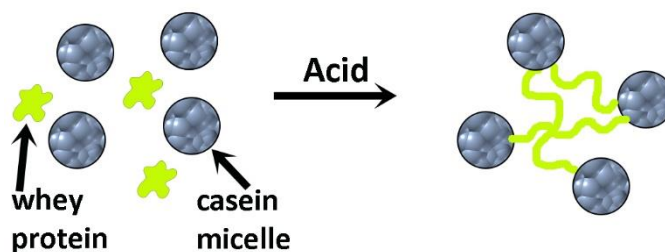


## pH and Denaturation

pH is a measure of the acidity of a solution. It reflects the relative concentration of hydrogen ( $H^+$ ) and hydroxide ions ( $OH^-$ ) in the solution. These ions potentially interfere with bonding between amino acids in a protein and can cause changes in the structure of the protein. Since protein function is determined by its structure, any change to the structure can directly impact function. Proteins are often described as having 4 levels of structure. The primary level of structure is the linear arrangement of amino acids in a peptide. The secondary level of protein structure (alpha helix, beta sheet) is created by the formation of hydrogen bonds between polar groups within the peptide chain. Hydrogen and hydroxide ions compete with these hydrogen bonds and lead to changes in the secondary structure. If there are enough ions or if the active site is compromised the enzyme is denatured. Hydrogen and hydroxide ions also impact the ionization state of some amino acid R-groups which can result in changes to tertiary structure.

This activity is a demonstration of denaturation of proteins, but not the denaturation of an enzyme. Milk is composed two major types of protein, casein (82% of protein) and whey proteins (18%). Casein forms micelles or little bubbles of protein. Whey is a globular protein; it is a peptide that folds into a glob or spherical shape. When acid is added to milk, the acid hydrogen ions compete with hydrogen bonds within the whey molecules. The whey proteins denature and unfold exposing amino acid R-groups that interact with the casein micelles forming an interconnected network.



### Materials

Sweetened condensed milk  
Lemon or lime juice  
Glass rod  
Graduated cylinder (100 mL)

Beaker (250 mL)  
Beaker (25 mL)  
Pipet

1. Use the graduated cylinder to obtain 50 mL of sweetened condensed milk. Pour the milk into the 250 mL beaker.
2. Rinse the graduated cylinder well and then pour 10 mL of lime or lemon juice into the graduated cylinder. Pour the lime juice into the 25 mL beaker.
3. Use a pipet to add 3 mL (60 drops) of lemon/lime juice to the beaker containing the sweetened condensed milk. Gently stir the milk with the glass rod.  
What changes are occurring to the milk? \_\_\_\_\_
4. If you do not observe any changes, add another 3 mL of lemon/lime juice to the beaker. Gently stir the milk with the glass rod.

Describe the milk in your beaker. \_\_\_\_\_

Acids frequently are used in pickled foods. Pickled herring for example is a fish dish made using acid and sugar. Pickled foods also rarely spoil. How do you explain this phenomenon?