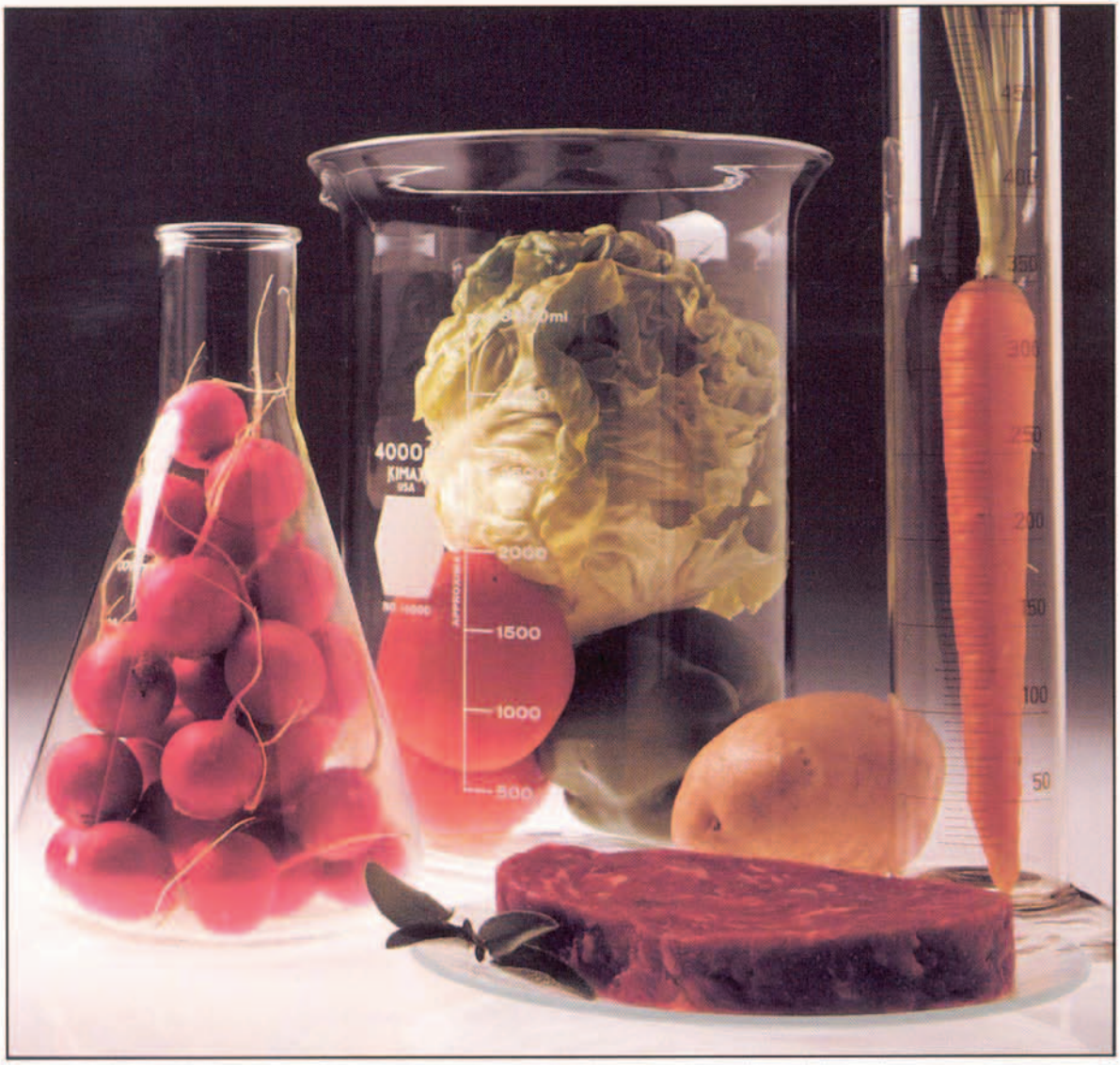
IFT Experiments in Food Science Series

Food Chemistry Experiments



Institute of Food Technologists
The Society for Food Science and Technology

Unit 3. PROTEINS

Teacher Activity Guide

Expected Outcomes

Students will learn about the sources of proteins and their uses in the food industry.

Activity Objective

In Part 1, the students will precipitate casein from milk using an acid. This method is used to make cottage cheese. In Part 2, the students will coagulate casein from milk using an enzyme. This method is used for making cheese. In Part 3, the student will coagulate soy protein from soymilk, using magnesium sulfate. This method is used to make tofu.

