

Chymosin Investigation

A. Objectives

The purpose of this lab is for you to

1. Apply your knowledge of enzyme kinetics to a real-world problem;
2. Practice designing and carrying out experiments using appropriate replicates and controls;
3. Evaluate your experimental design in light of your results and the work of your peers;
4. Practice team-work

B. Before coming to lab

Read part D. Background and answer the first review questions.

C. During lab

1. Work in a group of four. Imagine you are a research team working for SmellyCheese, Inc., located in California, the land of the happy cows. Your job is to optimize milk clotting, which is an early step in cheese making. You have heard that your competitor in Wisconsin, BlandCheese, Inc., uses a new product, chymosin, which really speeds up the clotting process. You don't know, however, under which conditions chymosin works best. Optimal performance would be defined as fast coagulation with good separation of coagulated milk and whey.
2. Agree on investigating **one factor** that might influence the performance of chymosin in milk coagulation. Your goal is to find optimal condition(s) for chymosin performance. Don't forget that your end-product is supposed to be edible, so don't put mercury in your test tubes, even though that might optimize chymosin's performance (well, actually, I doubt it; but can we be sure without testing that?). Consider also that we have limited time available to do this investigation. This means that you might have to do a pre-test to find out... *what?*
3. Write a protocol for your investigation.
4. Include a list of materials that you will need.
5. Check with your instructor to see whether those materials are available, otherwise, adjust your protocol.
6. Gather the materials you'll need and carry out your experiment. Perform replicates and controls as needed.
7. Document your results in form of a table or other appropriate organizing system.
8. Interpret your results.
9. Present your findings to the class.

D. Background

Milk is an emulsion - a mixture of fat and water held together by a natural emulsifying agent called casein. If casein is destroyed, coagulation of milk, also called milk clotting, occurs. In this case, the emulsion separates into its components and insoluble casein precipitates as curd. Curd is used directly (farmers cheese) or as a starting point for many cheeses.

Coagulation can occur as the result of microbial or enzymatic actions, or of the addition of acids such as vinegar. When milk coagulates, it separates into semi-liquid curd and liquid whey. You can observe this phenomenon if you leave milk at room temperature for an extended period of

time, or if you add vinegar to it. This process also takes place in the stomachs of many milk-producing species such as cows, goats, sheep, horses, zebras, and camels as one step of milk digestion. Calves produce the enzyme prochymosin, that is activated by the stomach acid and produces chymosin.

Chymosin is a protease (meaning that it breaks long amino acid chains into smaller ones) that reacts with the casein protein in milk. Chymosin reacts almost exclusively with one key member of the casein family, called kappa casein, which is therefore referred to as the natural substrate for chymosin. Chymosin cleaves the 169 amino acids long kappa casein polymer in only one place - between amino acids 105 and 106 in the chain, forming two large protein pieces (Figure 1).

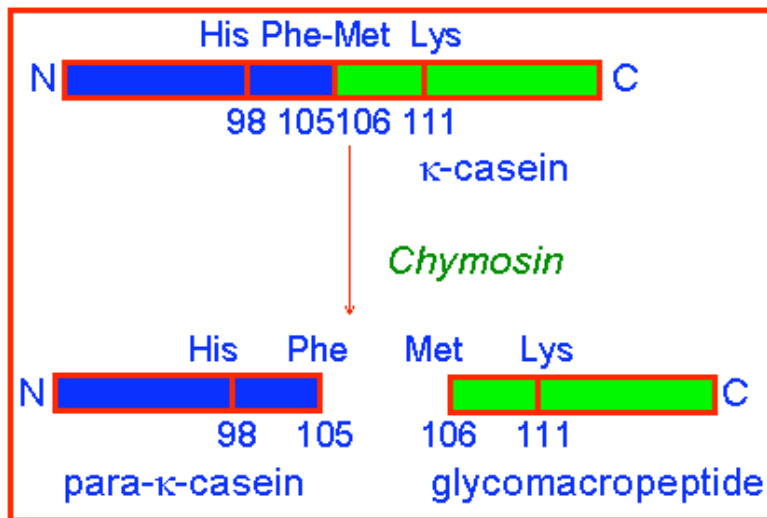


Figure 1: Chymosin reaction (Source: <http://dwb.unl.edu/Teacher/NSF/C08/C08Links/www.fst.rdg.ac.uk/courses/fs560/topic1/t1g/t1g.htm>)

