

The process of making cheese is an ancient craft that dates back thousands of years. People first started making cheese because it keeps better than milk and has the same nutritional value. Nutritionists consider cheese one of the best protein foods. In addition to protein, cheese contains essential vitamins A, D, E, and K, calcium, phosphorus, carotene and many others.

Cheese making is a complex biochemical process that involves both skill and patience. First, milk is pasteurized by heating it to 72°C for 15 seconds to destroy any harmful bacteria. Then, the milk is cooled and either a special blend of bacteria or an acidic solution- such as vinegar or lemon juice- is added. The milk is left for one and a half hours while the bacteria converts lactose (a sugar found in milk) into lactic acid. This causes the milk to sour and the pH to drop to approximately 4.5. A substance called "rennet" is then added to the milk and, within a short time, "curds" and "whey" are produced. Rennet is made up of a group of enzymes, such as pepsin and chymosin, that break up proteins. Rennet breaks up the milk protein called casein into a smaller protein called paracasein. The paracasein then combines with the calcium found in milk to form paracaseinate which separates out. Milk fat and some water combines with paracaseinate to form curds. The remaining liquid from the milk is called whey. After curdling, the whey is removed and the curds are processed into cheese.

Until recently, rennet was extracted from the lining of the fourth stomach of milk-fed calves where the rennet serves to digest their mother's milk. Scientists have identified that chymosin is the most important enzyme in rennet and have developed a method to produce chymosin that doesn't not come directly from calves. In March of 1990, the Food and Drug Administration approved the genetically engineered form of chymosin. This genetically engineered chymosin is exactly the same as the chymosin which comes from calves. To make it, scientists extract DNA-which contains the instructions for producing chymosin molecules- from calf stomach cells. The DNA is then inserted into Bacteria or fungi. As the microorganisms grow, they produce large amounts of chymosin, which is purified before use. Presently, over 70% of cheese made in the US is coagulated with genetically engineered chymosin.

Today you will be making your own cheese in order to learn about enzymatic function. You have already read above that the production of cheese is reliant on the efficient function of the enzyme called chymosin. This laboratory exercise will allow you to investigate the effect of environmental factors such as concentration, temperature, and pH on enzyme function. Good Luck!!

#### NECESSARY PRE-KNOWLEDGE

• You must be able to discuss how factors like concentration, temperature, and pH affect enzyme function.

### HOW TO MAKE THE CHEESE

- Mix approximately 50 mL of room temperature whole milk with 1 mL of vinegar in the glass 250 mL beaker provided. Record the actual (precise) volume of whole milk you actually use. Use a pH paper strip to determine the pH of the milk solution and vinegar and then record your results in the data table provided. To test the pH of vinegar – pour a very small volume into the small beaker at your station, then dip the paper partially into the vinegar in the beaker.
  - The chemical name for vinegar is acetic acid. Please explain why it may be necessary to add acid to the milk prior to starting. Yes, you do have to answer these questions in the procedure!
- Place the beaker with milk in the warm water bath set up by your teacher. The temperature should be maintained between 37°C and 42°C for 1 minute.
  - Please explain why a water bath at the temperature listed above may be necessary in this experiment. Hint....look at the next couple steps.
- Using a pipet, add 0.5 mL of chymosin to the milk mixture in the beaker. Swirl the beaker gently for approximately 10 seconds to mix.
- Place the beaker back into the warm water bath and leave it undisturbed for 10 minutes.
  - Please explain why the mixture needs to be left alone in the water bath for 10 minutes......what's the purpose of this step?

- Using a flat wooden stick, cut the curd formed in the cup into a "tic-tac-toe" pattern and return it to the warm water bath for approximately another minute. During this time, the curd will start to contract and release the whey.
- Be sure to weigh your cheesecloth <u>before</u> continuing with this step and record in table. Line a funnel with two layers of cheese cloth. Be sure that the cheese cloth layers hang over the cup. Pour the contents of the cup through the cheese cloths to separate the curds from the whey. Allow the whey to drain into the funnel and down into a different beaker (400mL) for 2-3 minutes. Then, twist the top of the cheese cloth closed and gently squeeze the whey from the cheese curds.
- Measure the volume of whey that was drained into the funnel and large beaker and determine its pH using a pH paper strip. Record your results in the data table provided.
- Open the cheese cloth to observe the cheese curd formed. Weigh the curds and cheesecloth together. Record. To
  get weight of the curds only subtract the mass of cheesecloth from curds and cheesecloth mass together. Observe
  their firmness and texture. Form into ball and measure its diameter. Record you observations in the data table
  provided.
- Dispose of your materials, clean up your work area, and wash your hands before leaving the lab.

DEPENDENT VARIABLES	MEASUREMENTS/OBSERVATIONS
pH of Acetic Acid	
pH of Milk	
pH of Milk + Acetic Acid	
pH of Whey (Waste)	
Total Volume of Milk + Acetic Acid + Enzyme solution (mL)	
Total volume of Whey (mL) (Waste)	
Total Mass of Curds and Cheesecloth (g)	
Mass of cheesecloth (g) (taken <u>before</u> experiment!)	
= Ending Curd Weight (grams)	
Curd Diameter Size (cm)	
Texture Description of Curd	
Other Observations	

## DATA TABLE

#### MATH ANALYSIS

• Please calculate the cheese production efficiency for this lab. In other words, use a number to describe how efficiently you turned the milk into cheese. Hint......you'll need to use your total volume calculations to determine this.

SHOW YOUR WORK IN THIS BOX:

PLACE YOUR ANSWER HERE:

# INQUIRY EXTENSION

Now that you know how to produce cheese, design a controlled experiment which tests the effect of 1 environmental factor on enzymatic function. To do this you must think about the following:

- What environmental factor do you want to investigate?
- What data will you take in order to draw conclusions after completion of the experiment?
- What changes will you have to make to the original procedure in order to successfully study the effect of your chosen environmental factor on enzyme function?

You will include the following items in a lab report. (see the sample lab report format posted on Bb)

- 1) A description which discusses the environmental factor you investigated in your experimental study and the hypothesis you generated prior to executing the experiment. Be sure to write your hypothesis in proper if/then format.
- 2) You must identify the portion(s) of the original cheese making procedure that you changed in order to carry out your investigation.
- 3) You must identify the independent and dependent variables in your experiment. Additionally, you must identify the control group, experimental groups, and things you kept constant between those groups. Lastly, you should identify any limitations which challenge the validity and application of your results to real life.
- 4) A carefully constructed data table which includes the measurements or calculations that clearly demonstrate the effect of a change in environmental factor on enzyme function.
- 5) A carefully constructed graph which provides a visual that demonstrates the effect of an environmental change on enzyme function.
- 6) A conclusion paragraph which uses specific references to experimental data in order to support or refute your original hypothesis. This paragraph should explain the effect of a change in environmental factor on the cheese making process. Lastly, you should explain what a real cheese maker can learn by reading your study.