

Remarkable rennet

CHEESE PRODUCTION has for thousands of years relied upon the action of enzymes which coagulate the proteins in milk, forming solid curds (from which cheese is made) and liquid whey. The process has two stages. Firstly, the enzyme breaks specific bonds in a glycopeptide on the surface of soluble calcium caseinate particles in the milk. Relatively insoluble calcium paracaseinate is formed, and in the presence of calcium ions this coagulates to form a continuous curd.

Traditional cheese-making has mainly involved the use of animal enzymes, although some cheeses in the UK were made with plant proteases. Today there are several enzymes available to the manufacturer — from animals, naturally-occurring fungi and animal-identical products from genetically-modified microbes.

Here we suggest investigations which can be done using one or several of these enzymes.

Materials

Rennet enzyme
e.g. calf rennet essence; Novo Nordisk *Rennilase*[®] (a fungal protease); or Gist-brocades *Maxiren* (pure calf chymosin from a genetically-modified yeast). Rennet essence is usually sold in supermarkets or health food shops. The two microbial enzymes are available from the NCBE.

Pasteurized milk
1 cm³ syringes (for measuring out enzymes)
10 cm³ syringes (for measuring out milk)
Test tubes
Stopclock
Water bath (optional)

ADDITIONAL INFORMATION

Madden, D. (1991) 'Milk-coagulating enzymes by accident and design' *NCBE Newsletter* Summer 1991. pp. 1–5.

Gill, J. and Saunders, T. (1987) 'Rennin - a neglected enzyme?' *Journal of Biological Education* 21, (4) 248–250.

Practical details

1. Dispense 10 cm³ of milk into test tubes.
2. Add 1 cm³ of rennet enzyme.
3. Record the time taken for the milk to coagulate. It may be possible to distinguish between a 'partial set' i.e. when particles adhere to the side of the tube when it is gently rocked from side to side, and a 'full set' i.e. when a solid curd is formed.

Safety

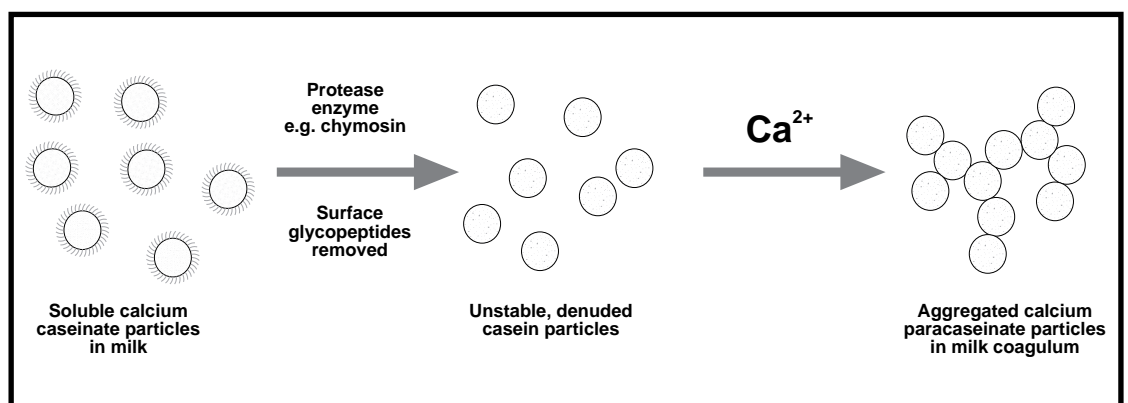
The products of this investigation should not be consumed (some daring home economics students have been known to try this!)

Further activities

1. Investigate the effect of temperature on the time taken for the curd to form.
2. Compare the activity of different types of enzymes.
3. Add calcium chloride to the milk, to investigate the effect of calcium ion concentration on the setting rate e.g. *Maxiren* requires a slightly greater calcium ion concentration than some other enzymes.
4. Alter the pH of the milk, using 1M hydrochloric or citric acid, 1M sodium hydrogen carbonate or sodium hydroxide. 1 cm³ of any of these reagents, added to 10 cm³ of Pasteurized milk will give approximate pHs of 1, 5, 8 and 14, respectively.
5. Dilute the enzyme with distilled water to investigate the effect of enzyme concentration.



Below:
Chymosin converts
milk protein (casein)
into a solid curd.



