Exploring the role of pectinase in making juice

Aim: To investigate the effect of pH on clarification of cloudy apple juice.

Background:
Extracting juice from fruits can be done manually or enzymatically. The use of enzymes increases the efficiency (in terms of speed and volume of juice). Pectinase, amylase and cellulase break down complex plant cell walls to release juice; a combination of these enzymes are frequently used for optimal results.

Fruit is made up of cells linked by middle lamellae which contain insoluble proto-pectin – this can be degrade by **pectinase**. The cell walls are composed largely of cellulose and hemicellulose – **cellulases** support the breakdown of these structures.

As cellular breakdown continues, various polysaccharides are found in the juice extract, which can contribute to cloudiness in the final juice preparation. This can be undesirable. **Pectinases** and **amylases** can breakdown these polysaccharides to release soluble sugars, which clarify the juice producing a clearer, sweeter product.

Enzymes are expensive products and juice manufacturers aim to minimise their costs by using the enzymes at their optimum conditions and therefore maximising their effectiveness, re-using the enzymes where possible.

Independent variable options:
There are various independent variables that can be investigated in this protocol. Pectinase, amylase and cellulase could be compared for their relative ability to extract juice from one type of fruit. A variety of factors that affect enzyme activity, e.g. temperature, pH, enzyme concentration, could be tested. In this investigation, the variety of apple used for juice extraction using pectinase will be explored.

Materials:

30 g apple	Knife
Chopping board	Balance
Blender	Weigh boat
Muslin	100 cm^3 Beaker
400 µl pectinase (Novozymes Pectinex)	Microcentrifuge
3 cm^3 plastic pipettes	Waterbath set to 60 °C
1000 µl automatic pipettes and tips	300 µl pH 4.0, pH 7.0 and pH 10.0 buffers
3x Microfuge tubes	25 cm^3 measuring cylinder
6 cm^3 distilled water	Microfuge tube foam rack
Colorimeter	4x cuvettes (smallest capacity)

Method:

1. **Prepare apple:** Peel and chop 30 g of apple. Add the apple to the blender, with 5 cm^3 distilled water. Blend the apple until it becomes a puree.

2. Pass the apple through muslin into a clean beaker to collect the juice.

3. Label 3 microfuge tubes: pH 4, pH 7, pH 10

4. Using the automatic pipette, add 100 µl of pectinase to 3 microfuge tubes.

5. Using the automatic pipette with a clean tip, add 300 µl of the appropriate pH buffer to the correct microfuge tube.

6. Place the microfuge tubes at 60 °C for 5 minutes.

7. Add 600 µl apple juice to each tube. Agitate to distribute the reaction mixture and incubate for 5 minutes.

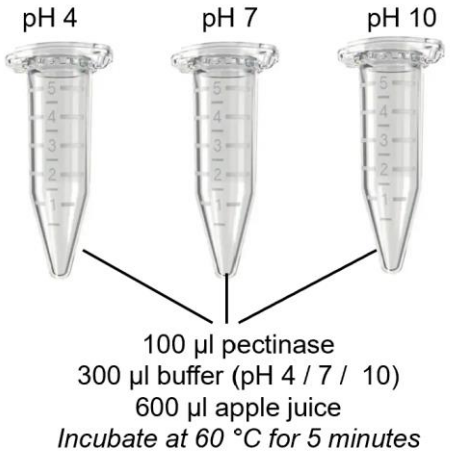

Figure 1. Preparation of 3 microfuge tubes for assays at different pH. Enzyme and buffer added, equilibrated to the optimum temperature, and then addition of apple juice. Reactions incubated at 60 °C for 5 minutes.

8. Centrifuge the reaction mixture for 1 minute – or simply allow to settle for a few minutes.

9. Transfer the supernatants to separate cuvettes using a plastic pipette.

10. **Colorimetric blank**: Set the colorimeter to measure light transmission with the blue diode (optimum will be around 435 nm – the blue diode will measure at 465 nm). To a clean cuvette, add 900 µl water + 100 µl pectinase. Calibrate / blank the transmission reading – this should say 100%. This is necessary because the enzyme has a colour and will affect the transmission of light through the sample.

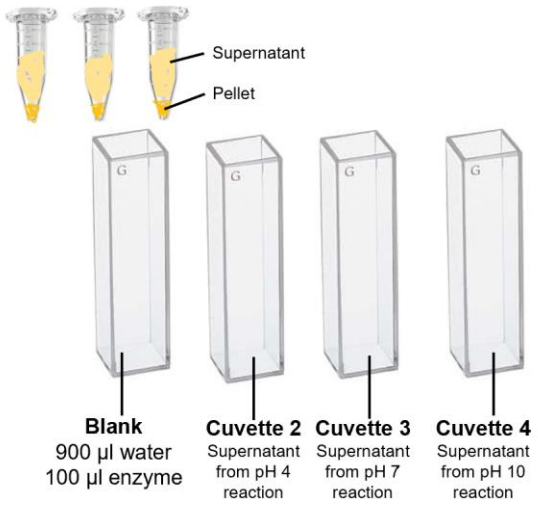

Figure 2. To measure the transmission of light through the enzyme-treated apple juice. Prepare the colorimetric blank to account for the effect of the coloured enzyme solution. Then decant the supernatant from each of the reactions into separate cuvettes. Measure the transmission of light through the samples using the blue diode.

11. Measure the transmission of light through each of the solutions. Share results with your colleagues and calculate the mean transmission value. What is the optimum pH of pectinase treatment?

Results

pH	Transmission of light through juice (%)			
	1	2	3	Average
4				
7				
10				

Supplier information:

Item	Supplier and cost
Pectinase	Pectinex® Available from NCBE University of Reading £24 for 100 cm^3
Blender	SSERC uses this one: Kenwood CH180 Mini Chopper Argos 423.2568 £25
Choice of apple	Golden delicious, Granny Smith, Jazz – all tried and tested. Any apple variety will work well, including those growing in gardens that learners could bring in.
Microcentrifuge	At SSERC, we use the Sprout Plus Mini Centrifuge from Philip Harris (product code: B8R09549) – cost £431.76 (inc. VAT). However, suitable alternatives include: Timstar / WF Education, Ultra minicentrifuge. £186.48 inc. VAT. Product code: CE190100
Microfuge tubes	We purchased these from: Philip Harris, Product Code: B8R05667 Pack of 500, £19.80 inc. VAT
Automatic pipettes & tips	Timstar, code: BT140565 Cost £114 inc. VAT

	Volume: 100 – 1000 µl
Colorimeter	This session uses the Mystrica colorimeter. Available from many suppliers including Breckland Scientific, Product code: COL-250-120 Cost: £156.92 inc. VAT Cuvette and cuvette rack comes with the colorimeter.