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More juice from apples

Pectinases and cellulases can be used to enhance the yield of juice from apples and similar fruits

Aim

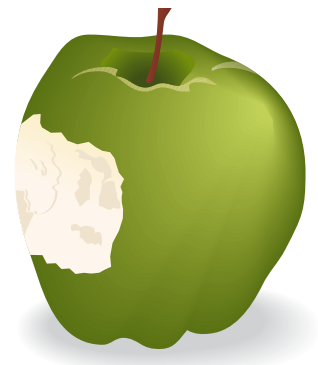
To investigate how enzymes may be used to enhance the yield of fruit juice from apples and similar fruits

Introduction

Enzymatic juice extraction from apples was introduced 35 years ago and today some 5 million tons of apples are processed into juice annually throughout the world. Where enzymes are used in commercial production, juice is extracted as follows:

- After they have been crushed, apples are usually left for 20–30 minutes so that enzyme inhibitors in the pulp are oxidised.
- The pulp is then heated to 30 °C before pectinases are added (this compares with a temperature of 50–60 °C which is needed if enzymes are not used). Typically, 130 mL of enzyme is added for every ton of apples.
- Enzyme treatment takes anything from 15 minutes to 2 hours, depending upon the exact nature of the enzyme, the dosage rate, the reaction temperature and the variety of apple used. Some varieties, like *Golden Delicious*, are very difficult to break down. During incubation, the pectinases degrade soluble pectin in the pulp, making the juice flow more freely.
- Next, the apples are pressed. Yields of juice may be increased by up to 20% by enzyme treatment, depending upon the age and variety of apple used and whether pre-oxidation is employed. Pectinase treatment is particularly effective with mature apples and those from cold storage. Significant increases in yield are not usually achieved from fresh, early season fruit.

This investigation presents a simplified version of the commercial production method, using relatively large amounts of enzyme so that results are obtained quickly. Try this procedure with one or more different enzyme preparations, or with different types of fruit.



Equipment and materials

Needed by each person or group

Equipment

- Knife (if pre-pulped apple is not used)
- Glass stirring rods or plastic spoons, 2
- 1 mL syringes, 2 (for measuring out enzyme and water)
- Filter funnels, 2
- 100 mL measuring cylinders, 2
- 100 mL beakers, 2
- Water bath or incubator, set to 40 °C
- Stopclock
- Access to a balance

Materials

- Apple or tinned apple purée, 100 g
- Pectinase, *e.g.*, *Novozymes Pectinex*[®], 1 mL. Dilute with an equal volume of distilled water immediately before use.
- OPTIONAL: Cellulase enzyme, *e.g.*, *Novozymes Celluclast*[®], 1 mL. Dilute with an equal volume of distilled water immediately before use.
- OPTIONAL (if starch-containing purée is used): Amylase, *e.g.*, *Novozymes Termamyl*[®] or Amyloglucosidase, AMG, 1 mL. Dilute with an equal volume of distilled water immediately before use.
- Coffee filter papers, 2
- Cling film

Procedure

- 1 Chop one medium-sized apple into small pieces, roughly 5 mm x 5 mm x 5 mm. Alternatively, tinned apple purée may be used.
- 2 Weigh half of the apple into one beaker and half into another (put about 50 g of apple in each).
- 3 Add 2 mL of diluted pectinase preparation to one of the beakers, and 2 mL of water to the other. OPTIONAL: Other enzymes may be used too, either by themselves or in combination.

Fig. 1

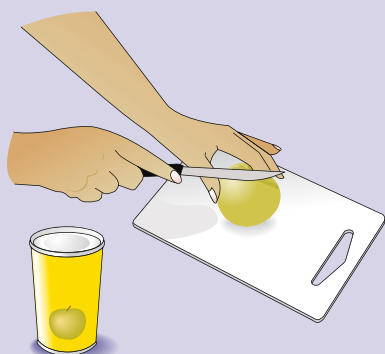


Fig. 2

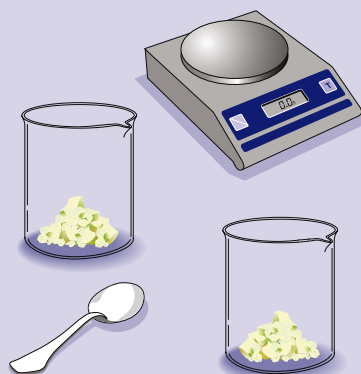
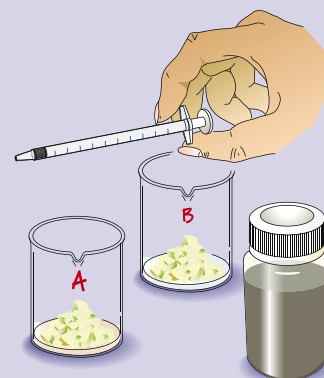


