set at 50 °C

Procedure

1. Label the tops of the four test tubes using a marker pen. *For example, if you were using pectinase and amylase, with a water control a suitable code might be as follows: P, A, P+A, W.*
2. Measure 1 cm^3 of pectinase into the first test tube. Place 1 cm^3 of amylase into the second test tube. Add a mixture of 0.5 cm^3 of pectinase and 0.5 cm^3 of amylase to the third tube. Finally, add 1 cm^3 of distilled water to the remaining tube. *You could try other enzymes too (e.g., AMG) either alone or in combination.*
3. Stir the apple juice well to distribute any suspended particles evenly through it. Add 10 cm^3 of apple juice to each test tube.
4. Agitate or stir the contents of the tubes to mix the enzymes throughout the juice.
5. Put all the tubes into the water bath at 50 °C.
6. Observe the tubes and record the appearance of their contents at 5-minute intervals over a half hour period.
7. Further clearing may occur if the tubes are left to stand overnight at room temperature.

Safety

Do not consume the fruit juice

The concentrations of enzymes suggested here are far greater than are normally used for clarifying fruit juice. In addition, the materials have not been handled aseptically. Therefore any fruit juice produced must *not* be consumed. *Please refer to the general enzyme safety guidelines on page 11.*

Further activities

It is relatively simple to devise investigations using different enzymes, individually or in combination, to clarify juices from various fruits. The following tests will help you to assess whether the enzyme treatment has been effective:

1. **Test for starch**
   Heat 10 cm^3 of juice to 70 °C in a test tube. Allow it to cool then add 2–3 drops of 1% iodine in 10% potassium iodide solution (the type used for food tests). A blue colour indicates the presence of starch; brown shows that the starch is partially broken down; yellow indicates no starch. *Note: some commercial apple juices contain practically no starch, whilst others have appreciable amounts. A few tests of different juice varieties will be needed.*

2. **Test for pectin**
   Add 1 part of juice to 1.5 parts of acidified ethanol (add 1 cm^3 of concentrated hydrochloric acid to 99 cm^3 of 96% ethanol). If flocculation occurs after 15 minutes, pectin is still present.

resources

The use of enzymes in the fruit juice industry (1985) Novo Ferment (Switzerland) Ltd.

An industrial enzyme brochure.

clarifying lemon and blackcurrant juices

Clarification of acidic juices

Unlike orange juice, that derived from lemons or blackcurrants is often sold as a clear liquid. Pectinases were first used in the early 1930s for clarifying syrups made from acidic fruits such as these. The traditional methods of clarifying lemon juice, for example, relied upon the fruit's natural pectin esterase content. The addition of calcium ions allowed suspended solids to be precipitated as calcium pectate, but this process can take some 4–16 weeks. A preservative must be added to the juice to prevent microbial spoilage during this period.

Fungal enzymes are available which exhibit pectolytic activity even in very acidic juices such as lemon or lime (pH 2.2–2.8). Their use enables clarification to be achieved in 6 hours without the need for preservatives. Because lemons are rich in the polysaccharide araban, special preparations of pectinase and arabanase are sold for clearing lemon juice.

1 Use a waterproof marker pen to label four test tubes with the following code letters:

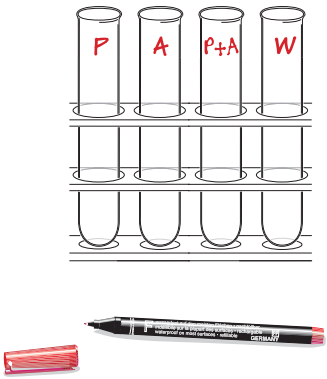
P – pectinase
A – amylase
P+A – pectinase + amylase
W – distilled water

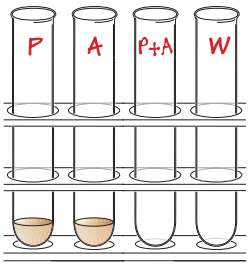
2 Using a clean syringe each time, measure:

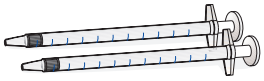
- 1 cm³ of pectinase into the first tube;
- 1 cm³ of amylase into the second tube;
- 0.5 cm³ of pectinase and 0.5 cm³ of amylase into the third tube;
- 1 cm³ of distilled water into the fourth tube.

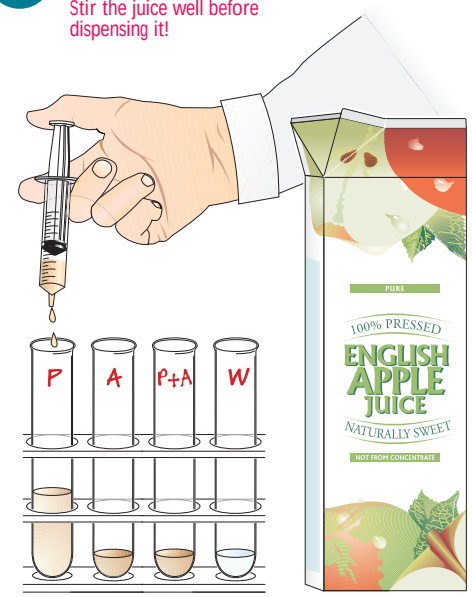
3 Now add 10 cm³ of cloudy apple juice to each tube.

