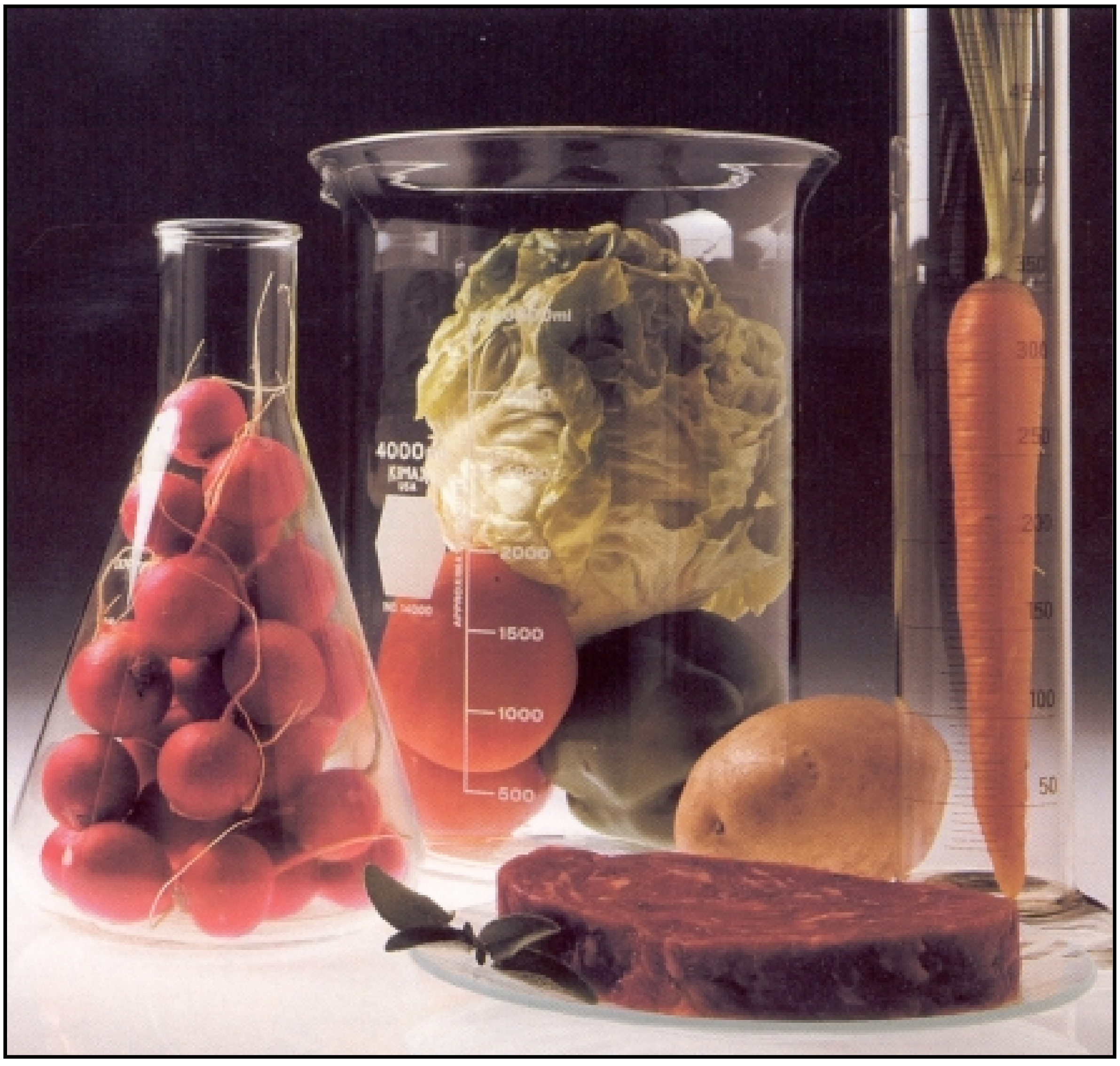
IFT Experiments in Food Science Series

Food Chemistry Experiments



Institute of Food Technologists
The Society for Food Science and Tchnology
221 N. LaSalle St., Suite 300, Chicago, IL 60601

FOOD CHEMISTRY EXPERIMENT BOOK

These experiments have multiple instructional applications and can be incorporated into currently used materials and activities. Some suggested uses are:

- Hands-on experiments in the classroom.
- Demonstrations.
- Ideas for science projects.
- Take-home assignments.
- Hands-on experiments in home schooling.

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Institute of Food Technologists Experiments in Food Science Series

The Institute of Food Technologists (IFT) is a scientific professional society with a membership of more than 28,000. The purpose of the Institute is to support improvement of the food supply and its use through science, technology, and education. Individual objectives of the Institute are to promote programs, implement proposals, and provide guidance consistent with and in support of the Institute.

The IFT Experiments in Food Science Series has been developed as a special project of the Career Guidance Committee of the Institute of Food Technologists, a scientific educational society with an interest in global concerns for providing a safe and wholesome food supply. This curriculum guide was developed for science teachers for grades 8 through 12 to enhance the learning in existing science-oriented courses. The following instructional materials contain educational hands-on activities to help students understand specific scientific facts and principles as they relate to the science of food.

For more information on the IFT Experiments in Food Science Series, contact the Professional Development Department, Institute of Food Technologists, 221 N. LaSalle Street, Suite 300, Chicago, IL 60601. Phone 312/782-8424, Fax 312/782-0045, Internet address *www.ift.org/careers/index.shtml*.

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TEACHER INTRODUCTION

Food chemistry is a major part of a larger discipline of study known as food science. Food science is an interdisciplinary study involving microbiology, biology, chemistry, engineering, and biotechnology. Food science is the application of science and engineering to the production, processing, distribution, preparation, evaluation, and utilization of food. Food chemistry encompasses the composition and properties of food components and the chemical changes they undergo during handling, processing, and storage. A food chemist must know chemistry and biochemistry and have knowledge of physiological chemistry, botany, zoology, and molecular biology to study and modify biological substances as sources of human food. Food chemists work with biological systems that are dead or dying (post-harvested plants and postmortem animal tissues) and study the changes they undergo when exposed to different environmental conditions. For example, during the marketing of fresh tomatoes, the food chemist must determine the optimal conditions to sustain the residual life in the tomatoes so the tomatoes will continue to ripen and arrive at the supermarket as a high-quality product for the consumer. Vital to understanding food science is the knowledge of the primary compounds in food. These compounds are carbohydrates, lipids, and proteins. The experiments and background information focus on the chemistry (functional properties) and structure of these compounds found in foods.

Food science also includes biotechnology, which is the use of biological processes to make new foods, enzymes, supplements, drugs, and vaccines. For thousands of years, people have been using microorganisms in the fermentation of beer and in the making of cheeses, wines, and breads. Today biotechnology also encompasses genetically engineered foods. In genetic engineering, scientists splice genetic material from plants, animals, or bacteria and insert this genetic material into the DNA of other organisms. These new organisms are called genetically modified organisms (GMOs).

Before we can discuss food chemistry, the students must understand basic chemistry concepts. The student introduction explains food chemistry. The introduction also includes general background information on chemical bonds.

Vocabulary

The **atom** is the smallest possible unit of an element, consisting of protons, neutrons, and electrons. The atom is the tiniest part of a chemical element that has all the properties of that element. The smallest speck that can be seen under an ordinary microscope contains more than 10 billion atoms.

A **chemical bond** is an attractive electrical force between atoms strong enough to permit them to function as a unit, called a molecule. Both positive (+) and negative (–) electrical charges attract, just as magnetic north and south poles attract.

The **molecule** is the smallest physical unit of an element or compound, consisting of one or more like atoms in an element and two or more different atoms in a compound.

This module, **Food Chemistry**, contains three major units: **Carbohydrates, Lipids,** and **Proteins.** Each unit includes a Teacher Activity Guide and a Student Activity Guide. **Teacher Activity Guides** present the teacher with all background information he or she will need to perform the experiments, as well as questions and answers and sample data tables. **Student Activity Guides** contain background information relevant to the unit, procedures, and key questions and data tables for the students to complete. Teachers may photocopy this section for distribution to the students.

The experiments in each unit are intended as demonstrations only. The food items and products produced should not be consumed.

There is a supplemental **CD-ROM** called **"The Pizza Explorer"** that the student can use as an independent study exercise on food chemistry. The Pizza Explorer is an interactive program that allows for further learning about food science at a student's own pace.

Molecular models provide a tangible, visual means of introducing the relationships between chemical structure and functional behavior of food molecules. The pictures of chemical structures illustrated in this experiment book were made with a molecular model kit. This kit is available from Molecular Modeling Kits (Molymod) by Spiring Enterprises, Ltd. For further information contact Philip Spiring, Beke Hall, Billingshurst, West Sussex, England RH14 9HF, Telephone + 01403 782387, E-mail *molymod@globalnet.co.uk*, Web site *www.molymod.com*. The kit contains the four basic atoms found in foods: carbon, oxygen, hydrogen, and nitrogen. The kit can be used to build sugars (glucose and fructose), fatty acids and triacylglycerols, and amino acids. The models can also demonstrate stereochemical principles, such as cis and trans double-bond configurations in fatty acids, alpha and beta glycosidic linkages in sugars, and the L and D configurations of amino acids.

STUDENT INTRODUCTION

Food chemistry is a major part of a larger area of study known as food science. Food science is an interdisciplinary study involving microbiology, biology, chemistry, and engineering. Food science is the production, processing, distribution, preparation, evaluation, and utilization of food. Food chemistry encompasses the composition and properties of food components and the chemical changes they undergo during handling, processing, and storage. A food chemist must know chemistry and biochemistry and have knowledge of physiological chemistry, botany, zoology, and molecular biology to study and modify biological substances as sources of human food. Food chemists work with biological systems that are dead or dying (post-harvested plants and postmortem animal tissues) and study the changes they undergo when exposed to different environmental conditions. For example, during the marketing of fresh tomatoes, the food chemist must determine the optimal conditions to sustain the residual life in the fruit so they will continue to ripen and arrive at the supermarket as a high-quality product for the consumer. Vital to understanding food science is the knowledge of the primary components or compounds in food. These compounds are carbohydrates, lipids, and proteins. A major focus of this experiment book is to learn the chemistry and structure of these compounds in foods.

Food science also includes biotechnology, which is the use of biological processes to make new foods, enzymes, supplements, drugs, and vaccines. For thousands of years, people have been using microorganisms in the fermentation of beer, and in the making of cheeses, wines, and breads. Today biotechnology also encompasses genetically engineered foods. In genetic engineering, scientists splice genetic material from plants, animals, or bacteria and insert this genetic material into the DNA of other organisms. These new organisms are called genetically modified organisms (GMOs).

The experiments in each unit are intended as demonstrations only. The food items and products produced should not be consumed.

GENERAL BACKGROUND ON CHEMICAL BONDS

How are food molecules held together? The atoms are connected by **chemical bonds.** The chemical bond forms the chemical structure of the molecules and affects the functional behavior of the molecules. This is the reason why carbohydrates and fats, which are made of the same elements, have different physical and chemical properties. A chemical bond formed by the sharing of one or more electrons, especially pairs of electrons, between atoms is called a **covalent bond**. There are different types of bonds that hold the atoms of molecules together, but we will restrict our discussion to covalent bonds. The following structures are examples of covalent bonds. Carbon is the black atom, hydrogen is white, oxygen is red, and nitrogen is blue.

