Edible Enzyme Essentials: Learning the properties of enzyme function through cheesemaking (and eating)

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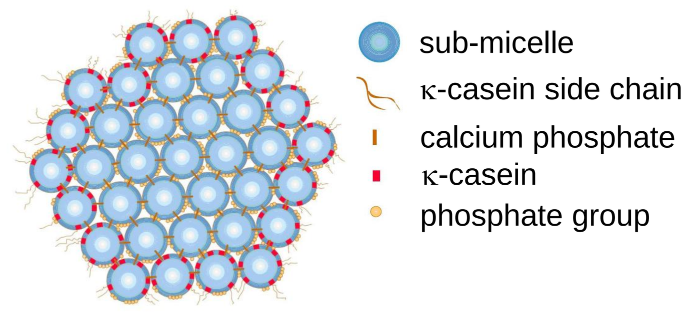
Why Cheesemaking?

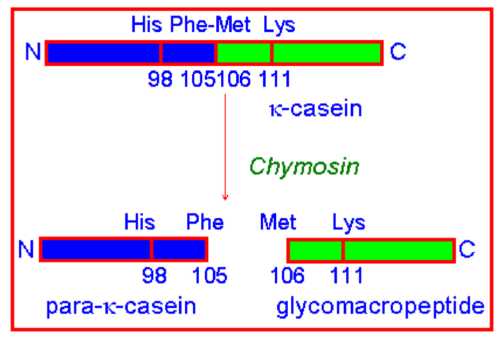
Often in general biology labs enzyme properties are explored through the use of spectrophotometer activities. While these offer the benefit of quantitative data that can be used for graphic functions, if the primary goal is to have students to really understand the roles of temperature, pH, concentration, and substrate type in enzyme function, the technical aspects of the spectrophotometry labs can sometimes cause students to miss the important points and often require multiple lab sessions to master. However, the practical application of cheese production offers a qualitative approach to exploring enzyme properties that may aid student conceptual understanding, and can be completed in two hours. In this lab activity, the enzyme chymosin is used, in the form of rennet, to make cheese from store-bought milk. By allowing students to experiment with temperature, pH, enzyme concentration, and substrate type, they are able to see (and taste) the practical results of enzyme properties.

Enzyme Reaction

The protease, chymosin, is produced in the stomach of young mammals to coagulate milk proteins so they do not pass too quickly through the digestive system, avoiding absorption. As chymosin is active in the stomach, it operates best under acidic conditions (pH 3.5-5.5 depending on source of enzyme).



Casein micelle (Food-info.net)



Chymosin reaction (D.W. Brooks)

Caseins are a major class of milk proteins (76%) that will precipitate in the presence of calcium. However, α & β caseins are hydrophobic and are stabilized by κ-caseins that form micelles, keeping the proteins soluble. Chymosin (in rennet), when activated by the appropriate pH environment, cleaves κ-casein making it unable to bind the α & β caseins, causing them to precipitate, forming the curd.

General Supplies Needed

Because this lab is intended to be edible, you will need non-general lab supply equipment. I wash down all lab tables and then cover them with tablecloths.

Supplies for each group:
- saucepan
- wooden spoon
- set of measuring spoons
- measuring cup
- thermometer
- hot plate
- glass bowl
- strainer
- cheesecloth or flour sack
- large bowl or bucket
- ½ gallon whole milk

Supplies for whole lab:
- enzyme (rennet)
- citric acid
- salt
- microwave(s)
- food grade gloves

Procedure: Demonstration & Experimentation

This lab can be accomplished in one 2-hour block or two 1-hour blocks, however, 2.5 hours would be more ideal.

First half of lab
- Review of enzyme function and the roles of pH, temperature, and enzyme concentration
- Demonstration of cheese making process following protocol exactly (I often have two students go through the process in front of the lab while I talk them through it and explain steps.)
- Discussion of temperatures and pH used and their biological significance

Second half of lab
- Groups answer questions about enzyme function in relation to lab
- Groups identify one variable to change in the cheese-making process
  - ➢pH (more or less citric acid)
  - ➢temperature (lower or higher)
  - ➢enzyme concentration
  - ➢enzyme source
  - ➢substrate type (soy vs. cow milk)
- Groups complete cheese-making process under altered condition
- Whole lab compares results of changing variables and discusses