Activity Length

45 minutes

Scientific Principles

Milk protein consists of 80% casein and 20% whey protein. There are four major types of casein molecules: alpha-s1, alpha-s2, beta, and kappa. Milk, in its natural state, is negatively charged. The negative charge permits the dispersion of casein in the milk. When an acid is added to milk, the H^+ concentration neutralizes the negatively charged casein micelles. When milk is acidified to pH 4.7, the isoelectric point (the point at which all charges are neutral) of casein, an isoelectric precipitate known as acid casein is formed. Cottage cheese and cream cheese manufacture involves an acid precipitation of casein with lactic acid or lactic acid–producing microorganisms. Acid casein is used in the chemical industry and as a glazing additive in paper manufacturing.

Casein also can be coagulated with the enzyme rennin, which is found in rennet (an extract from the stomach of calves). Rennin works best at body temperature (37°C). If the milk is too cold, the reaction is very slow, and if the milk is too hot, the heat will denature the rennin, rendering it inactive. The mechanism for the coagulation of the casein by the rennin is different from the acid precipitation of casein. The coagulation of the casein by rennin is a two-stage process. In the first stage, rennin (a proteolytic enzyme) splits a specific bond in the amino acid chain of the kappa-casein macromolecule converting it into a para-kappa-casein and a glyco-macropeptide. This causes an imbalance in the intermolecular forces in the milk system, and the hydrophilic (water-loving) macropeptides are released into the whey. Unlike kappa-casein, the para-kappa-casein does not have the ability to stabilize the micellular structure to prevent the

calcium-insoluble caseins from coagulation. In the second stage, colloidal calcium phosphate bridges within the casein micellular structure are formed in the presence of the soluble calcium, resulting in the three-dimensional curd structure. The rennin coagulum consists of casein, whey protein, fat, lactose, and the minerals of the milk, and has a fluffier and spongier texture than the acid precipitate. Rennet is used in the manufacture of cheese and cheese products, and rennet casein is used in the plastics industry.

Casein is solubilized with sodium hydroxide and calcium hydroxide to produce sodium caseinate and calcium caseinate, respectively. Caseinates are added to food products to increase their protein content and are key ingredients in non-dairy coffee creamers and *Cool Whip®*.

Approximately 90% of soybean proteins are classified as globulins, based on their solubility in salts. More specifically, the proteins are conglycinin (a glycoprotein) and glycinin. Tofu is manufactured by coagulating proteins in soymilk with magnesium sulfate. As bonding occurs between the positively charged magnesium ions and negatively charged anionic groups of the protein molecules, the proteins coagulate.

Vocabulary

Amino acids contain carbon, hydrogen, nitrogen, and sometimes sulfur and serve as the monomers to make peptides and proteins. Amino acids have a basic structure that includes an amino group (NH_2) and a carboxyl group (COOH) attached to a carbon atom. This carbon atom also has a side chain (an "R" group). There are twenty amino acids, found in the body. Eight of them are essential for adults and children, and nine are essential for infants.

Casein is a milk protein. There are four major types of casein molecules: alpha-s1, alpha-s2, beta, and kappa.

Coagulation is the transformation of a liquid into a soft semisolid or solid mass. In the coagulation of milk, it refers to the aggregation or clumping together of proteins.

Colloid is a suspension of finely divided particles in a continuous medium in which the particles do not settle out of the substance rapidly, and are not readily filtered.

Denatured means changed from its natural state. In a denatured protein, its characteristics or properties have been altered in some way, by heat, chemicals, or enzymatic action, resulting in the loss of its biological activity.

Digestion is the chemical breakdown of large food compounds into smaller molecules that can be absorbed by the intestines in the human and animals. The smaller food molecules travel in the blood and are used by cells to make other components or produce energy needed by the body. Digestion begins in the mouth as salivary amylase begins to break down starch into simple sugars.

Enzymes are protein catalysts, which control specific chemical reactions in living systems (plants and animals). Enzymes are active at low concentrations and are substrate specific. The enzyme rennin catalyzes the coagulation of casein in milk, but is not effective in any other chemical reaction.

Isoelectric means having equal electric potential.

Kappa-casein is one of the four major types of casein molecules. Kappa-casein self-associates into aggregates called micelles. The alpha- and beta-caseins are kept from precipitating by their interactions with kappa-casein.

Micelle is a submicroscopic aggregation of molecules, as a droplet in a colloidal system.

Peptide bonds are covalent bonds between two amino acid molecules.

Precipitation is the removal of insoluble material from solution.

Proteins are complex polymers composed of amino acid monomers, and are considered to be the primary structure of all living organisms. Some examples of protein are muscle, hair, skin, hormones, and enzymes.

Proteolysis is the hydrolysis of proteins into peptides and amino acids by cleavage of their peptide bonds. This occurs during digestion and when rennin is used to coagulate milk.

Rennet is an extract from the inner lining (membrane) of the fourth stomach (abomasum) of the calf. The abomasum is the gastric stomach of ruminant animals such as the cow. The lining is used to make cheese because it contains the enzyme rennin.