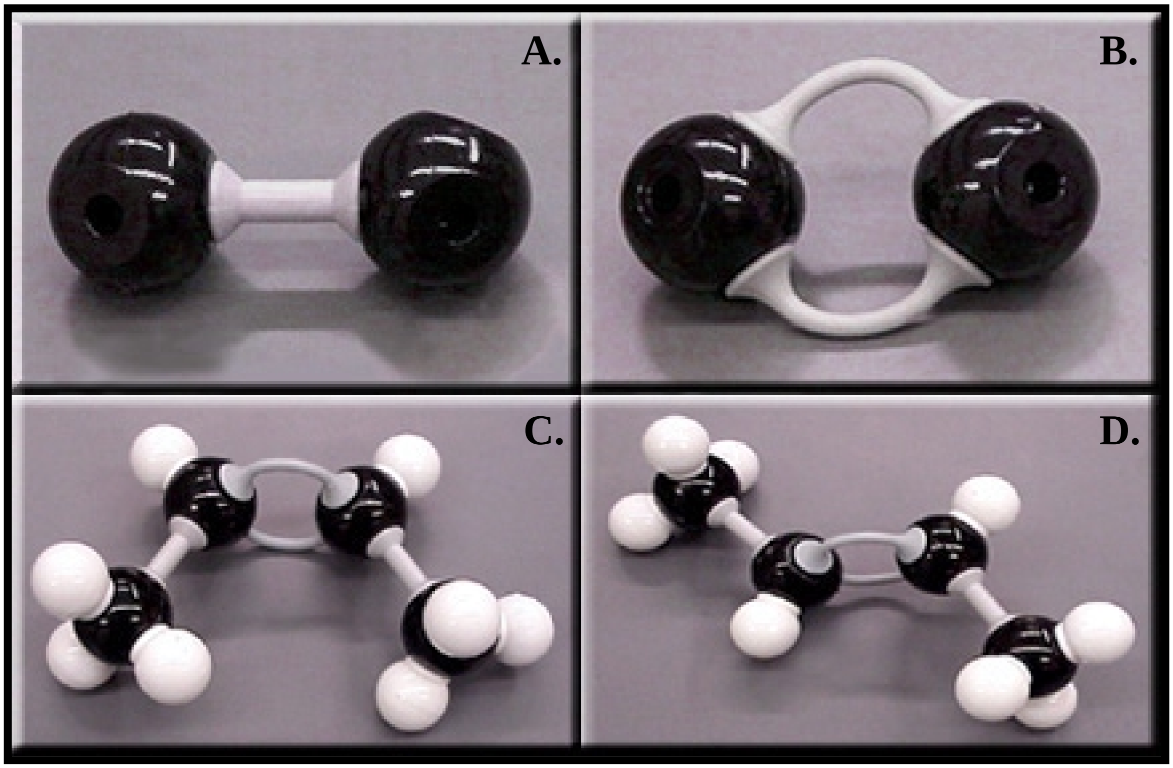


Figure 1

A. A single covalent bond between two carbon atoms. There is only one pair of shared electrons between two adjacent atoms. The two atoms are free to rotate 360^{o}.

B. A double covalent bond between two carbon atoms. There are two pairs of shared electrons between two adjacent atoms. This bond brings the atoms closer together and is stronger than a single bond. There is no free rotation between the two atoms.

C. A cis double bond is where the hydrogen atoms are on the same side of the double bond. The word "cis" means located near or on the same side.

D. A trans double bond is where the hydrogen atoms are on the opposite sides of the double bond. The word "trans" means located across or away from.

Unit 1. CARBOHYDRATES

Teacher Activity Guide

Expected Outcome

The student will learn about the sources of carbohydrates and their uses in the food industry. The students will be able to use a carbohydrate to modify another food substance and explain how food chemistry was involved.

Activity Objective

Students will use pectin in conjunction with an acid and sugar to form jelly. By varying the sugar concentrations, the students will observe that there is an optimum ratio for the creation of this spreadable gel.

Activity Length

Part 1 - 20 minutes
Part 2 - 20 minutes
Part 3 - 20 minutes

Scientific Principles

Pectin solutions form gels when an acid and sugar are added. As the pH is decreased by the addition of acid, the carbohydrate chains of the pectin molecule join together to form a polymer network, which entraps the aqueous solution. The formation of these junction zones is aided by high concentrations of sugar, which allow the chains to interact with one another by dehydrating (pulling water away from) the pectin molecules. This increases the strength and rigidity of the gel.

Vocabulary

Amylase is an enzyme that hydrolyzes starch polymers to yield glucose and maltose. Salivary amylase begins the chemical breakdown of large starch molecules into smaller sugar molecules.

Carbohydrate is a compound of carbon and water with the basic formula $C_nH_2O_n$. [Note: condensation products, such as sucrose, have one less H_2O and a formula $C_nH_2O_{(n-1)}$]. Carbohydrates are the most abundant of all carbon-containing compounds, composing nearly three-fourths of the dry mass of all plant life on earth. Examples of carbohydrates include glucose, sucrose (table sugar), starch, and cellulose.

Cellulose is a polymer of glucose, linked by beta-1,4-glycosidic bonds. It is a complex carbohydrate similar in structure to starch. Examples are cotton, wood, and paper. As part of the human diet, cellulose helps prevent constipation and fights colon cancer.

Fructose is a sugar occurring naturally in a large number of fruits and honey. It is the sweetest of all common sugars. It is a simple carbohydrate with the formula $C_6H_{12}O_6$.

Galactose is a simple sugar having the same chemical formula ($C_6H_{12}O_6$) as glucose and fructose, but a different arrangement of its atoms. It is an isomer of glucose with a hydroxyl group on carbon 4 reversed in position. Galactose is often found in carbohydrates used in cellular recognition, such as blood types and neural receptors.

Glucose is a simple sugar ($C_6H_{12}O_6$) and the primary source of energy for all mammals and many plants. It is also known as dextrose, grape sugar, and corn sugar. It is about half as sweet as table sugar.

Hydrolysis is a chemical process whereby a compound is cleaved into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond that is cleaved. During digestion, the intestinal enzyme sucrase hydrolyzes (adds water to) sucrose ($C_{12}H_{22}O_{11}$) to produce glucose ($C_6H_{12}O_6$) + fructose ($C_6H_{12}O_6$) in the intestinal tract.

Hemiacetal is a product of the addition of an alcohol to an aldehyde. An aldehyde is a compound containing the radical CH=O, reducible to an alcohol (CH_2OH) and oxidizable to a carboxylic acid (COOH).

Isomers are two or more molecules with the same number and kind of atoms, but different arrangements of those atoms.

Lactase is an enzyme that hydrolyzes lactose into glucose and galactose, which can be absorbed into the bloodstream.

Lactose is a disaccharide composed of galactose and glucose linked by a beta-1,4-glycosidic bond. Lactose is found in cow's milk and other dairy products.

Maltose is a disaccharide composed of two molecules of glucose linked by an alpha-1,4-glycosidic bond. It is obtained from the hydrolysis of starch, and is used to flavor some candy. Maltose must be hydrolyzed to glucose before it can be absorbed and taken into the bloodstream.

Polymers contain two or more monomers. Starch is a polymer of the monomer glucose. Protein is a polymer of amino acids.

Starch is a polymer of glucose, linked by alpha-1,4-glycosidic bonds. Starch is a complex carbohydrate found in green plants, and an important source of energy for animals and humans. During the day, green plants store energy by converting glucose to starch. At night, plants convert starch back to glucose for growth.

Stereochemistry is the branch of chemistry concerned with the spatial three-dimensional relations of atoms in molecules. For example, stereochemistry refers to the relative positions of atoms or groups of atoms in the molecule or compound and the effect of these positions on its properties.

Sucrose ($C_{12}H_{22}O_{11}$) is a disaccharide made up of glucose and fructose. Sucrose is obtained from cane sugar, sorghum, and sugar beets. Sucrose is the name for common table sugar, which can't be used by the body unless it is broken down by the enzyme sucrase into monosaccharides by the process of digestion. Absorption of glucose and fructose occurs in the small intestine.

Materials Required

Sure-Jell®	Heatproof gloves
Concentrated fruit juice (apple, grape), thawed, if frozen	Balance or scale
Granulated sugar	Graduated cylinder
Water	Heatproof pad
600-milliliter beakers	Stirring rod/spoon/wooden *Popsicle* stick
Bunsen burner w/ stands or hot plate	

Instructional Strategies and Procedures

You will be able to complete and observe the entire experiment in one class period, if you divide the class into three groups and each group does one part of the experiment.

Teaching Tips

- **The foods produced in these experiments are not to be consumed.**
- Purchase the regular *Sure-Jell*. It contains pectin, acid and dextrose (glucose).
- You can use either frozen juice concentrate or the nonrefrigerated, aseptically processed juice concentrates found in the fruit juice section of the supermarket.
- Caution the students against overheating the jelly. Once the jelly starts to boil, it will bubble up and over the top of the beaker.

SAMPLE DATA TABLE - JELLY CONSISTENCY

EXPERIMENT	JELLY	CONSISTENCY *
Part 1	Normal	Firm
Part 2	Half sugar	Runny; viscosity is like glue
Part 3	Twice sugar	Has some firmness, will not hold a shape

*Jelly results based on the use of *Mott's®* *In-A-Minute* Unfrozen Grape Concentrate.

<u>Key Questions and Answers</u>

1. How did the consistency of the jelly change when you changed the ratio of sugar to pectin?

   When you used half the normal amount of sugar, the jelly was runny; when you used twice the sugar, the jelly was soft and did not hold its shape.

2. Why did the consistency change when you changed the ratio of sugar to pectin?

   There is an optimum ratio for jelly formation. The addition of sugar increases the firmness of the gel by aiding in the formation of polymer junctions. The addition of too much sugar, however, interferes with the gelling process. Although the mechanism for this reaction is not known, it is thought that very high concentrations of sugar dehydrate the pectin molecules to such an extent that some of the entrapped water is pulled out of the gel and back into solution. The result would be a softer gel that would not hold its shape.

Web sites for more information on carbohydrates

www.ag.iastate.edu/departments/agronomy/cornpage.html - Iowa State University. Contains general, technical, and production information on corn.

http://205.156.215.10/ - A. E. Staley Manufacturing Company. Information on food and industrial starches, sweeteners, and corn wet milling processing.

http://osu.orst.edu/instruct/nfm236/starch/index.htm - Oregon State University. Information on starch, its uses and composition.

Student Activity Guide

CARBOHYDRATES

Carbohydrates make up a group of chemical compounds found in plant and animal cells. They have the empirical formula $C_nH_{2n}O_n$, or $(CH_2O)_n$. An empirical formula tells the atomic composition of the compound, but nothing about structure, size, or what chemical bonds are present. Since this formula is essentially a combination of carbon and water, these materials are called "hydrates of carbon", or carbohydrates for short.

Carbohydrates are the primary products of plant photosynthesis. The simplified light-driven reaction of photosynthesis results in the formation of a carbohydrate: $nH_2O + nCO_2 \rightarrow -(CH_2O)_n- + nO_2$. This type of carbohydrate is found in the structures of plants and is used in the reverse reaction of photosynthesis (respiration) or is consumed as fuel by plants and animals.

Carbohydrates are widely available and inexpensive, and are used as an energy source for our bodies and for cell structures. Food carbohydrates include the simple carbohydrates (sugars) and complex carbohydrates (starches and fiber). Before a big race, distance runners and cyclists eat foods containing complex carbohydrates (pasta, pizza, rice and bread) to give them sustained energy.

Carbohydrates are divided into **monosaccharides**, **disaccharides**, and **polysaccharides.** As shown in the following molecular model structures, carbohydrates may be found as hexagon (6-sided, see **Figure 1A**) and pentagon (5-sided, see **Figure 1B**) shaped rings.

Monosaccharides

Monosaccharides are single-molecule sugars (the prefix "mono" means one) that form the basic units of carbohydrates. They usually consist of three to seven carbon atoms with attached hydroxyl (OH) groups in specific stereochemical configurations. The carbons of carbohydrates are traditionally numbered starting with the carbon of the carbonyl end of the chain (the carbonyl group is the carbon double-bonded to oxygen). The number of carbons in the molecule generally categorizes monosaccharides. For example, three-carbon carbohydrate molecules are called **trioses**, five-carbon molecules are called **pentoses**, and six-carbon molecules are called **hexoses**.

Ribose and 2-deoxyribose are pentoses, and both have a crucial role in reproduction as polymers known as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). One of the most important monosaccharides is glucose (dextrose). This molecule is the primary source of chemical energy for living systems. Plants and animals alike use this molecule for energy to carry out cellular processes. Mammals produce peptide hormones (insulin and glucagon) that regulate blood glucose levels, and a disease of high blood glucose is called diabetes. Other hexoses include fructose (found in fruit juices) and galactose.

