Cheese please!

A limiting factor in many biology experiments is finding a reliable end point in a practical investigation or experiment. One such practical activity involves the milk clotting time associated with cheese making.

There are several steps in the manufacture of cheese. First, the milk is pasteurised to kill most of the bacteria, then other bacteria are added to convert the milk sugar (lactose) into lactic acid. Enzymes are added to clot the milk proteins.

Rennet is a mixture of enzymes produced in the stomachs of some animals. Chymosin, the key component of rennet, is a protease enzyme that curdles the casein in milk helping young mammals to digest their mother's milk. Chymosin can also be used to separate milk into solid curds used for cheese making, and liquid whey (Figure 1). In addition to chymosin, rennet contains other important enzymes such as pepsin. Originally the rennet used for cheese making came from the stomachs of young mammals such as calves.

Some people find it unacceptable to eat products from animals and alternative sources of milk clotting enzymes have been developed. Some fungi produce enzymes which clot milk proteins. These fungi are grown in large quantities in fermenters and the enzymes are then extracted.

With the development of genetic engineering, it is now possible to isolate the rennet coding genes from animal stomachs and insert them into certain bacteria, fungi, or yeasts making them produce chymosin during fermentation. The genetically modified microorganism is killed after fermentation and chymosin is isolated from the fermentation broth. This means that the fermentation-produced chymosin used by cheese producers does not contain any GM component or ingredient.

Determining the clotting time of the milk after the rennet is added has long been a part of many suggested biology investigations but it has often been difficult (and messy!) to accurately determine the exact clotting point.

The procedure described in this article is very simple to carry out and gives an accurate clotting time. The method involves placing a microscope slide into the clotting mixture until such time that the clots can be seen clearly on the slide.

Method

The original investigation can be found on the University of Guelph website [1] and further information such as a teacher/technical guide, help cards and an investigation sheet can be found on the SSERC website [2].

The basic method is summarised as:

1) Place 20 cm^3 of milk into a small beaker and measure the pH.
2) Add 2 cm^3 of 0.02% calcium chloride solution.
3) Stir the beaker and warm to 30°C.
4) Add 0.2 cm^3 of rennet enzyme, stir and measure the pH.
5) Every 30 s dip a clean microscope slide into the milk and record when flecks of curd appear on the slide (the clotting time).
6) Record the pH .

This method could form the basis of many different investigations where learners could vary:

- the type of rennet used;
- the starting pH of the production mixture;
- the temperature of the milk;
- the type of milk used;
- the concentration of calcium chloride used in the production mixture;

***Figure 1** - The whey begins to seperate from the curds.*

- the quantity of salt which is added to the production mixture (common salt is added as a regular component of cheese and whilst the standard protocol above does not include such salt it could be added as an additional variable).

Other resources

There are a number of on-line resources which one might use to support learning and teaching in respect of this topic [3].

Figure 2 - *After a certain time the clots can be seen clearly on the microscope slide.*