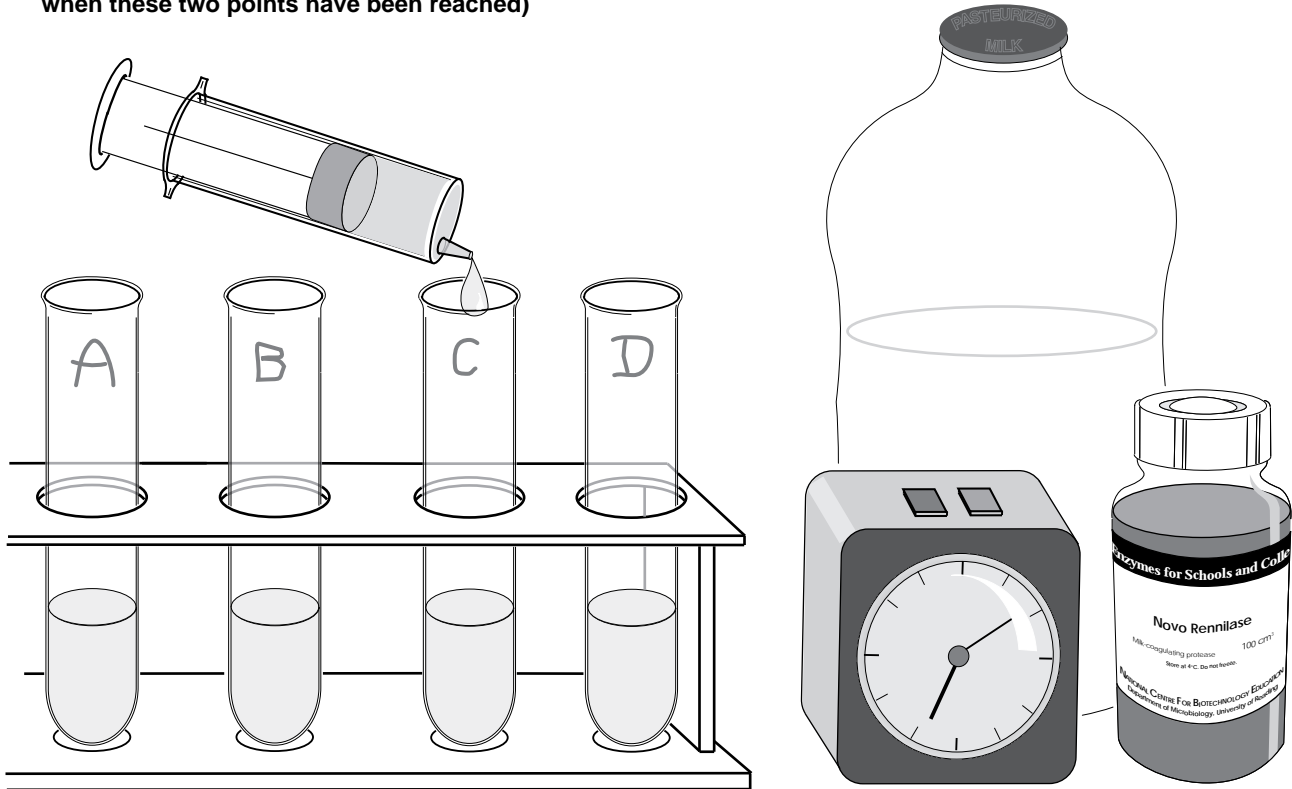
Remarkable rennet

Use 1 cm³ of enzyme to 10 cm³ of Pasteurized milk in a test tube

Record how long it takes for the milk to set :

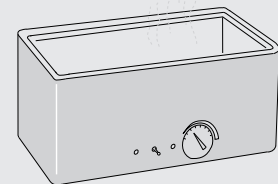
a) partially; and b) completely

(You will need to think carefully about how you decide when these two points have been reached)



Extend the investigation by trying:

Different temperatures;
(use a water bath)



Different enzymes;
(for example, compare shop-bought calf rennet (for making junket), fungal enzyme and pure chymosin from genetically-modified yeast)



Different pH values;

(1 cm³ of 1M hydrochloric acid added to 10 cm³ of milk plus enzyme gives a pH of about 1; 1 cm³ of 1M citric acid, pH 5; 1M sodium hydrogen carbonate, pH 8; 1M sodium hydroxide, pH 14 and distilled water, pH 7)