Different structures are possible for the same monosaccharide. Although glucose and fructose are identical in chemical composition ($C_6H_{12}O_6$), they are very different in structure (see molecular models). Such materials are called **isomers**. Isomers in general have very different physical properties based on their structure.

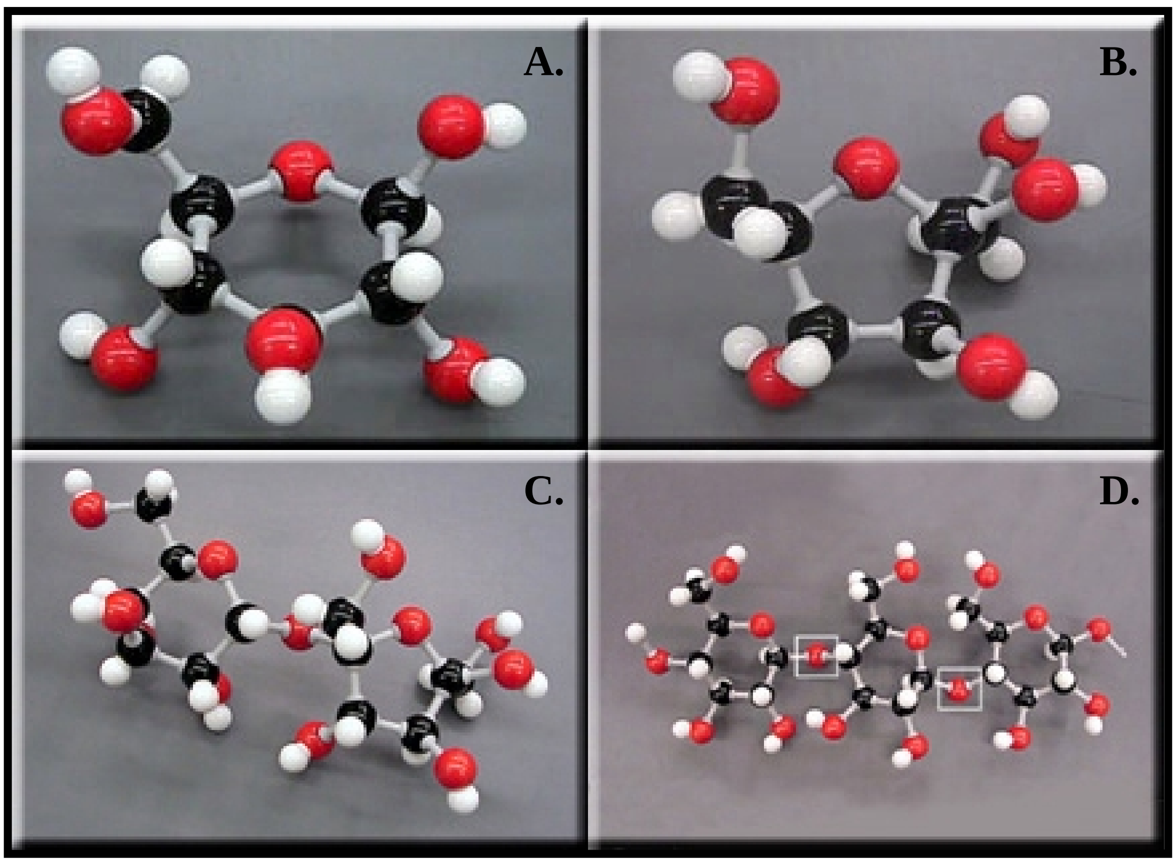


Figure 1

A. Glucose, a six-membered ring monosaccharide. B. Fructose, a five-membered ring monosaccharide. C. Sucrose, a disaccharide containing glucose and fructose. D. Molecular representation of starch illustrating the alpha-glycosidic linkages joining monosaccharides to form the polysaccharide structure.

Disaccharides

Disaccharides are two monosaccharide sugar molecules that are chemically joined by a **glycosidic linkage** (- O -) to form a "double sugar" (the prefix "di" means two). When two monosaccharide molecules react to form a glycosidic bond (linkage), a water molecule is generated in the process through a chemical reaction known as condensation. Therefore, condensation is a reaction where water is removed and a polymer is formed. The most well known disaccharide found in nature is sucrose, which is also called cane sugar, beet sugar, or table sugar (see **Figure 1C**). Sucrose is a disaccharide of glucose and fructose. Lactose or milk sugar is a disaccharide of glucose and galactose and is found in milk. Maltose is a disaccharide composed of two glucose units. Disaccharides can easily be hydrolyzed (the reverse of condensation) to become monosaccharides, especially in the presence of enzymes (such as the digestive enzymes in our intestines) or alkaline catalysts. **Invert sugar** is created from the hydrolysis of sucrose into glucose and fructose. Bees use enzymes to create invert sugar to make honey. Taffy and other invert sugar type candies are made from sucrose using heat and alkaline baking soda.

Disaccharides are classified as oligosaccharides (the prefix "oligo" means few or little). This group includes carbohydrates with 2 to 20 saccharide units joined together. Carbohydrates containing more than 20 units are classified as polysaccharides.

Polysaccharides

Polysaccharides (the prefix "poly" means many) are formed when many single sugars are joined together chemically. Carbohydrates were one of the original molecules that led to the discovery of what we call polymers. Polysaccharides include starch, glycogen (storage starch in animals), cellulose (found in the cell walls of plants), and DNA.

Starch is the predominant storage molecule in plants and provides the majority of the food calories consumed by people worldwide. Most starch granules are composed of a mixture of two polymers: a linear polysaccharide called amylose and a branched-chain polysaccharide called amylopectin. Amylopectin chains branch approximately every 20-25 saccharide units. Amylopectin is the more common form of starch found in plants. Animals store energy in the muscles and liver as glycogen. This molecule is more highly branched than amylopectin. For longer-term storage, animals convert the food calories from carbohydrates to fat. In the human and animals, fats are stored in specific parts of the body called adipose tissue.

Cellulose is the main structural component of plant cell walls and is the most abundant carbohydrate on earth. Cellulose serves as a source of dietary fiber since, as explained below, humans do not have the intestinal enzymes necessary to digest it.

Starch and cellulose are both homopolymers ("homo" means same) of glucose. The glucose molecules in the polymer are linked through **glycosidic covalent bonds**. There are two different stereochemical configurations of glycosidic bonds—an alpha linkage and a beta linkage. The only difference between the alpha and beta linkages is the orientation of the linked carbon atoms. Therefore, glucose polymers can exist in two different structures, with either alpha or beta linkages between the glucose residues. Starch contains **alpha linkages** (see **Figure 1D**) and cellulose contains **beta linkages.** Because of this difference, cornstarch has very different physical properties compared to those for cotton and wood. Salivary amylase only recognizes and catalyzes the breakdown of alpha glycosidic bonds and not beta bonds. This is why most mammals can digest starch but not cellulose (grasses, plant stems, and leaves).

Food Uses of Carbohydrates

Carbohydrates are widely used in the food industry because of their physical and chemical properties. The sweet taste of sucrose, glucose, and fructose is used to improve the palatability of many foods. Lactose is used in the manufacture of cheese food, is a milk solids replacer in the manufacture of frozen desserts, and is used as a binder in the making of pills/tablets.

Another useful aspect of some carbohydrates is their chemical *reducing* capability. Sugars with a free hemiacetal group can readily donate an electron to another molecule. Glucose, fructose, maltose, and lactose are all **reducing sugars**. Sucrose or table sugar is not a reducing sugar because its component monosaccharides are bonded to each other through their hemiacetal group. Reducing sugars react with the amino acid lysine (see **Unit 3, Proteins, Figure 1A**) in a reaction called the **Maillard reaction**. This common browning reaction produced by heating the food (baking, roasting, or frying) is necessary for the production of the aromas, colors, and flavors in caramels, chocolate, coffee, and tea. This non-enzymatic browning reaction differs from the enzymatic browning that occurs with fresh-cut fruit and vegetables, such as apples and potatoes.

Carbohydrates can protect frozen foods from undesirable textural and structural changes by retarding ice crystal formation. Polysaccharides can bind water and are used to thicken liquids and to form gels in sauces, gravies, soups, gelatin desserts (*Jell-O*®), and candies like jelly beans and orange slices. They are also used to stabilize dispersions, suspensions, and emulsions in foods like ice cream, infant formulas, dairy desserts, creamy salad dressings, jellies and jams, and candy. Starches are used as binders, adhesives, moisture retainers, texturizers, and thickeners in foods.

In the following experiment we will be investigating pectin. Pectin is a polysaccharide that is found in green apples and in the peel of limes and lemons. Pectin forms a gel when heated with an acid and sugar, and is used to make high-sugar jellies, jams, and marmalade.

Pectin solutions form gels when an acid and sugar are added. The acid will reduce the pH of the solution and cause the carbohydrate molecules to form junctions. From these junctions a network of polymer chains can entrap an aqueous solution. The sugar increases junction formation. The pectin makes the gel, and the low pH and the amount of soluble solids adjusts the rigidity. The optimum conditions for jelly strength are 1% pectin, a pH of 3.2, and a sugar concentration of 55% (by weight).

<u>Activity Objective</u>

To observe how pectin can be used to form a gel and the effects of too little and too much sugar on gelling.

<u>Materials Required</u>

Sure-Jell ®
Concentrated fruit juice (apple, grape), if frozen, thawed
Granulated sugar
Water
600-milliliter beakers
Bunsen burner with stand or hot plate
Heatproof gloves
Balance or scale
Graduated cylinder
Heatproof pad
Stirring rod/spoon/wooden *Popsicle* stick

<u>Experimental Procedure</u>

Part 1

1. Measure out 53 grams (1/4 cup) of sugar.
2. Put 18 milliliters (0.75 fluid ounce) of fruit juice concentrate, 60 milliliters (1/4 cup) of water, and 7 grams (3 teaspoons) of *Sure-Jell* into a 600-milliliter beaker.
3. Place the beaker on a hot plate or Bunsen burner and stir constantly over a high heat until bubbles form all around the edge.
4. Add the sugar. Bring the mixture to a boil and boil hard, while stirring, for one minute. Be sure to adjust the heat source so that the liquid does not boil up the sides of the beaker. Caution! This can boil over very quickly if it's not carefully watched.
5. Using gloves, remove the beaker from the heat source. Place the beaker on a heatproof pad to cool. Allow the jelly to cool. Use a spoon to skim off the foam on the top.
6. Record your results.

Part 2

1. Measure out 26 grams (1/8 cup) of sugar.
2. Repeat steps 2, 3, 4, and 5 in Part 1.
3. Record your results.

Part 3

1. Measure out 106 grams (1/2 cup) of sugar.
2. Repeat steps 2, 3, 4, and 5 in Part 1.
3. Record your results.

DATA TABLE - JELLY CONSISTENCY

EXPERIMENT	JELLY	CONSISTENCY
Part 1	Normal	
Part 2	Half sugar	
Part 3	Twice sugar	

Questions

1. How did the consistency of the jelly change when you changed the ratio of sugar to pectin?

2. Why did the consistency change when you changed the ratio of sugar to pectin?

NAME__________________________CLASS___________________PERIOD_______

Cryptic Carbohydrates

Fill in the blank spaces with the appropriate terms to complete the sentences. Solve the hidden message by entering the boxed letters in the spaces at the bottom of the page.

1. [__] __ __ __ __ __ __
are identical in chemical composition but differ structurally.

2. [__] __ __ __ __ __ __ __ __
is a polymer of glucose and serves as a source of dietary fiber for humans.

3. __ __ __ __ __ __ __ __ __ __ __ [__] __
are an inexpensive and widely available source of energy for our bodies.

4. __ __ [__] __ __ __ __
is a disaccharide found in cow's milk.

5. __ __ __ [__] __ __ __
is a disaccharide composed of glucose and fructose.