Stages of the cheesemaking process

Step 1. Heat milk to 55ºF while stirring

Step 2. Add citric acid (1 tsp. dissolved in water for ½ gallon milk) and check pH

Step 3. Heat to 88ºF while stirring

Step 4. Add rennet (1/8 tsp. in water)

Step 5. Continue stirring while heating to 105ºF

Step 6. Separate the curds from the whey by straining and squeezing (you can save the whey for other use)

Step 7. Collect the curds into a glass bowl and microwave for about 20 sec.; use spoon or gloved hands to press cheese and remove excess whey; add salt (about 1 tsp.) then repeat microwaving and pressing

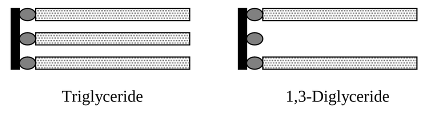
Step 8. Taste test

Reflection & Assessment

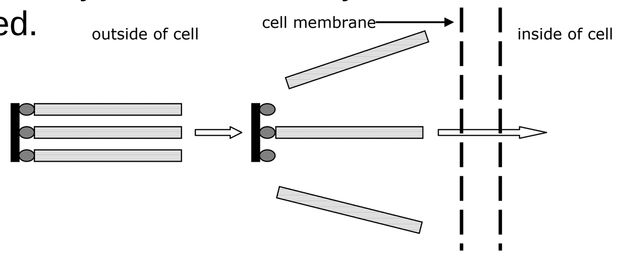
Once all groups have finished making their cheese, you can set up a taste test where students can compare taste, texture, look of the cheeses. Each group should also describe anything different about their reaction (*e.g.*, how long the reaction took, what the curds looked like).

Some questions for the groups to then discuss include:

- With your knowledge of chymosin, draw the optimum pH and temperature ranges of this enzyme. Imagine instead we were working with an enzyme found in the blood of a deep sea fish. Draw the optimum pH and temperature ranges you would expect for this enzyme.
- What happened, or what would you expect to happen, when chymosin is used with soy milk instead of cow milk? How do you think soy cheese is made?
- What makes proteins different? How can different enzymes have different optimal temperatures and work with different substrates?
- The company that makes the cooking oil Enova™ claims that less of their oil is stored as fat when you eat it as compared to other oils. Most cooking oils are made primarily of triglycerides while Enova™ is made primarily of 1,3-diglycerides.



When you eat fats, an enzyme breaks off the chains from the first and third carbons and all the components are transported across the cell membranes from inside your intestine into your body cells where enzymes reassemble the fat and then package it to be stored.



The company that makes Enova™ claims that the diglycerides in their oil are disassembled and transported in the same way as triglycerides, but cannot be reassembled. Therefore, instead of being packaged for fat storage, the unassembled pieces are sent to the liver to be used immediately for energy by breaking them down through cellular respiration. Why do you think the disassembled diglycerides cannot be reassembled once they cross the membrane into the cell?

Other Related Topics

As with any topic, this lab can be related to other biology content besides just enzyme function, making it possible to refer back to the lab throughout the semester, reinforcing learning.

- **Cell Biology** – Exploring the cells that make chymosin (chief cells) and the regulation of chymosin production
- **Anatomy & Physiology** – Examining the location and timing of chymosin production in mammals
- **Evolution** – Comparison of chymosin sequences among mammals
- **Biotechnology** – Recombinant enzyme production (*e.g.,* with *E. coli* and *Kluveromyces lactis)*
- **Biochemistry** – Exploring protein structure in greater detail, including the chemical properties of amino acids

Resources

Bowen, R. (1996) Chymosin (rennin) and the coagulation of milk. Online: http://www.vivo.colostate.edu/hbooks/pathphys/digestion/stomach/rennin.html

Brooks, D.W. (nd) Recombinant Chymosin. Online: http://dwb4.unl.edu/Chem/CHEM869N/CHEM869NLinks/www.fst.rdg.ac.uk/courses/fs560/topic1/t1g/t1g.htm

Goff, D. (nd) Dairy Chemistry and Physics. Online: http://www.foodsci.uoguelph.ca/dairyedu/chem.html

Wageningen University (2010) Milk Proteins. Online: http://www.food-info.net/uk/protein/milk.htm

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