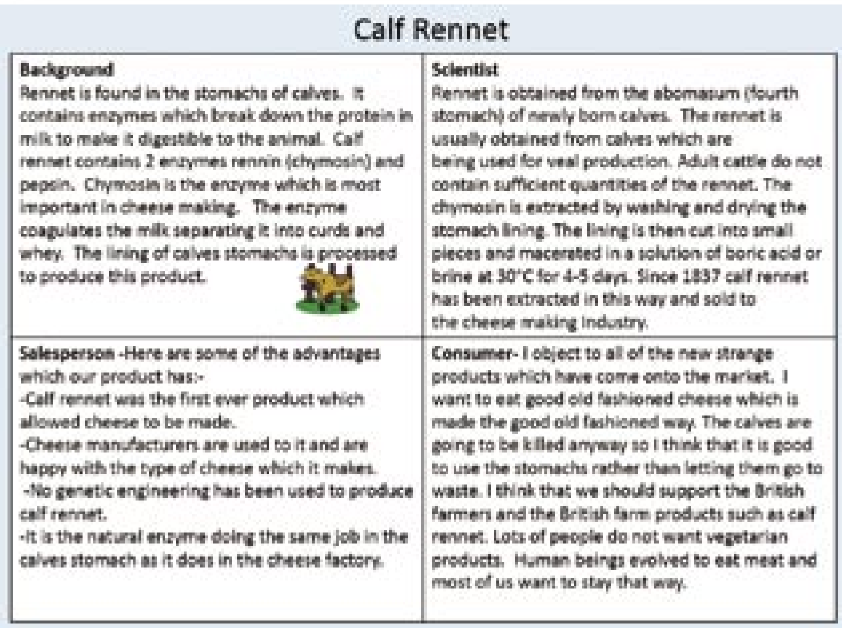
Curriculum links

This milk clotting practical can easily be explored at different levels within the curriculum. For example:

- National 4: Cell Biology: 4. Properties of enzymes and use in industries; Carry out experiments with rennet. Make cheese/visit cheese factory. Investigate the history and ethics of rennet.
- *CfE* Higher Biology also has an outcome which fits well with this practical activity: CELL BIOLOGY: 3. Metabolism in microorganisms:
  - Recombinant DNA technology.
  - Use of recombinant yeast cells.
  - Ethical considerations in the use of microorganisms, hazards and control of risks.

We have gathered a group of relevant resources together on the SSERC website [4].

In addition to the practical activity and investigation, a discussion activity [4] has been developed to complement the practical aspect and to explore some of the moral and ethical issues associated with the use of GM products. Discussion cards are available for each type of rennet and learners take on the role of the scientist, the salesperson or the consumer and have to consider the pros and cons of each type of rennet as shown in the example on the left (Figure 3).



Calf Rennet

Background Rennet is found in the stomachs of calves. It contains enzymes which break down the protein in milk to make it digestible to the animal. Calf rennet contains 2 enzymes rennin (chymosin) and pepsin. Chymosin is the enzyme which is most important in cheese making. The enzyme coagulates the milk separating it into curds and whey. The lining of calves stomachs is processed to produce this product.	**Scientist** Rennet is obtained from the abomasum (fourth stomach) of newly born calves. The rennet is usually obtained from calves which are being used for veal production. Adult cattle do not contain sufficient quantities of the rennet. The chymosin is extracted by washing and drying the stomach lining. The lining is then cut into small pieces and macerated in a solution of boric acid or brine at 30°C for 4-5 days. Since 1837 calf rennet has been extracted in this way and sold to the cheese making industry.
Salesperson -Here are some of the advantages which our product has:- -Calf rennet was the first ever product which allowed cheese to be made. -Cheese manufacturers are used to it and are happy with the type of cheese which it makes. -No genetic engineering has been used to produce calf rennet. -It is the natural enzyme doing the same job in the calves stomach as it does in the cheese factory.	**Consumer**- I object to all of the new strange products which have come onto the market. I want to eat good old fashioned cheese which is made the good old fashioned way. The calves are going to be killed anyway so I think that it is good to use the stomachs rather than letting them go to waste. I think that we should support the British farmers and the British farm products such as calf rennet. Lots of people do not want vegetarian products. Human beings evolved to eat meat and most of us want to stay that way.

Figure 3

Health & safety

There are no particular safety concerns associated with this activity although it should be emphasised that the cheese produced under the conditions outlined here is not suitable for human consumption.

References

[1] University of Guelph, https://www.uoguelph.ca/foodscience/book-page/rennet-activity.

[2] http://www.sserc.org.uk/index.php/biology-2/biology-resources/biology-national-4149/n4-cell-biology/3372-properties-of-enzymes-and-use-in-industries2.

[3] An overview of the cheese making process can be found at www.rsc.org/chemistryworld/2013/11/cheese-chemistry and a suitable film showing the cheese making process can be found at www.youtube.com/watch?v=RlfRnjf1CCM.

[4] http://www.sserc.org.uk/index.php/biology-2/biology-resources/biology-national-4/n5-cell-biology/3402-proteins-and-enzymes.