6. __ [__] __ __ __ __
is a starch that has gelling properties and is used in making jams and preserves.

7. Glucose is a
__ __ __ __ __ [__] __ __ __ __ __ __ __ __ .

8. The [__] __ __ __ __ __ __ __
reaction is a nonenzymatic browning reaction that occurs when foods are roasted or baked.

9. __ __ __ __ __ __ __ __ __ __
bonds chemically join two or more monosaccharide molecules.

10. Carbohydrates are the primary products of plant
__ __ __ __ __ __ __ __ __ __ __ __ __ __ .

HIDDEN MESSAGE:

A polysaccharide called carrageenan is a seaweed extract. Carrageenan is used as a stabilizer in what popular frozen dessert product?

__ __ __   __ __ __ __ __

Solution for Cryptic Carbohydrates

1. ISOMERS
2. CELLULOSE
3. CARBOHYDRATES
4. LACTOSE
5. SUCROSE
6. PECTIN
7. MONOSACCHARIDE
8. MAILLARD
9. GLYCOSIDIC
10. PHOTOSYNTHESIS

HIDDEN MESSAGE: ICE CREAM

NAME______________________________CLASS_________________PERIOD____

Cool Carbs

Find the words listed below in the word search. After all the words are found, the letters that are not used reveal a hidden message at the bottom of this sheet.

M	A	I	L	L	A	R	D	S	C	H	O	O	H	P
S	E	E	R	A	V	A	A	R	T	G	I	C	E	O
T	Y	T	O	E	F	F	O	G	L	N	R	O	D	L
S	W	A	I	T	M	H	I	U	U	A	A	N	E	Y
A	C	R	H	F	O	O	C	O	T	S	D	L	G	S
R	O	D	U	E	P	O	S	S	F	K	G	Q	P	A
G	L	Y	C	O	S	I	D	I	C	B	O	N	D	C
A	S	H	B	E	B	O	J	E	F	O	R	G	E	C
A	Y	O	L	D	Z	M	L	R	V	E	N	Q	S	H
I	F	B	T	V	K	E	U	U	M	L	M	N	O	A
R	P	R	J	Z	D	C	Y	Y	L	C	N	P	T	R
O	J	A	P	B	T	P	L	N	T	L	A	K	C	I
F	M	C	O	O	X	O	N	I	T	C	E	P	A	D
L	E	G	S	Q	P	E	N	E	R	G	Y	C	L	E
Y	J	E	B	N	J	E	W	W	J	H	G	O	U	R

CARBOHYDRATE	CELLULOSE	ENERGY	FRUCTOSE
GEL	GLYCOSIDIC BOND	GLUCOSE	ISOMER
LACTOSE	MAILLARD	PECTIN	PLANTS
POLYMER	POLYSACCHARIDE	STARCH	SUGAR

HIDDEN MESSAGE: We should _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
_ _.

Institute of Food Technologists, IFT Experiments in Food Science Series

Solution to Cool Carbs

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

(Over, Down, Direction)

CARBOHYDRATE (3, 13, N)
ENERGY (7, 14, E)
GEL (3, 14, W)
GLYCOSIDIC BOND (1, 7, E)
LACTOSE (14, 14, N)
PECTIN (13, 13, W)
POLYMER (6, 14, NE)
STARCH (9, 6, NE)
CELLULOSE (13, 14, NW)
FRUCTOSE (10, 8, SW)
GLUCOSE (11, 2, SW)
ISOMER (9, 7, NW)
MAILLARD (1, 1, E)
PLANTS (14, 6, NW)
POLYSACCHARIDE (15, 1, S)
SUGAR (11, 5, NW)

HIDDEN MESSAGE:
We should choose a variety of foods within each food group.

Unit 2. LIPIDS

Teacher Activity Guide

Expected Outcomes

The student will learn about sources of lipids and their uses in the food industry.

Activity Objective

Students will make visual observations of fat and then extract and examine the invisible fat from chocolate, potato chips, and sunflower seeds.

Activity Length

Part A - 20 minutes.
Part B - 40 minutes to perform the experiment, dry overnight, 10 minutes the next day to record observations.

Scientific Principles

In this experiment, acetone is used to extract the invisible fats, since lipids are sparingly soluble in water but soluble in organic solvents. When the extraction is complete, the students will be able to see, touch, and smell the lipid in the Petri dishes and be able to determine if it contains saturated or unsaturated fatty acids. The cocoa butter found in chocolate chips is a saturated fat and will be solid at room temperature. The oils used to fry the potato chips are unsaturated and will be liquid at room temperature. The oil from the sunflower seeds also is unsaturated and will be liquid at room temperature.

Vocabulary

Acetone is a clear, colorless, flammable, fruity-smelling organic (carbon-containing) liquid used to make many other chemical compounds. It is also formed in diabetic people, and its presence in urine is one symptom of this disease.

Emulsion is a property where two liquids are evenly spread out in each other, yet not dissolved in each other. Oil and water form the most common emulsions, and milk is an emulsion of butterfat in water. Emulsions are important in the production of foods that contain water and fat, such as mayonnaise or margarine. These products require the addition of an **emulsifier**, such as the food lipid lecithin, to stabilize food emulsions.

A **fatty acid** is a carboxylic acid derived from or contained in an animal fat or vegetable oil. All fatty acids are composed of alkyl groups or hydrocarbon chains containing from 4 to 22 carbon atoms and characterized by a terminal carboxyl group COOH. Fatty acids are the building blocks of fats, having hydrogen atoms attached to chains of carbon atoms. Fatty acids are found in every cell of the human body.

Insoluble means not capable of being dissolved. Fats are insoluble in water (fat is non-polar, and water is very polar). Fats are soluble in like solvents. As an example, fats are soluble in non-polar solvents such as acetone and ether. On the other hand, sugar will not be soluble if more is added than what a certain volume of water can dissolve, which means that the solvent has become saturated with sugar.

Lipase is a generic name given to a group of enzymes that catalyze the hydrolysis of lipids. For example, a lipase that works on food lipids breaks down triacylglycerol into glycerol and fatty acids.

Lipids are compounds of fatty acids and glycerol. Lipids are the most efficient source of fuel in living things; they are stored beneath the skin in animals and the human, and mostly in the seeds of plants. Food lipids are divided into **fats**, which come from animal sources and are solid at room temperature, and **oils**, which come from plant sources and are liquid at room temperature. Another type of lipid is cholesterol. Cholesterol is a sterol compound made by animals and is used to make certain steroid hormones in the body. It is not found in plants.

Mass is the quantity of atoms or matter in an object. As the mass of an object increases, so does the degree of difficulty to change the motion (or lack of motion) of an object. This is also referred to as a measure of inertia. A locomotive has more atoms than a car and therefore more mass. It also has much greater inertia, requiring a much greater force to change its movement.

Organic means related to the branch of chemistry dealing with carbon compounds. Though all living things contain carbon and thus are considered to be organic, other carbon-containing compounds have been produced in the laboratory.

Quantitative describes a measurable amount or number value for something. For example, a white, round (qualitative) piece of filter paper weighs 1.32 grams (quantitative).

Soluble means capable of being dissolved. Gases or solids that dissolve are called **solutes**, while the liquid that does the dissolving is called the **solvent**. Like substances are usually soluble in like solvents.

A **triacylglycerol** is a lipid compound consisting of three fatty acids linked to one glycerol molecule. This compound is an important source of energy for the human body. To utilize this compound for energy, enzymes called lipases must first hydrolyze it to liberate the fatty acids that are chemically bonded to glycerol.

Materials Required

Chocolate chips (semi-sweet)
Sunflower seeds
Potato chips
Acetone
100-mm Petri dishes
100-and 600-milliliter beakers
Graduated cylinder
Balance or scale
Microwave
Paper towels
Foil
Hammer
Safety goggles
Latex or rubber gloves

Instructional Strategies and Procedures

This activity could be conducted over one class period if the students are assigned to groups that focus on the separate aspects of the lipid experiments.

Teaching Tips

- **The foods produced in these experiments are not to be consumed.**
- Use acetone purchased from a local hardware store. Acetone is normally used as a thinner for epoxies, lacquers, and adhesives. The acetone will evaporate in one hour from the chocolate and potato chips. You will have to smell the sunflower seeds to determine if the acetone is gone. Do not use nail polish remover or rubbing alcohol because they contain too much water to extract the lipid.
- You may substitute mineral spirits or denatured alcohol purchased from the hardware store for the acetone.
- Have the students wear latex gloves and safety goggles when handling the acetone solvent.
- Perform this experiment in a well-ventilated area.
- The acetone is highly flammable. Be sure there are no flames or pilot lights on in the room.
- Semi-sweet chocolate chips contain more fat than milk chocolate chips.
- You may substitute walnuts for sunflower seeds.
- Try to use low-salt potato chips; do not use low-fat or baked potato chips because these products have much less lipid.

Part A SAMPLE DATA TABLE – VISUAL OBSERVATIONS OF FAT

Food	**Describe what you see on the paper towel**
Chocolate chips	Grease spot spreads beyond the chocolate
Potato chips	Large, wet grease spot on the paper towel
Sunflower seeds	Grease spot is only where the seeds touch the paper

Part B SAMPLE DATA TABLE – EXTRACTION OF LIPIDS

Food	**Weight of beaker**	**Weight of beaker with raw food**	**Weight of raw food**	**Weight of beaker with dried food**	**Weight lost from food**	**% Lipid extraction**
Chocolate chips	49 g	53.4 g	4.4 g	53.2 g	0.2 g	4.5%
Potato chips	48.6 g	53.2 g	4.6 g	52.7 g	0.5 g	10.9%
Sunflower seeds	51.1 g	55.5 g	4.4 g	54.8 g	0.7 g	15.9%

(weight of beaker with raw food) – (weight of beaker) = weight of raw food

(weight of beaker with raw food) – (weight of beaker with dried food) = weight lost from the food

$$\frac{\text{weight lost from food}}{\text{weight of raw food}} \times 100 = \text{\% lipid extracted}$$

Part B SAMPLE DATA TABLE – DESCRIPTION OF FAT

Food	**Color ***	**Texture ***	**Odor ***	**Viscosity ***
Chocolate chips	Light brown	Waxy	Smells like chocolate	Hard, dry
Potato chips	Light yellow	Oily	Corn	Thick oil
Sunflower seeds	Yellow	Oily	Sunflower seeds	Thick oil

*Results reported were obtained using the following products: *Nestlé*® semi-sweet chocolate chips, *Jays*® *Potato Chips* (fried in native corn oil), and *Pic-A-Nut*® sunflower seeds (shelled).

Key Questions and Answers

1. How can you tell that the dark wet spot on the paper towel is fat and not water?

   The paper is transparent, and it's greasy to the touch.

2. Rank from most to least the percentage of lipid extracted from all three foods. Look at the Nutrition Facts label on the packages of all three foods and rank them. Did your ranking agree with the ranking of the product labels?

   Sunflower seeds, potato chips, and chocolate chips. From the Nutrition Facts labels: 28 grams of sunflower seeds contain 15 grams of fat; 28 grams of potato chips contain 10 grams of fat; and 28 grams of chocolate chips contain 8 grams of fat. Yes, the rankings are consistent.

3. Determine which lipids contained saturated and unsaturated fatty acids in this experiment, based on your descriptions of the fats in the Petri dishes.

   The lipid extracted from the chocolate contained saturated fatty acids. The lipid extracted from the potato chips contained unsaturated fatty acids. The lipid extracted from the sunflower seeds contained unsaturated fatty acids.

Web site for more information on lipids

www.centralsoya.com/lecithin.html - Central Soya - Information about lecithin, sources of lecithin, and how it is used in foods and other applications.

LIPIDS

Lipids include fats, oils, waxes, cholesterol, other sterols, and most steroids. In the body, fat serves as a source of energy, a thermal insulator and cushion around organs, and an important cellular component. The fat-soluble vitamins are A, D, E, and K. You are probably most familiar with the nutritional aspects of dietary fats and oils. Since fats have 2.25 times the energy content of carbohydrates and proteins, most people try to limit their intake of dietary fat to avoid becoming overweight. The food industry has a big market for low-fat and non-fat foods. Just take a look around your local grocery stores!

