Curdling milk to cheese and breaking down lactose with enzymes

Pauliina Lankinen, Antti Savin and Sari Timonen
Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences

Objective

The objective of this exercise is to become familiar with microbial enzymes produced that are used in the dairy industry to make different milk products.

Background

Almost all enzymes used in many different industries are produced by microbes. The industrial enzymes are produced by cultivating microbes in a liquid growth medium. From there the enzymes can be collected, purified and concentrated.

The food industry has one of the biggest industrial uses of microbial enzymes. In the dairy industry enzymes are used for making cheese, yoghurt and low-lactose or lactose free products. During the production of cheese milk must be precipitated first so that the milk solids, or **curd**, can be separated from the remaining liquid, **whey**.

The precipitation of milk is usually done with rennet. **Rennet contains enzymes that catalyze the degradation of casein proteins in milk.** About 80 % of protein in milk is casein, the rest is whey proteins.

In cheese manufacturing, the amount of whey formed is almost 90 % of the amount of milk used. That is, out of 10 liters of milk, you get 1 kilogram of cheese and 9 kg of whey. Whey is a side product of dairy industry that without further processing is a waste product. Whey is used as the starting material for industrial production of lactose, both for fodder as well as a common raw material for the food industry. Whey proteins isolated from whey are used in nutrient supplements that are favoured in particular by athletes.

Traditionally rennet has been isolated from stomachs of butchered calves. The growing demand of cheese and decreasing supply of calves has driven the development of alternative rennets ever since the 1960's. The more economical and ethical ways of production have also played a role in developing new kinds of rennet.

In addition to the animal-based rennet there now is microbiological rennet and rennet produced by microbes. Fungi that naturally produce proteolytic, or protein-degrading, enzymes

are mainly used for producing microbiological rennet. In particular, enzymes produced by fungus *Rhizomucor miehei* are used as microbiological rennet.

The development of genetic engineering and its methods has opened up new possibilities for producing enzymes on an industrial scale. With recombinant DNA the chymosin gene of cattle has been transferred to micro-organisms, where producing chymosin is both cheaper and more efficient. Chymosin is now mostly produced with fungal or bacterial cultures. With the help of microbes it is possible to produce rennet that has mainly chymosin, whereas animal-based rennet has other enzymes as well. The fungi *Aspergillus niger* and *Kluyveromyces lactis* and bacterium *Escherichia coli* are the most common microbes used to produce chymosin.

Also the sugar in milk, lactose, can be broken down with enzymes to get low-lactose or lactose-free products. Lactose is a disaccharide that consists of a single glucose and a single galactose monomer. Lactose is broken down to smaller units by the lactase enzyme produced by the small intestine.

It's common in mammals that lactase secretion deteriorates or ceases altogether after infantry. During human evolution a mutation of the lactase gene has occurred in part of the population. The mutation enables the lactase secretion throughout adulthood. The mutation is common particularly in the European population.

The state, in which lactase is not secreted or the secretion has weakened, is called lactase deficiency. In the food industry, low-lactose and lactose-free products are produced with enzymes. A food product that contains less than 1 g of lactose / 100 g of product can be called low-lactose. Lactase products meant to be taken before digesting a lactose-containing meal, are also sold in pharmacies.

Principles

The meaning of this exercise is to apply the methods used in making of cheese and lactose-free products. The precipitation of milk into curd and whey is done with microbiological rennet and the lactose in the separated whey is then broken down with a lactase product. The degradation of lactose into glucose is shown with a glucose determination strip. In a positive reaction the strip changes colour depending on the glucose concentration, but no change occurs in a negative reaction.

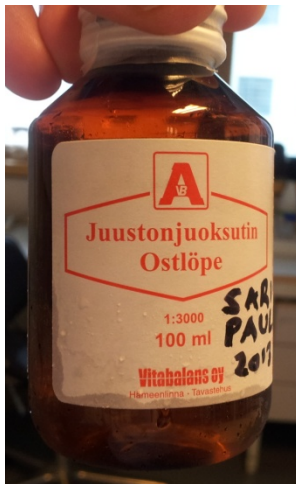

Figure 1 Microbiological rennet

Lab safety

Good laboratory working methods should be applied. It is recommended that gloves and a lab coat are worn during the (exercise).

Time needed

Ca. 45 – 60 minutes.

Equipment

- Milk (not lactose free)
- Rennet (from a pharmacy)
- 100 ml measuring cylinder or something similar
- 100 ml laboratory flask or other heat resistant glass container
- Water bath
- Thermometer
- Gauze
- Funnel
- 2 ml syringe
- Spoon
- Pasteur pipet
- Lab coat and gloves

Figure 2 Precipitation of milk

Instructions

1. Measure 50 ml of milk into the 100 ml (flask) and heat the milk to 30–60 °C in the water bath. Control the temperature rise with a thermometer to avoid overheating.
2. Add 1 ml of rennet into the milk and stir for a few minutes (Figure 2).

TEMPERATURE = _____

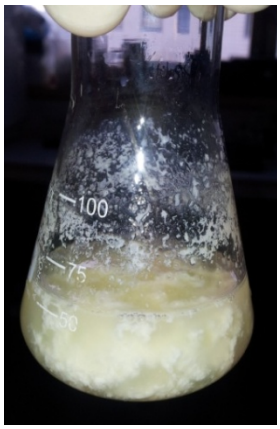

Figure 3 Formation of the curd

3. Let the milk sit for 10–20 minutes and watch/observe the precipitation of the milk's proteins. At this stage the milk starts to separate to curd and whey (figure 3). After the precipitate has formed, stir with a spoon to a grainy consistency so that the whey is separated from the curd.
4. Cut a (right size) piece of the gauze and filter the mixture to a clean beaker so that the curd is separated from the whey (figure 4).
5. Describe your cheese:

DESCRIPTION = _____

_____

Figure 4 Separating the curd and the whey.

6. Divide the whey into two containers. Add the contents of a lactase capsule to the container and stir. The lactase starts to break down the lactose in the whey.

7. Using a pipette, add a few drops of the enzyme-treated whey on the glucose determination strip and observe the colour change. As a comparison, do the same with the untreated whey. Note! Read the results as instructed for your determination strips.

COLOUR OF THE STRIP IN THE ENZYME-TREATED WHEY=
_____

COLOUR OF THE STRIP IN THE UNTREATED WHEY =
_____

Observations and additional methods

- Why should the milk not be heated to more than 40 °C? Try higher temperatures and compare the results.
- Does the temperature matter if the milk is precipitated with different methods (such as acid)?
- Try to precipitate the milk with cold milk. What did you observe?