Rennin is a proteolytic enzyme that is used to coagulate milk to make cheese. Rennin is typically used in the form of rennet, a commercial preparation taken from the abomasum (fourth stomach) of young calves, but because its demand is great and supply limited, the cheese industry has been increasingly turning to microbial rennin produced from genetically engineered microorganisms. Rennin is also known as chymosin.

Substrate is the name of a reactant molecule for enzymes. A substrate is the substance on which an enzyme acts. Using the analogy of a lock and key, the lock is the substrate, and the key is the enzyme.

Materials Required

Distilled white vinegar (acetic acid), 5% acidity
Pasteurized whole milk
Soymilk
Rennet tablets (*Junket*®)
Epsom salt (magnesium sulfate)
Cheesecloth
Rubber bands
Stirring rod/wood *Popsicle* sticks
Heatproof gloves
Weigh boats
Hot plate/Bunsen burner
Beakers
Graduated cylinder
Balance
Thermometer
Foil
Hammer
Eyedroppers
Heatproof pad

Instructional Strategies and Procedures

If you divide the class into three groups and have each group perform one part of the experiment, you will be able to complete the entire experiment in one class period.

For the biuret test: Wear gloves when you make the reagent for the biuret test. Make a 10% sodium hydroxide solution (10 grams of NaOH dissolved in 100 milliliters of water). As the pellets dissolve, the solution will get hot. (This reaction is an example of exothermic dissolution.) Do not heat this solution to dissolve the pellets. This solution will be clear. Prepare a 5% copper (II) sulfate solution (5 grams of anhydrous cupric sulfate, $CuSO_4$ (M.W. 159.6) dissolved in 100 milliliters of water). This solution will be a blue color.

How the biuret method works: Substances containing two or more peptide bonds (three or more amino acids) form a purple-violet complex with copper salts in alkali solution. The nature of the color is probably due to the formation of a tetra-coordinated cupric ion (Cu^{+2}) with amino groups. Use foods that will provide a negative response to the biuret test, such as potato chips, raw potato, or bread, for students to compare with the positive response of the precipitates. A light blue color indicates a negative response, a purple-violet color a positive response.

Teaching Tips

- **The foods produced in these experiments are not to be consumed.**
- Soymilk (located near the canned milk section in the grocery store) contains more protein than the soy drink beverage (located in the health food section).
- Do not use evaporated milk, since the gums and stabilizers in the milk will not allow the protein to precipitate.
- You may use dry powdered milk, but allow it more time to precipitate. It works especially well with the rennet.
- Do not use rice milk. No precipitation will occur.
- Rennet tablets can be found at your supermarket. Ask for *Junket Rennet Tablets*. It's used to make custard and ice cream.
- If the soymilk splatters while boiling, drop boiling chips or marbles into the beaker.
- If you are not able to purchase soymilk, you can make your own homemade soymilk before class with the following recipe.

Instructions to make homemade soymilk

Ingredients: 350 grams (2 cups) of soybeans and 2.8 liters (12 cups) of water

Method: Soak 350 grams (two cups) of dry soybeans overnight. Cover with plenty of water. The beans will swell quite a bit. On the next day, drain the water off the beans and rinse the beans with fresh water in a colander. Take ½ of the beans and put them in a blender. Add 0.70 liter (three cups) of cold water and blend on high until the beans are finely ground. Pour the bean mixture into a large pot—a 4.5-liter (1-gallon) size is good. Blend the remaining beans with 0.70 liter of water and add them to the pot. Add 1.4 liters (six cups) more of cold water to the pot. Put the pot on the stove and bring it to a boil, stirring frequently to keep the bottom from scorching. If the mixture threatens to boil over, reduce the heat. Let the beans simmer for seven minutes, then sprinkle a small amount of cold water over the mixture until boiling stops. Let the mixture come to a simmer again. Repeat the cold water/simmer treatment two more times. Take the mixture off the heat. Pour the slurry through a sieve (lined with two to four layers of fine cheesecloth) into another pot. The strained liquid is soymilk. This procedure makes about 2.8 liters (three quarts) of soymilk. Put the soymilk into containers and let cool before refrigerating. The soymilk keeps for about one week in the refrigerator and 3 to 6 months in the freezer.

SAMPLE DATA TABLE – MILK AND SOYMILK CURDS

	Weight of milk/soymilk	**Weight of curd**	**Describe the curd (color, texture)**
Milk + acid	114.4 g	19 g	White, fine granules
Milk + rennet	114.4 g	34.1 g	White, fluffy, spongy, thick
Soymilk + Epsom salt	123.6 g*	29.2 g*	Light brown, fine granules*

*Tofu results based on the use of *Original Edensoy*® organic soymilk.