Lipids are classified as organic compounds that are soluble (dissolvable) in organic solvents, but only sparingly soluble in water. Lipids are biologically important for making barriers (membranes of animal cells), which control the flow of water and other materials into a cell.

Fats and **oils** make up 95% of food lipids and phospholipids, and sterols make up the other 5%. Traditionally, fats were considered to be solid at room temperature, and oils were considered to be liquid. However, this designation is often used to distinguish between fats and oils from animals and plants, respectively. Animal fats are found in meats (beef, chicken, lamb, pork, and veal), milk products, eggs, and seafood (fish oil). Plant (vegetable) oils come from nuts (peanuts), olives, and seeds (soybean, canola, safflower, and corn). We use lipids for flavor (butter and olive oil), to cook foods (oils and shortening), to increase the palatability of foods by improving the texture or "mouthfeel" (cakes, creamy ice cream), and in food processing (emulsifiers).

Fatty acids are generally long, straight chains of carbon atoms with hydrogen atoms attached (**hydrocarbons**) with a carboxylic acid group (COOH) at one end and a methyl group (CH_3) at the other end. These long, straight chains combine with the **glycerol** molecule (see **Figure 1A**) to form lipids (glycerol lipids).

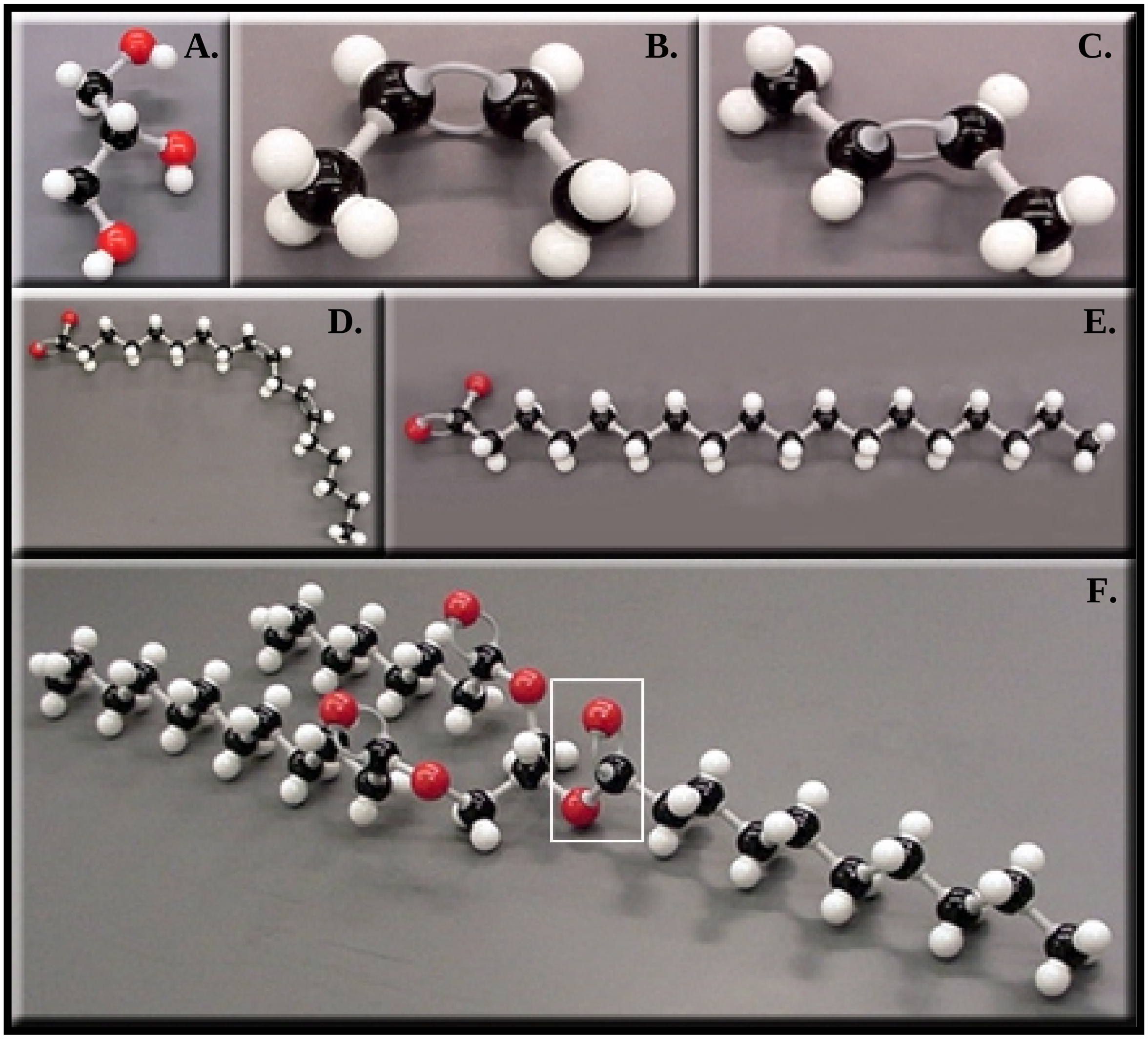


Figure 1

A. Glycerol molecule is the backbone of a glycerol lipid. The triacylglycerol contains three fatty acids attached at the oxygen atoms of glycerol.

B. Configuration of a *cis* double bond.

C. Configuration of a *trans* double bond.

D. Linoleic acid is an essential fatty acid containing two double bonds. It is needed for growth and health.

E. Stearic acid is a saturated fatty acid found in foods from animal and plant sources.

F. Milk fat triacylglycerol molecule illustrating the ester bonds between fatty acids and glycerol.

Most naturally occurring fatty acids contain an even number of carbon atoms. The 18-carbon fatty acids are the most abundant in our food supply; examples are linoleic acid (an omega-6 fatty acid) found in corn oil and linolenic acid (an omega-3 fatty acid) found in flaxseed oil. Linoleic and linolenic acids are considered **essential fatty acids** because they are needed for normal physiological functions and our body cannot make them. We need to get these fatty acids from food sources. These fatty acids are found in the vegetable oils used in several different food products.

Structure of Lipids

Most of the carbon–carbon bonds in fats are single bonds, which allow the carbons to freely rotate, making the attached groups chemically identical. However, the number of unsaturated bonds (double bonds) may vary from one to many in the hydrocarbon part of the fatty acid. Since double bonds do not allow free rotation between the attached carbons, any attached chemical groups are fixed in their respective positions.

There are two possible orientations for groups attached to the carbons in a double bond. If they are on the same side of the double bond (close together), they are in the **cis** conformation. The opposite of the cis conformation is the **trans** conformation, where the residues at ends of the double bond are farther apart. Double bonds in natural vegetable oils and in animal fats are mostly in the cis conformation (see **Figures 1B** and **1C**); however, a few exceptions are known where the trans conformation is present.

The **presence of the double bonds is responsible for the liquid properties** of native vegetable oil. Because the cis double bonds are "kinked", they disrupt the physical interactions between fatty acid molecules, preventing them from packing together tightly to form crystals (see **Figure 1D**, structure of linoleic acid). This disruption keeps the fatty acid molecules from associating with each other, resulting in a liquid structure. **If the double bonds are removed** by adding hydrogen (hydrogenation), the kinks are removed, allowing the fatty acid molecules to more easily associate with each other (see **Figure 1E**, structure of stearic acid). The result is **crystallization** (solid fat) at room temperature.

Depending on how the various fatty acid chains associate, the crystalline structure of the solid fat can have different appearances, such as a smooth, shiny solid or a rough, puffy solid. These crystalline forms also have different light-reflection characteristics and physical hardness. This difference in physical properties is used when making **shortening**, which is **crystallized into a very white, soft crystalline form** at the factory. However, upon melting and re-solidification, it becomes more translucent and grayer, due to the formation of a different crystal structure.

Nomenclature for Fats

If all the bonds are single, the fatty acid molecule is **saturated,** because the maximum number of hydrogen atoms is associated with the carbon atoms. Some examples are tallow (beef fat), lard (pork fat), and butter (milk fat). If there is a double bond among the carbon atoms, the fatty acid molecule is **unsaturated**. Examples of unsaturated fats are canola oil, corn oil, cottonseed oil, and soybean oil. If there are multiple double bonds (two or more), it is called **polyunsaturated**. You may recall seeing the saturated, unsaturated, and polyunsaturated terms with respect to nutritional aspects of oils. Corn and soybean oils are some of the most important food sources of polyunsaturated fatty acids in our food supply. Shown below are the shorthand notation used to describe some important food sources of 18-carbon (C18) fatty acids.

- C18:0 is a fully saturated 18-carbon fatty acid called stearic acid.
- C18:1 has one double bond, between carbons 9-10, (18:1n9) counting from the COOH end, and is called oleic acid.
- C18:2 has two double bonds, between carbons 9-10 and 12-13, counting from the COOH end, and is called linoleic acid (9,12-octadecadienoic acid or 18:2n6).
- C18:3 has three double bonds at carbons 9-10, 12-13, and 15-16, counting from the COOH end, and is called linolenic acid (9,12,15-octadecatrienoic acid or 18:3n3).

The number of fatty acids joined to the glycerol molecule also plays a part in how the molecule is named. If only one fatty acid is connected, the general name for the molecule is a **monoacylglycerol**. If two are joined, the molecule is called a **diacylglycerol,** and if three are joined, a **triacylglycerol**. The bond between the fatty acid and the glycerol also has a special name. It is called an **ester bond** (see **Figure 1F**). The carboxyl end (COOH) of the fatty acid molecule attaches to one of the -OH groups of the glycerol molecule. Because of this combination, an -OH group and -H are left, which combine to form a water molecule.

Since triacylglycerols have three fatty acids, you can get mixed-fatty-acid triacylglycerols, in which there are different fatty acids on each of the glycerol bonds. Naturally occurring soybean oil is a mixed triacylglycerol, containing saturated, monounsaturated, and polyunsaturated fatty acids. Soybean oil contains more monounsaturated and polyunsaturated fatty acids than saturated fatty acids.

Surfactant is a short term for surface-active agent. Polar lipids, like lecithin in soybean oil, serve as specialized surfactants known as **emulsifiers**. By interacting with water on one end of the molecule and repelling water on the other end, emulsifiers keep fat globules dispersed in water or water droplets dispersed in fat. Lipid surfactants are important to our own cellular functions, as well as useful in stabilizing specific food products. **Lecithin** is a phospholipid, which functions as a surfactant. Lecithin and other phospholipid emulsifiers are found in food from animal and plant sources. The food sources of lecithin are eggs, milk, cheese, and soybean oil. These chemical properties of lecithin are used in the food industry to prevent fats from separating out of chocolate, mayonnaise, peanut butter, and salad dressings.

The fats that you see in raw beef, chicken, and pork are known as visible fats. These fats are in plain view and are solid at room temperature. Vegetable oils are also visible fats. The fats that are in snack foods, cookies, desserts, and candy are known as invisible fats. Although you cannot see them, they can add extra calories to your diet.

Activity Objective

In this experiment, we will be extracting and examining the fat in chocolate, potato chips, and sunflower seeds. In chocolate, sugar and cocoa are dispersed in a crystallized fat matrix. To keep the fat from separating out of the chocolate, an emulsifier called lecithin is used. The fat in the potato chip is mostly on the surface of the chip from the frying process. The fat in the sunflower seed is in the seed itself. The cooking oils that we use come primarily from nuts and seeds. Examples of these fat sources are corn, soybean, and peanut oils.

Materials Required

Chocolate chips (semi-sweet)
Sunflower seeds
Potato chips
Acetone
100-mm Petri dishes
100-and 600-milliliter beakers
Graduated cylinder
Balance or scale
Microwave
Paper towels
Foil
Hammer
Safety goggles
Latex or rubber gloves

Experimental Procedure

Part A. Visual evidence of invisible fats from foods

Part 1. Chocolate Chips

1. Measure out 2 grams of chocolate chips and place on a paper towel.
2. Microwave for 40 seconds on high.
3. Fold the paper towel over the chocolate chips and gently press the chocolate chips flat with your fingers.
4. Allow it to sit for 5 minutes. Open up the paper towel. Record your results.