"Microorganisms have been modified genetically to yield chymosin that is identical to the enzyme obtained from animals. This can be used to produce better-quality cheese than the fungal or other animal (non-calf) rennets. The first scientists to make chymosin in this way in 1981 used bacteria. Numerous groups have since followed their lead, using other microbes, so that chymosin has now been obtained from food yeasts. 1

In 1988, chymosin was the first enzyme from a genetically-modified source to gain approval for use in food. Figure 2 illustrates the process of modifying yeast genomes to produce chymosin. Three such enzymes are now approved in most European countries and the USA. These proteins behave in exactly the same way as calf chymosin, but their activity is more predictable and they have fewer impurities. Such enzymes have gained the support of vegetarian organizations and of some religious authorities. Chymosin obtained from recombinant organisms has been subjected to rigorous tests to ensure its purity.

Today about 90% of the hard cheese in the UK is made using chymosin from genetically modified microbes. Notice that the cheese is NOT made using a GMO, but rather the product of a GMO (the enzyme). Consequently, ALL cheeses on sale are in fact 'GMO free'. Another important point is that the enzyme does not remain in the finished cheese. Like all enzymes it is required only in very small quantities and because it is a relatively unstable protein

it breaks down as the cheese matures. Indeed, if the enzyme remained active for too long it would adversely affect the development of the cheese, because it would degrade the milk proteins too much." (Source: National Center for Biotechnology Education 2006)

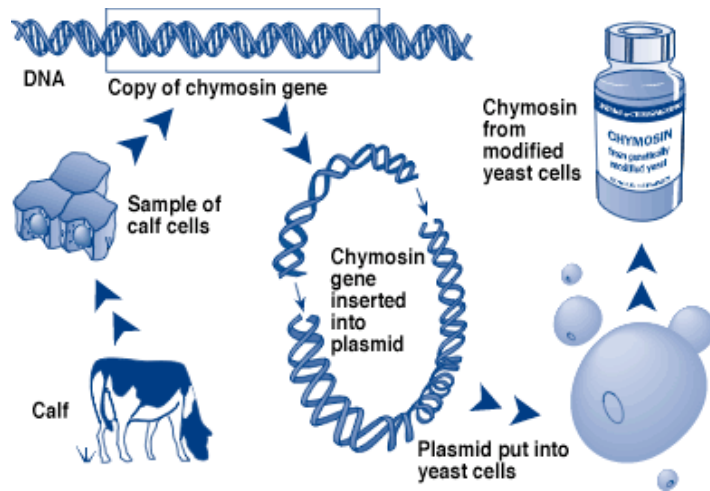


Figure 2: Recombinant Chymosin production ([dwb.unl.edu/.../ NCBE/GMFOOD/chymosin.html](http://dwb.unl.edu/.../NCBE/GMFOOD/chymosin.html))

E. After lab

If you are not electing to write a report on this lab:

In your laboratory notebook summarize the findings of the class. Which influences on chymosin performance were investigated? What were the results? Can you explain these results (do they make sense in light of your knowledge of enzymes)?

Write another paragraph critiquing your experimental design. Do you trust your results? Why or why not? Did you have adequate replications and controls? What factors besides the one you investigated could have influenced your results?

F. Review questions

To answer in your lab notebook before lab

1. Which variables influence enzyme performance in general?
2. With these variables in mind, hypothesize: What are the optimal conditions for the performance of chymosin? (Hint: you might investigate the **normal** conditions under which substances like chymosin are active).
3. Briefly describe an investigation aimed at optimizing one variable in chymosin performance.