Fig. 3



- 4 Stir the beakers' contents with a clean glass rod or spoon.
- 5 Cover the beakers with cling film, then incubate them in a water bath or incubator at 40 °C for 15–20 minutes.
- 6 Filter the juice from the apple pieces or purée, using coffee filter papers in funnels placed in measuring cylinders.
- 7 Record the volume of juice obtained from both lots of apple pulp at 5-minute intervals, and plot a graph of the results.

Fig. 4

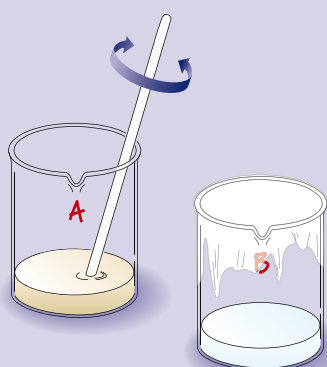
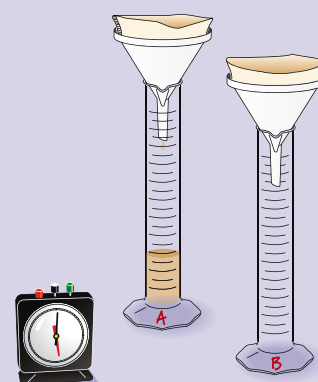


Fig. 5



Fig. 6



Safety guidelines



Do not consume the fruit juice

The enzymes suggested for this work are safe to use, provided they are handled appropriately. While all the enzymes suggested are food-grade products, juice prepared with them should not be consumed. There are two reasons for this. Firstly, the proportions of enzyme products used here are far greater than are normally used in the food industry. Secondly, the enzymes have not been handled aseptically, so they (and the products made using them) may have been contaminated.

Readers are advised to refer to any local safety guidelines and to carry out their own risk assessment for any practical work.

General enzyme safety guidelines

As enzymes are water-soluble, water should always be used for their removal if they are spilt.

Do not let liquid enzyme preparations dry up

If liquid preparations are allowed to dry up, there is a risk of dust formation. In susceptible people the repeated inhalation of such dust may provoke asthma or a reaction similar to hay fever. Any spillage — on equipment, on the floor or bench — should immediately be rinsed away with water.

Avoid the formation of aerosols

If enzyme-containing aerosols are formed, there is a risk of inhalation of the enzyme. In susceptible people the repeated inhalation of such aerosols may provoke asthma or hay fever. For this reason enzyme preparations should never be sprayed.

Avoid direct skin and eye contact

If, by accident, you get liquid enzyme on your skin or in your eyes, the remedy is plenty of tap water. The same applies to clothing. In the event of a spill on clothes, rinse with water then wash as usual. This treatment will generally prove sufficient, but if symptoms develop in the respiratory passages, on the skin or in the eyes, consult a doctor immediately.



Knives

Care should also be taken when handling knives during this practical procedure. Prepared apple pulp (e.g., tinned) may be substituted for fresh apple if desired.