Part 2. Potato Chips

1. Measure out 2 grams of potato chips and place on a paper towel.
2. Microwave for 25 seconds on high.
3. Fold the paper towel over the potato chips and crush the chips with a hammer.
4. Allow it to sit for 5 minutes. Open up the paper towel. Record your results.

Part 3. Sunflower Seeds

1. Measure out 2 grams of sunflower seeds and place on a paper towel.
2. Microwave for 25 seconds on high.
3. Fold the paper towel over the sunflower seeds and crush the seeds with a hammer.
4. Allow it to sit for 5 minutes. Open up the paper towel. Record your results.

DATA TABLE – VISUAL OBSERVATIONS OF FAT

Food	**Describe what you see on the paper towel**
Chocolate chips	
Potato chips	
Sunflower seeds	

<u>Part B. Quantitative measurement of invisible fats from foods</u>

Part 1. Extraction of Fat from Chocolate Chips

1. Weigh out 5 grams (9 chips) of chocolate chips. Crush the chocolate between two sheets of foil with a hammer.
2. Label the beakers that you are using to put the food in, one each for chocolate chips, potato chips, and sunflower seeds. Record the weights of the labeled beakers.
3. Using the beaker that is labeled for chocolate chips and place the crushed chocolate chips in the beaker. Record the weight with the crushed chocolate chips.
4. Add 10 milliliters of acetone to the crushed chocolate chips in the beaker.
5. Swirl for 1 minute in a hood, or stir with a glass rod (in a well ventilated area).
6. Carefully decant the acetone into the Petri dish, making sure the chocolate remains in the beaker.
7. Add 10 milliliters of acetone to the chocolate and repeat steps 5 and 6.
8. Allow the acetone in the Petri dish to dry overnight in a hood (or a well ventilated area) to visualize the lipid that was extracted.
9. Allow the beaker with the chocolate to dry overnight. Weigh the beaker with the chocolate.

Part 2. Extraction of Fat from Potato Chips

1. Weigh out 5 grams of potato chips. Break into dime-size pieces with your fingers.
2. Repeat steps 2–9 in Part 1.

Part 3. Extraction of Fat from Sunflower Seeds

1. Weigh out 5 grams of sunflower seeds. Crush the seeds between two pieces of foil with a hammer.
2. Repeat steps 2–9 in Part 1.

DATA TABLE – EXTRACTION OF LIPIDS

Food	Weight of beaker	Weight of beaker with raw food	Weight of raw food	Weight of beaker with dried food	Weight lost from food	% Lipid extraction
Chocolate chips						
Potato chips						
Sunflower seeds						

(weight of beaker with raw food) **–** (weight of beaker) = weight of raw food

(weight of beaker with raw food) **–** (weight of beaker with dried food) = weight lost from food

$$\frac{\text{weight lost from food}}{\text{weight of raw food}} \times 100 = \%\ \text{lipid extracted}$$

DATA TABLE – DESCRIPTION OF FATS

Food	Color	Texture	Odor	Viscosity
Chocolate chips				
Potato chips				
Sunflower seeds				

Questions

1. How can you tell that the dark wet spot on the paper towel is fat and not water?

2. Rank from most to least the percentage of lipid extracted from all three foods. Look at the Nutrition Facts label on the packages of all three foods and rank them. Did your ranking agree with the ranking of the product labels?

3. Determine which lipids contained saturated and unsaturated fatty acids in this experiment, based on your descriptions of the fats in the Petri dishes.

NAME______________________________CLASS_________________PERIOD____

Freaky Fats

Fill in the blank spaces with the appropriate terms to complete the sentences. Solve the hidden message by entering the boxed letters in the spaces at the bottom of the page.

1. __ __ __ __ __   __ [__] __ __ __
   are long chains of carbon and hydrogen atoms that combine with glycerol molecules to form a lipid.
2. [__] __ __ __
   extracted from olives, canola, and corn are plant lipids and are liquid at room temperature.
3. __ __ [__] __ __ __ __ __
   is a phospholipid emulsifier found in eggs and soybean oil.
4. __ __ __ __ __   __ [__] __ __ __
   are found between the fatty acids and the glycerol molecules in triacylglycerols.
5. __ __ __ __ __ __ [__] __ __ __
   is a crystalline form of a solid fat.
6. __ __ [__] __ __ __ __ __ __ __ __
   are used to prevent the cocoa butter from separating out of chocolate bars.
7. __ __ __ __ __ __ __ __ [__] __ __ __ __ __ __
   fatty acids have multiple double bonds.
8. __ __ __ __ __ __ [__] __
   molecules form the backbone of attachment for fatty acid molecules.
9. __ __ [__] __ __ __
   fat is saturated because it contains all single bonds and is solid at room temperature.
10. __ __ __ __ __ __ __ [__] __
    oils like corn and soybean are important food sources of polyunsaturated fatty acids in our food supply.

HIDDEN MESSAGE:
In the United States, this once popular oil is no longer used by the food industry as an ingredient because it was reported to increase blood cholesterol level. It was used primarily in cookies, cakes, and snack foods.

__ __ __ __ __ __ __   __ __ __

Institute of Food Technologists, IFT Experiments in Food Science Series

Solution for Freaky Fats

1. FATTY ACIDS
2. OILS
3. LECITHIN
4. ESTER BONDS
5. SHORTENING
6. EMULSIFIERS
7. POLYUNSATURATED
8. GLYCEROL
9. ANIMAL
10. VEGETABLE

HIDDEN MESSAGE: COCONUT OIL

NAME_____________________________CLASS________________PERIOD____

Phats

Find the words listed below in the word search. After all the words are found, the letters that are not used reveal a hidden message at the bottom of this sheet.

S	E	A	T	A	D	L	P	V	A	R	T	I	E	T
L	T	Y	O	F	I	A	O	F	R	U	R	I	E	T
S	I	E	V	E	C	T	L	G	E	T	I	A	S	B
L	S	P	A	E	A	S	Y	S	A	N	A	D	T	G
S	R	T	I	R	C	Y	U	A	I	N	C	S	E	D
O	I	A	N	D	I	R	N	I	L	Y	Y	E	R	F
F	R	C	D	A	E	C	S	Z	L	Y	L	M	B	S
X	A	G	E	A	L	S	A	N	G	M	G	U	O	E
S	S	I	A	L	O	P	T	C	L	U	L	L	N	A
N	Z	Y	N	N	N	A	U	U	I	Z	Y	S	D	F
S	T	A	E	M	I	I	R	A	N	D	C	I	R	O
Q	A	C	L	F	L	C	A	E	I	V	E	F	A	O
S	D	I	C	A	Y	T	T	A	F	B	R	I	W	D
A	I	O	R	L	I	O	E	V	I	L	O	E	F	L
V	F	D	I	K	E	K	D	B	L	W	L	R	K	C

CIS	CRYSTAL	EMULSIFIER	ESTER BOND
FATTY ACIDS	LARD	LINOLEIC ACID	LIPID
MEATS	OLIVE OIL	ORGANIC	POLYUNSATURATED
PLANTS	TRIACYLGLYCEROL	SEAFOOD	STEARIC ACID

HIDDEN MESSAGE: We should __ __ __ __ __ __ __ __ __ __ __ __ __ __ __
__ __ __ __ __ __, __
__ __ __ __ __.

Solution to Phats

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

(Over, Down, Direction)

CIS (3, 7, NW)
EMULSIFIER (13, 6, S)
FATTY ACIDS (10, 13, W)
LINOLEIC ACID (6, 12, N)
MEATS (5, 11, W)
ORGANIC (1, 6, SE)
POLYUNSATURATED (8, 1, S)
STEARIC ACID (1, 1, SE)

CRYSTAL (7, 7, N)
ESTER BOND (14, 2, S)
LARD (6, 12, SW)
LIPID (1, 2, SE)
OLIVE OIL (12, 14, W)
PLANTS (7, 9, NW)
SEAFOOD (15, 7, S)
TRIACYLGLYCEROL (12, 1, S)

HIDDEN MESSAGE:

We should eat a variety of fruits, vegetables, and grains daily.

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.

4. How did the biuret test indicate the presence of proteins?

There was a color change from blue to a purple-violet in the presence of proteins. There was no color change if proteins were not present.

Web site for more information on proteins

www.talksoy.com - United Soybean Board. Contains information pertaining to soy protein, phytochemicals, isoflavones (technical information), and the effects of soy products on human health.

Student Activity Guide

Proteins

Body builders and football players eat a lot of protein (eggs, cheese, and meat) to build muscle mass. You have probably seen protein-enriched drinks and protein-enriched foods (power bars) at the supermarket.

Proteins are the most complex and important group of molecules because they possess diverse functionality to support life. Every cell that makes up plants and animals requires proteins for structure and function. Your body and plants also have enzymes. These specialized proteins catalyze chemical reactions that are necessary for metabolism and cell reproduction. Your muscles are made from a variety of proteins, and these proteins allow your muscles to contract, facilitating movement. Other types of proteins in your body are the **peptide hormones**; insulin and glucagon are two common examples.

Proteins are complex polymers composed of **amino acids**. Amino acids contain carbon, hydrogen, nitrogen, and sometimes sulfur and serve as the monomers for making 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 (see **Figure 1A**). This carbon atom also has a side chain (an "R" group). This side chain can be as simple as an -H or a $-CH_3$, or even a benzene group.

The R groups on an amino acid are analogous to an athlete's clothing and sports equipment. By changing clothing or equipment, an athlete can become more effective as a soccer, football, or baseball player. Although this person is still an athlete, the change can make the athlete more effective in a particular activity or function. The same is true with amino acids. They are still amino acids regardless of the attached R group, but different R groups produce different functions and different properties.

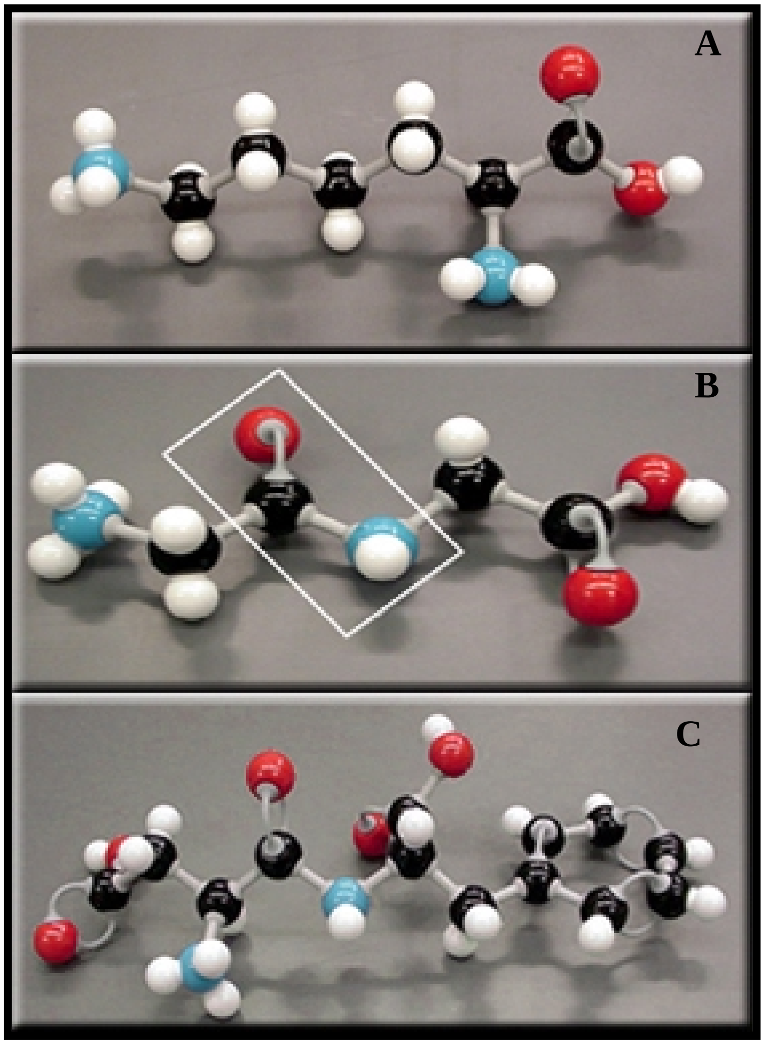


Figure 1

A. Lysine is an essential amino acid (notice the blue nitrogen atom and the carboxyl group).

B. Glycylglycine is a dipeptide containing two glycine molecules connected by a peptide bond.

C. Aspartame is a dipeptide containing aspartic acid and phenylalanine.

There are twenty amino acids found in the body. Eight of these amino acids are essential for adults and children, and nine are essential for infants. Essential means that we cannot synthesize them in adequate quantities for growth and repair of our bodies, and therefore, must be included in the diet.