The weight of beaker with milk – weight of beaker = weight of milk

SAMPLE DATA TABLE – BIURET TEST ON FOODS

	Biuret test – positive or negative
Milk + acid precipitate	Purple, positive
Milk + rennet coagulum	Purple, positive
Soymilk + Epsom salt coagulum	Purple, positive
Potato chip	Blue, negative
Raw potato	Blue, negative
Bread	Blue, negative

Results for variations of experiments

Part 2. Rennet at low and high temperatures

Rennet has the highest activity at body temperature (37°C). Coagulation will occur very slowly with cold milk. High temperatures will denature the rennet, so no coagulation will occur.

Part 3. Effect of rennet on soymilk and Epsom salt on milk

No coagulation should occur when rennet is added to soymilk, because the rennet is specific for casein. No coagulation should occur when Epsom salt is added to whole milk, since magnesium sulfate does not coagulate casein.

Key Questions and Answers

1. Compare the weights of the curds from the milk (acid and rennet) with that from the soymilk.

   The milk + rennet curd weighed the most; the soymilk + Epsom salt curd weighed less; and the milk + acid curd weighed the least.

2. Why did the casein that was coagulated with the rennet weigh more than the casein that was precipitated with the acid?

   The rennet coagulum contains milk protein and fat, while the acid precipitate contains only casein.

3. Compare the amount of acid casein precipitated from the whole milk with the amount of soy protein coagulated from the soymilk. How do your results compare with the Nutrition Facts label for each product?

   Less casein precipitated from the whole milk than soy protein precipitated from the soymilk. If you look at the Nutrition Facts label on the milk and soymilk, you will see that the milk contains 8 grams of protein per 240 milliliters, while soymilk contains 10 grams of protein per 240 milliliters. Therefore, the results for the precipitates are consistent with the labels.

Solution to Powerful Proteins

1. PEPTIDES
2. POLYMERS
3. AMINO ACIDS
4. HORMONES
5. RENNIN
6. CASEIN
7. DIGESTION
8. AMYLASE
9. COAGULATION
10. EGG

HIDDEN MESSAGE: ELMER'S GLUE

Solution to Puzzling Proteins

S	***T***	I	***A***	N	***Y***	***P***	***H***	***Y***	***S***	P	D	***I***	C	***C***
A	***L***	N	***L***	O	***Y***	***A***	***C***	***T***	D	R	N	***I***	H	***V***
E	***T***	S	I	I	***H***	***R***	***O***	E	***U***	E	O	R	E	***G***
H	***O***	U	***U***	T	***T***	***Y***	N	P	***O***	C	B	E	E	***U***
R	***L***	L	***I***	A	R	A	***F***	R	***E***	I	E	N	S	+
+	+	I	+	L	T	O	+	O	+	P	D	N	E	+
+	+	N	+	U	+	+	G	T	+	I	I	I	+	+
+	+	+	R	G	+	+	+	E	+	T	T	N	+	+
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H	O	R	M	O	N	E	S	N	N	T	E	+	+	E
+	M	U	S	C	L	E	+	+	+	I	P	+	N	+
A	M	I	N	O	A	C	I	D	T	O	E	Z	+	+
E	N	I	S	Y	L	+	+	O	+	N	Y	S	+	+
+	+	+	+	+	+	+	F	+	+	M	+	+	A	+
+	+	+	+	+	+	U	+	+	E	+	+	+	+	C

(Over, Down, Direction)
AMINO ACID (1, 12, E)
CASEIN (15, 15, NW)
CHEESE (14, 1, S)
COAGULATION (5, 11, N)
DENATURE (10, 2, SW)
ENZYME (15, 10, SW)
HORMONES (1, 10, E)
INSULIN (3, 1, S)
LYSINE (6, 13, W)
MUSCLE (2, 11, E)
NITROGEN (3, 2, SE)
PEPTIDE BOND (12, 11, N)
PRECIPITATION (11, 1, S)
PROTEIN (9, 4, S)
RENNIN (13, 3, S)
TOFU (10, 12, SW)

HIDDEN MESSAGE:
You should stay physically active throughout your life!

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Once completed you may mail it to:

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Thank you for your help!

*** *** *** *** *** ***

1. What grade(s) and subjects do you teach?

   Grade(s):_____ Subject(s):_____

2. How many students participated in this lesson? _____

3. Please enter the name of the experiment or workbook you are evaluating.

   _____

4. On a scale of 1 – 10 (10 = most acceptable), how do you evaluate the relationship of the materials to understanding of science concepts? (Circle one.)

   1 2 3 4 5 6 7 8 9 10

5. On a scale of 1 – 10 (10 = most acceptable), how do you evaluate the understanding of the materials by your students? (Circle one.)

   1 2 3 4 5 6 7 8 9 10

6. Please describe any unusual or interesting way in which you presented this material to your students.

   _____
   _____
   _____
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7. We would appreciate any additional comments or suggestions you may have…

   _____
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Thank You!

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