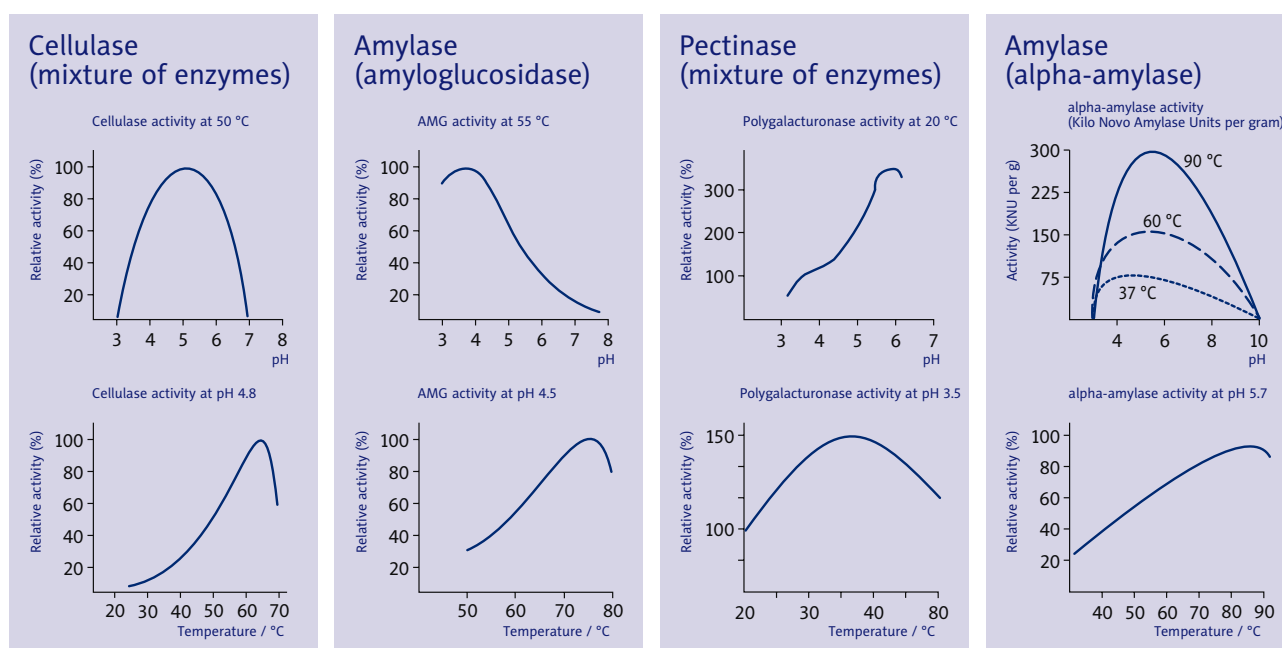
Preparation and timing

This activity takes about 50 minutes. The apple pulp can be prepared beforehand or bought as tinned purée.

Troubleshooting

Some tinned purée contains starch however, and may not yield much juice — or it may require the addition of other enzymes (e.g., amylase).

Enzyme performance data



DATA COURTESY NOVZYMES A/S

Open-ended investigations

This procedure presents several opportunities for open-ended practical investigations. For example, students can:

- Compare the yield of juice from different varieties of apples (or other fruits or vegetables).
- Investigate the effects of enzyme dosage and incubation temperature on juice yield.
- Compare the yield of juice from pulp which has or has not undergone pre-oxidation (oxidation before pressing prevents certain enzyme inhibitors from working).
- Does the addition of cellulase to the purée increase the yield of juice? Do pectinase and cellulase in combination further enhance yields?

Suppliers

The NCBE supplies *Novozymes* enzyme products to schools and colleges in the UK. Similar arrangements may exist in other countries.

Storage of materials

The enzyme preparations should be stored, undiluted, at 3–4 °C.

Other sources of information

In a jam and out of juice by Dean Madden (2000) National Centre for Biotechnology Education. ISBN: 0 7049 1373 9.

The current protocol is adapted from one in this publication.

Voragen, A.G.J. and L.A.M. van den Broek (1991) 'Fruit juices' In: *Biotechnological innovations in food processing* (M.C.E. van Dam-Mieras, et al. [Eds]) 187–210. Butterworth-Heinemann. ISBN: 0 7506 1513 3.

This chapter in a textbook provides a comprehensive review of the use of enzymes in fruit juice production.

Some useful, albeit slightly dated, reviews are given by the following papers:

Rombouts, F.M. and W. Pilnik (1978) Enzymes in fruit and vegetable juice technology. *Process Biochemistry*, 13 (8) 9–13.

Kilara, A. (1982) Enzymes and their uses in the processed apple industry: A review. *Process Biochemistry*, 17 (4) 35–41.

Web site

Novozymes A/S, Denmark

<http://www.novozymes.com>

Acknowledgement



This practical protocol was adapted for the *Volvox* project, which is funded under the Sixth Framework Programme of the European Commission.