Amino acids are linked together by a **peptide bond** in which the carboxyl carbon of one amino acid forms a covalent bond with the amino nitrogen of the other amino acid (see **Figure 1B**). Short chains of amino acids are called **peptides**. Longer chains of amino acids are called **polypeptides.** Although the term polypeptides should include proteins, chains with less than 100 amino acid residues are considered to be polypeptides, while those with 100 or more amino acid residues are considered to be proteins.

Many of the major hormones in the body are peptides. These hormones can influence enzyme action, metabolism, and physiology. Insulin, which is given to a person with a specific type of diabetes, is an example of a peptide hormone. Certain antibiotics and a few anti-tumor agents are also peptides. The artificial sweetener aspartame (*Equal*®) is a dipeptide composed of aspartic acid and phenylalanine with a methyl group attached at the carboxyl terminal group (L-aspartyl-L-phenylalanine methyl ester) (see **Figure 1C**).

The sequence of amino acid residues in a polypeptide chain is critical for biological function. For example, a genetic disease (mutation of a single base pair in DNA) called sickle cell anemia is caused by the substitution of one amino acid (glutamate) with another (valine) in a structural protein called beta-globulin, which is a part of hemoglobin in red blood cells. Hence, a single structural change resulted in a dramatic alteration in physiological function. The ability of an enzyme to catalyze a particular reaction depends on its specific shape. It's a lot like a key and lock—if the key is broken or in a different shape, it won't open the lock. The receptor sites on cell surfaces must be in a specific shape for polypeptide hormones to interact with the cell. With twenty different amino acids and each polypeptide consisting of hundreds of amino acids, it is no wonder that proteins play such a variety of roles in the human body.

Chemistry of Proteins

The protein backbone is formed from the peptide bonds created from the amino and carboxyl groups of each monomer that repeat the pattern -N-C-C- or C-C-N-. The number and sequence of amino acids in a polypeptide chain is referred to as the **primary structure** of a protein. The free amino group and carboxyl group on opposite ends of a polypeptide chain allow proteins to act as pH buffers (resist changes in pH) inside the cell. The amino group (NH_2) accepts a proton and becomes (NH_3^+), and the carboxyl group (COOH) donates a proton and becomes dissociated (COO^-).

As noted previously, each amino acid residue in the polymer may have a different side chain or chemical group attached to it, such as hydroxyl (OH), amino (NH_2), aromatic ring (conjugate rings such as the phenol ring in phenylalanine), sulfhydryl (SH), carboxyl (COOH), or various alkyl (CH_n). This variety of side chain groups on the polymer backbone gives proteins remarkable chemical and physical properties. For example, carboxylate groups can function as carboxylic acids (COO^-), or amino groups can behave as bases (NH_3^+). This allows protein polymers to be multifunctional molecules, with both acidic and basic behavior at the same time! Additionally, the presence of hydroxyls, carboxylates, sulfhydryls, and amino groups allows hydrogen bonding, and the alkyl groups provide hydrophobic interactions, both within the protein polymer itself and between separate protein molecules.

In the case of macromolecules, such as proteins, the polymeric structure of the macromolecule allows it to simultaneously carry many different charges (on different amino acid residues). However, unlike the small single molecules, the amino acid residues are constrained by linear peptide linkages and thus cannot move freely to randomly associate with other charged molecules. Assuming that charged residues will seek to bond with the nearest convenient counter ion, it is most likely that oppositely charged amino acid residues located at different points within a single protein chain will bond. These structural differences result in the folding of proteins into a **three-dimensional structure**, which is, in part, responsible for their functional properties as biocatalysts, structural materials, muscles, and chemical receptors. Proteins can be shaped as long flat sheets or in globular spheres. This leads to the names fibrous or globular for **protein shapes**. Most enzymes are globular proteins.

In standard acid–base chemistry, students learn that molecules carry electrostatic charges based on the type of atoms that make up a molecule and the environment of the molecule. Given that opposite charges attract, cationic and anionic atoms can combine to form covalent bonds, in which electrons are shared between atomic orbitals, or form ionic bonds, in which only electrostatic attractions exist. In solution with smaller molecules, such as HCl (an acid) or NaOH (a base), protein molecules can freely move around and associate with each other on a more-or-less random basis.

Protein polymers extend the simple acid–base charged chemical species concepts to explain how biological systems have greater levels of complexity and can utilize simple, monomeric chemical structures (like amino acids) to create exquisitely complex biological structures like antibodies, muscle, and skin. Protein polymers have physical structure, even when dissolved in liquids. The charged and hydrophobic residues within a protein tend to associate, causing the protein to fold up. When you unfold the protein molecule (called **denaturation**), its charged residues can reassociate with other charged molecules (**precipitation or coagulation**). Protein precipitation is widely used to recover recombinant protein products, enzymes, or in the production of many common foods. Cheeses and soybean tofu are examples of coagulated protein food products.

Enzymes

Enzymes are protein polymers that possess the ability to specifically "recognize" biological molecules, bind to them, and catalyze a chemical reaction. In contrast to non-protein catalysts, enzymes are specific catalysts—they usually react with only one **substrate**. Since all biochemical reactions are enzyme catalyzed, many different enzymes must exist. An *Escherichia coli* bacterium, one of the simplest biological organisms, has more than 1,000 different enzymes working at various times to catalyze the reactions necessary to sustain life of the bacterium.

The complex molecules that are contained in food provide the energy needed by living organisms to carry out all life functions. These molecules are not useful to the organism unless they are first broken down into smaller, simpler forms through **digestion**. Digestion involves the hydrolysis (breakdown) of proteins to amino acids, starches to monosaccharides, and fats to fatty acids and glycerol. Unfortunately, hydrolysis at body temperature occurs at a rate that is too slow to be useful to the organism. To speed up (catalyze) the hydrolysis reaction, living organisms produce and use enzymes.

Carbohydrate digestion begins in the mouth with an enzyme called salivary amylase. This enzyme is an **alpha-amylase** whose function is to reduce starch, a complex carbohydrate, to simple sugars. Starch is initially reduced to maltose and then to glucose. The glucose is absorbed by the intestines and used to supply energy for the body.

Food Uses of Proteins

Proteins also serve important roles in the processing of food products. They are used for their thickening, gelling, emulsifying, and water-binding properties in meats (sausages), bakery products, cheese, desserts, and salad dressings. Proteins are used for their cohesive and adhesive properties in sausage making, pasta, and baked goods. Egg proteins are used for their foaming properties in desserts, cakes, and whipped toppings. Milk, egg, and cereal proteins are used as fat and flavor binders in low-fat bakery products. Proteins are used for texture and palatability in bakery products (breads, cakes, crackers, and pizza crust) and sausages.

Milk protein consists of 80% casein and 20% whey proteins. 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^{o}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 the 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 the 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.

Activity Objective

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

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

Experimental Procedure

Part 1. Precipitation of casein from milk with an acid (vinegar)

1. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of milk in the beaker. Record the weight of the milk in the data table (weight of beaker with milk – weight of beaker = weight of milk).
2. Place the beaker with the milk on a hot plate. Heat the milk to 21^{o}C (70^{o}F). Turn off the hot plate and remove the beaker.
3. Add 11 milliliters (2 teaspoons) of vinegar to the warm milk and stir for 2 minutes, then allow the milk to sit for 5 minutes. The casein will precipitate into heavy white curds.
4. Cut out a piece (2–3 layers) of cheesecloth large enough to cover the top and 2 inches down the sides of a beaker. Using the rubber band, fasten the cheesecloth over the top of the beaker. Pour the curdled milk into the beaker, collecting the curds (casein) in the cheesecloth and allowing the vinegar and whey to drain off into the bottom of the beaker.
5. Gather up the cheesecloth with the casein and rinse in cool water by dipping into another beaker containing water.
6. Squeeze the casein until almost dry, then spread out the cheesecloth to let the casein dry for 5 minutes.
7. Weigh the precipitate. (Do not weigh the cheesecloth with the precipitate). Record your results.

Part 2. Enzymatic coagulation of the casein from milk with rennet

1. Place 1/2 of a crushed rennet tablet into a beaker. To crush the tablet, place it between two pieces of foil and hit the tablet with a hammer.
2. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of milk in the beaker. Record the weight of the milk in the data table (weight of beaker with milk – weight of beaker = weight of milk).
3. Place the beaker with the milk on a hot plate. Heat the milk to 43°C (110°F). Pour the hot milk over the rennet tablet, stir for 2 minutes, and allow the milk to sit on the lab bench for 5 minutes.
4. Collect the curds by pouring the curds and liquid into a beaker covered with cheesecloth (2–3 layers) (see step 4 in Part 1). Gather up the cheesecloth and squeeze out the liquid whey from the curds. Spread out the cheesecloth to allow the curds to dry for 5 minutes.
5. Weigh the curds. (Do not weigh the cheesecloth with the curd.) Record your results.

Variations:
Test the effect of low temperature on the activity of rennet. Repeat the experiment with cold milk at 4°C (40°F). Record your results.

Test the effect of high temperatures on the activity of rennet. Repeat the experiment with hot milk heated to 70°C (160°F). Record your results.

Part 3. Coagulation of protein from soymilk using a salt (magnesium sulfate)

1. Weigh the empty beaker and record the weight. Weigh and record the weight of 120 milliliters (1/2 cup) of soymilk in the beaker. Record the weight of the soymilk in the data table (weight of beaker with soymilk – weight of beaker = weight of soymilk).
2. Place the beaker with the soymilk on a hot plate.
3. Bring the soymilk to a boil and turn off the heat. Monitor this step closely; do not allow the soymilk to boil over the top of the beaker.
4. Add 1.6 grams (1/4 teaspoon) of Epsom salt (magnesium sulfate, a mineral salt) to the hot soymilk and stir.
5. Wait until the curds are floating in an almost clear liquid.
6. Fasten a piece (2–3 layers) of cheesecloth over the top of a beaker with a rubber band. Pour the soy curds and liquid into the beaker, collecting the curds in the cheesecloth and allowing the liquid to drain into the bottom of the beaker.
7. Gather up the cheesecloth and squeeze out as much water as possible. Spread out the cheesecloth to allow the curds to dry for 5 minutes.
8. Weigh the curds. (Do not weigh the cheesecloth with the curd.) Record your results.

Variations:
Test to see if rennet can coagulate soy protein. Add 1/2 rennet tablet to 120 milliliters (1/2 cup) of warm soymilk (43°C). Record your results.

Test to see if Epsom salt can coagulate casein. Add 1.6 grams (1/4 teaspoon) of Epsom salt to 120 milliliters (1/2 cup) of boiled milk. Record your results.

DATA TABLE – MILK AND SOYMILK CURDS

	Weight of milk/soymilk	**Weight of curd**	**Describe the curd (color, texture)**
Milk + acid			
Milk + rennet			
Soymilk + Epsom salt			

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

Biuret Test
This test is used to indicate the presence of proteins.

Place a pea-size sample of the milk and soymilk curds on a Petri dish. Place 1 milliliter of sodium hydroxide on each curd. Add 5 drops of copper sulfate to each curd. Record the color of the reagent and whether your results are positive or negative.