Stir the juice well before dispensing it!

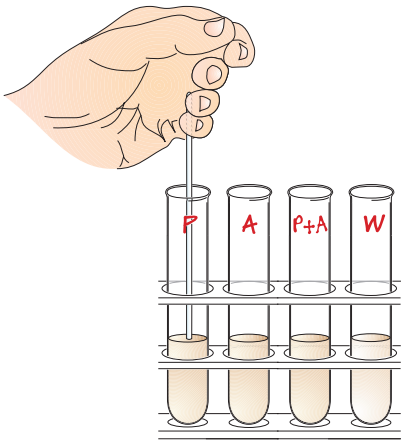






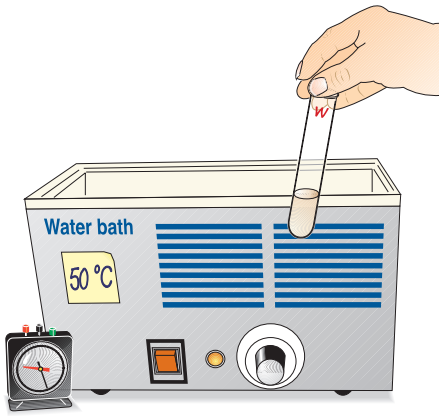


4 Stir the contents of each tube with a clean glass rod.



5 **6** Stand the tubes in a water bath or incubator set at 50 °C.

Examine the juice at 5-minute intervals, and record its appearence.



These graphs show the activity of four commercial enzyme products:

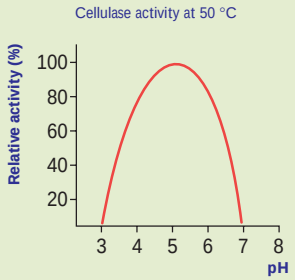
Celluclast®, a mixture of cellulases;
Amyloglucosidase, a type of amylase;
Pectinex™, a pectinase preparation that contains several enzymes, the chief one being polygalacturonase; and
Termamyl®, an α-amylase.

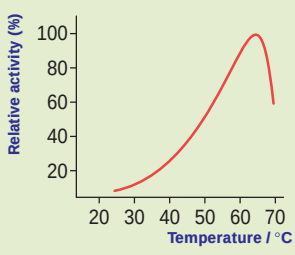
The data may help you to plan further investigations of your own.

Note:
These graphs can only be used as a guide, as the enzyme activity will vary according to many factors, as well as temperature and pH, including:

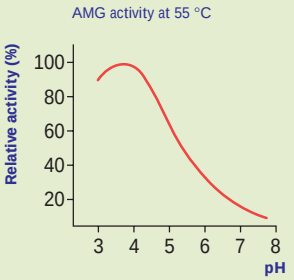
- the exact substrate used;
- the reaction time;
- the presence of inhibitors (or in the case of *Termamyl*®, the stabilising effect of Ca^{2+} ions).

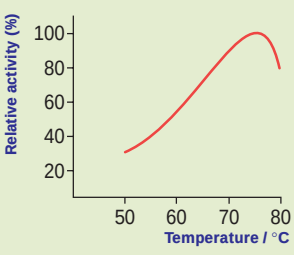
Cellulase (mixture of enzymes)



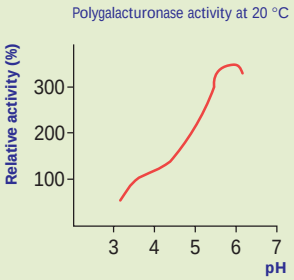


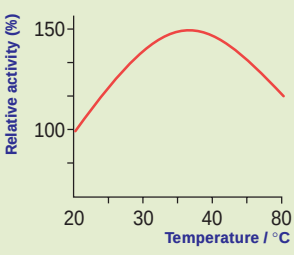
Amylase (amyloglucosidase)



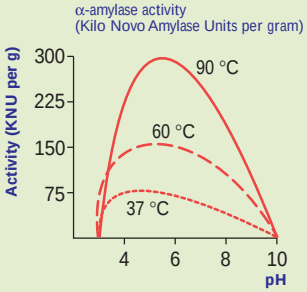


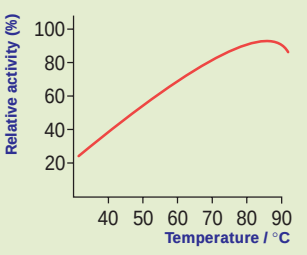
Pectinase (mixture of enzymes)





Amylase (α-amylase)





Data from Novozymes A/S.

Dean Madden