Repeat the procedure with a potato chip, raw potato, or piece of bread.

DATA TABLE – BIURET TEST ON FOODS

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

Questions

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

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

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?

4. How did the biuret test indicate the presence of proteins?

NAME________________________CLASS_________________PERIOD_______

Powerful Proteins

Fill in the blank spaces with the appropriate terms to complete the sentences. Solve the hidden message by entering the boxed letters in the spaces at the bottom of the page.

1. __ [__] __ __ __ __ __ __
are short chains of amino acids.

2. __ __ [__] __ __ __ __ __
of amino acids make up protein molecules.

3. __ [__] __ __ __   __ __ __ __ __
are the building blocks of proteins.

4. __ __ __ __ __ __ [__] __
can influence enzyme action, metabolism, and physiology.

5. [__] __ __ __ __ __
is a proteolytic enzyme that is used to make cheese.

6. __ __ [__] __ __ __
is a milk protein.

7. __ __ [__] __ __ __ __ __ __
involves the hydrolysis of proteins to amino acids.

8. __ __ __ [__] __ __ __
is a protein enzyme that breaks down starch in the mouth.

9. __ __ __ __ [__] __ __ __ __ __ __ __
is the transformation of a liquid into a soft semi-solid or solid mass.

10. [__] __ __
proteins are used for their foaming properties in desserts, cakes, and whipped toppings.

HIDDEN MESSAGE:

Elementary school children frequently use this casein-based adhesive that was introduced by *Borden*® over 50 years ago. Hint: There is a picture of Elsie the cow on the container.

__ __ __ __ __'__   __ __ __ __

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

*NAME*______________________________*CLASS*_________________*PERIOD*____

Puzzling Proteins

Find the words listed below in the word search. After all the words are found, the letters that are not used reveal a hidden message at the bottom of this sheet.

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
O	G	I	N	L	T	O	K	O	W	P	D	N	E	N
C	T	N	W	U	U	Z	G	T	J	I	I	I	N	S
R	C	G	R	G	Y	W	C	E	R	T	T	N	N	Z
M	P	E	S	A	V	D	N	I	N	A	P	Y	O	U
H	O	R	M	O	N	E	S	N	N	T	E	Z	E	E
Y	M	U	S	C	L	E	R	S	J	I	P	R	N	V
A	M	I	N	O	A	C	I	D	T	O	E	Z	I	F
E	N	I	S	Y	L	E	I	O	Z	N	Y	S	F	P
C	T	W	X	O	O	H	F	K	C	M	F	X	A	K
B	R	K	E	X	V	U	F	G	E	M	C	U	S	C

AMINO ACID	CASEIN	CHEESE	COAGULATION
DENATURE	ENZYME	HORMONES	INSULIN
LYSINE	MUSCLE	NITROGEN	PEPTIDE BOND
PRECIPITATION	PROTEIN	RENNIN	TOFU

HIDDEN MESSAGE: You should

— — — —   — — — — — — — — — — —   — — — — — — —

— — — — — — — — — — —   — — — —   — — — —!

Institute of Food Technologists, IFT Experiments in Food Science Series

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	+	+
+	+	E	+	A	+	+	+	I	N	A	P	+	+	+
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

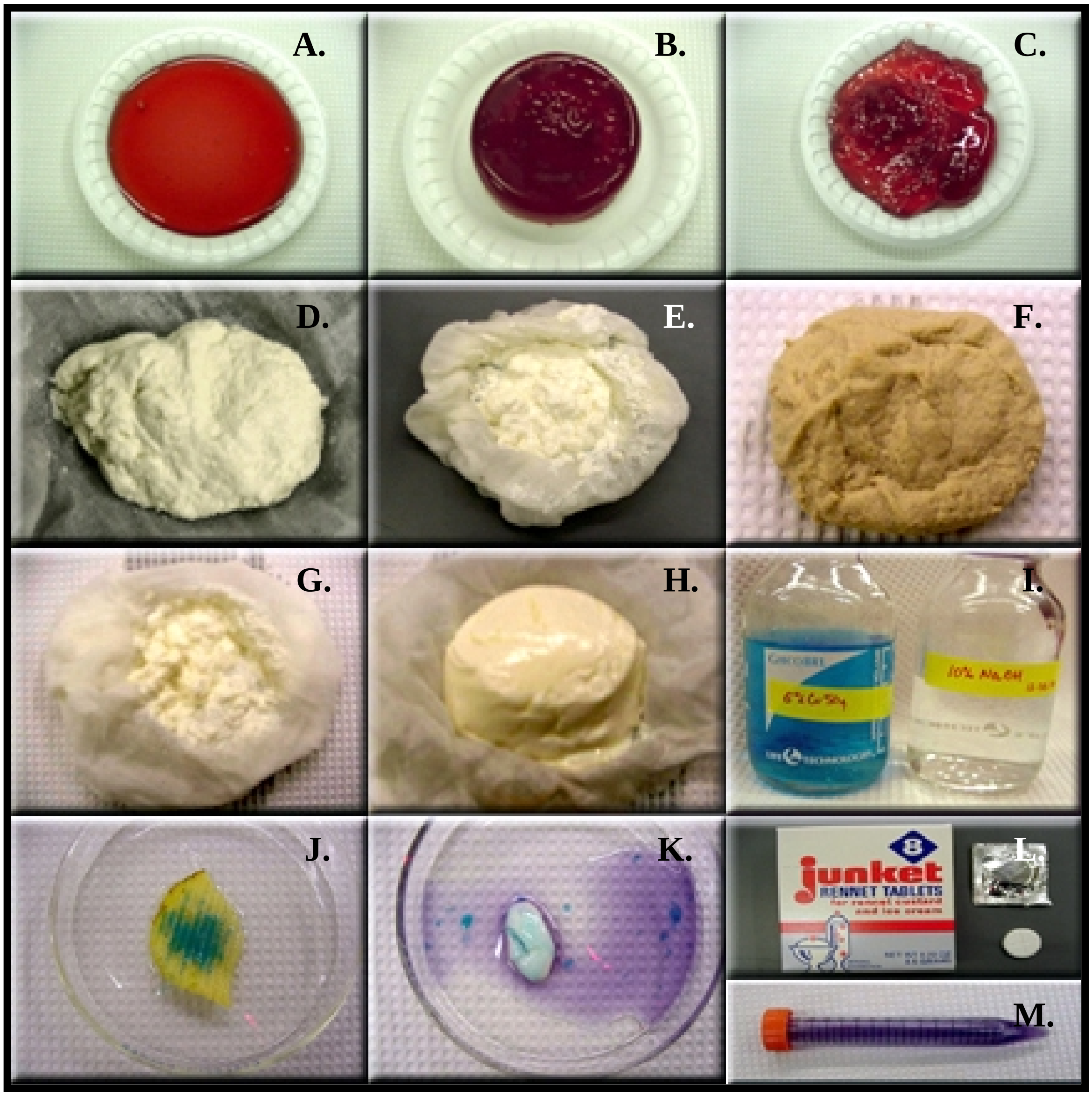
(Over, Down, Direction)

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

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

APPENDICES Teacher's Supplement

Pictures Illustrating Experimental Outcomes



Unit 1. Carbohydrates. **A. Normal jelly. B. Jelly made with half the amount of sugar. C. Jelly made with twice the amount of sugar.**

Unit 3. Proteins. **D. Milk + Acid. E. Milk + Rennet. F. Soymilk + Epsom Salt. G. Dry Milk + Acid. H. Dry Milk + Rennet. I. Reagents for biuret test. J. Biuret test on potato chip (negative reaction). K. Biuret test on milk curd (positive reaction). L. *Junket.* M. Biuret test on whey (positive reaction).**

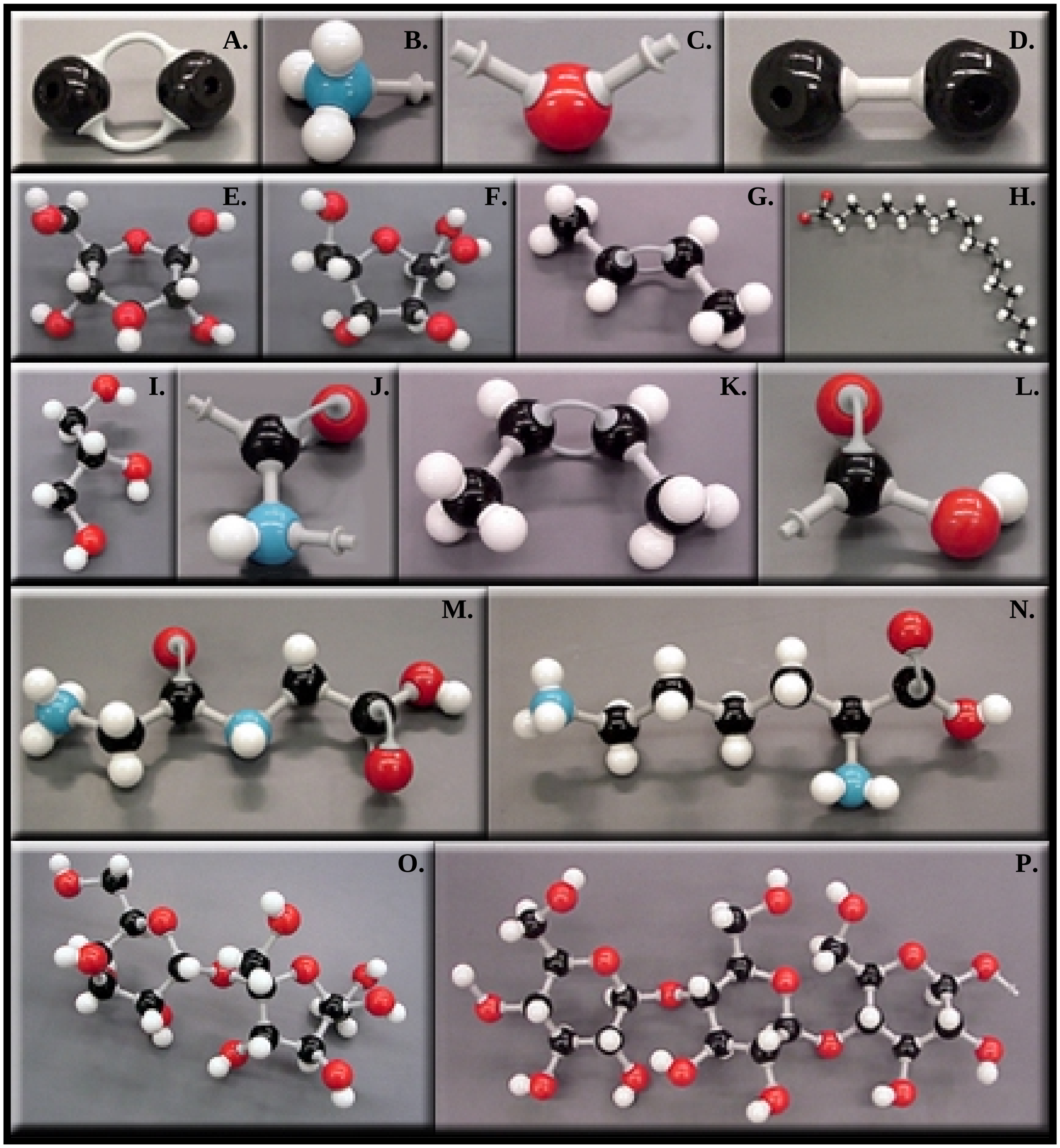
NAME________________________CLASS________________PERIOD________

MOLECULAR MATCHING

Write the letter from the correct structure on the following page with the correct name on this page.

MATCHING

1. Glucose _________
2. Glycerol _________
3. Fructose _________
4. Lysine _________
5. Linoleic acid _________
6. Sucrose _________
7. Starch _________
8. Peptide linkage _________
9. Carboxyl group _________
10. Amino group _________
11. Glycosidic linkage _________
12. Cis double bond _________
13. Single bond _________
14. Trans double bond _________
15. Double bond _________


A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.
M.
N.
O.
P.