Dolan
DNA Learning Center
www.dnalc.org

# Industrial Enzymology:

## Juice Production

**Background**

Most of the 100 trillion cells in the human body (except mature red blood cells and reproductive cells) contain the entire human genome--all the genetic information necessary to build a human being. This information is encoded in 3.2 billion base pairs, which are subunits of DNA. Inside the cell nucleus, 2 meters (about 6 feet) of DNA are packaged into 23 pairs of chromosomes (one chromosome in each pair is inherited from each parent). Human cells have 46 chromosomes that contain the DNA for approximately 30,000 individual genes (the units of heredity). Each gene is a segment of double-stranded DNA that holds the recipe (or code) for making a specific molecule, usually a protein.

In eukaryotes, DNA never leaves the nucleus. For the DNA instructions to reach the rest of the cell, an enzyme called RNA polymerase must make an RNA copy of a gene, called mRNA (messenger RNA). The mRNA copy leaves the nucleus and travels to a ribosome where the message is read. The ribosome then makes the protein coded for by that gene. To make a strand of RNA, the DNA first unzips, or pulls apart. RNA polymerase, an enzyme, builds an RNA strand complementary to one half of the unzipped DNA. For every C in the DNA strand the RNA polymerase inserts a G; for every G a C; for every T an A. For every A, RNA polymerase inserts a U, or Uracil. RNA does not contain Thymine. The ribosome "reads" the message 3 bases at a time. Each combination of bases letters is called a "codon." For example, "UCG" is an mRNA codon. Codons code for amino acids, the building blocks of proteins. The UCG codon codes for the amino acid Serine (Ser). Transfer RNA (tRNA) molecules attach and deliver the proper amino acids to the ribosome to create a long chain. This chain of amino acids folds up to form a protein.

**Proteins**

There are four main types of proteins: structural proteins, transport proteins, messenger proteins and enzymes. Structural proteins provide our bodies with support. Examples include actin and myosin, which make up much of our muscle tissue, and keratin, which is found in both hair and fingernails. Transport proteins carry molecules throughout our bodies. One example includes hemoglobin, the protein in red blood cells that carries oxygen. Messenger proteins allow cells in different parts of the body to communicate. Some hormones, such as insulin and human growth hormone (HGH) are messenger proteins.

Enzymes act as the construction workers of the protein world. They can build molecules as well as break them apart by catalyzing (speeding up) chemical reactions. The molecule that a given enzyme breaks down is called its substrate. Like a lock and key, enzymes will only react with substrates that fit their shape correctly. Once the substrate is broken apart or digested, the enzyme is free to catalyze further chemical reactions. Unless the shape of the enzyme is altered, it can catalyze reactions indefinitely. The process that changes the shape of an enzyme is called denaturing. When an enzyme is denatured, it usually cannot alter its shape to function again. Some factors that can cause denaturing include changes in acid levels or pH and temperature fluctuations.

## Human Digestive Enzymes

Enzymes are produced throughout the digestive system. Each digestive enzyme targets a specific substrate. For example, lipases target fats, amylases target starch, and proteases target proteins.

Most of the chemical digestion of food occurs in the small intestine by enzymes produced in the pancreas or in the intestine itself. **Lactase** enzyme is secreted in the lining of the small intestine. Individuals who are lactose intolerant have a lactase deficiency, and as a result, the dairy sugar lactose doesn't get digested. Normally, lactose is digested into its two constituent ingredients, glucose and galactose. When lactose isn't digested, it can cause abdominal discomfort and bloating, vomiting and or diarrhea. This enzyme deficiency can be treated through supplementation. Individuals who are lactose intolerant can take a pill containing the enzyme when they eat to aid in the digestion of the dairy sugar lactose. Individuals can also purchase lactose reduced dairy products, such as milk or ice cream. Harnessing the reusable nature of enzymes, with very small amounts of lactase large quantities of lactose free milk can be produced.

Surprisingly, it is quite normal for lactase levels to decrease with age. Lactose intolerance is actually an ancestral trait. As mammals, the natural production of this enzyme is only necessary in infancy when a baby nurses from its mother. After this stage, most mammals discontinue ingesting lactose, and the enzyme production drops and/or stops. Naturally, because humans are mammals, this happens to us too! In many of us though, lactase production continues beyond infancy thanks to our nomadic ancestors. When humans began to collect and drink milk from other animals, lactase production began to change.

**Catalase** is another example of a human digestive enzyme. It is produced in the liver. As blood is filtered through the organ, catalase digests toxic hydrogen peroxide that is present in low levels as a byproduct of cellular respiration. The products of this chemical reaction are water and oxygen. This reaction can easily be reproduced in the classroom, but with calf's liver instead of human liver! There is a visible reaction when liver is placed in hydrogen peroxide. Violent bubbling is a sign that gas is being released, and an increase in temperature is a direct indicator of an exothermic reaction taking place.

## Enzymes in Industry

In cheese production, enzymes are used to separate the curds and whey in milk. Cheese is the solid portion of milk (curd) that has been separated from the liquid portion (whey). The cheese-making process was actually discovered by our nomadic ancestors. They would travel great distances, carrying liquids in the stomachs of dead animals. When milk is placed in a stomach the enzymes of the stomach cause the milk to curdle, thus producing clumps of curds or cheese. By combining the enzyme **emporase** with milk, this chemical reaction can easily be observed.

Enzymes are also used in the production of juice. In the flesh of an apple, apple juice is stored in tiny particles surrounded by walls of pectin. Pectin is a sugar molecule, and must be destroyed to release the juice trapped within. The enzyme **pectinase** is used to perform this chemical reaction in juice factories today. Years ago, apples would be pressed and crushed to extract their juices. Today, with the help of pectinase, juice factories are able to increase their yield.

**Enzymes and Health**

The absence of a single enzyme can have a drastic effect on an individual's health. For example, a single enzyme deficiency in white blood cells can lead to immune disorders such as SCIDS (Severe Combined Immunodeficiency Syndrome) also known as the "boy in the bubble" disease. Sadly, David Vetter, the young boy for whom the disease was nicknamed passed away following a bone marrow transplant at the age of 11. Since his passing, 2 young girls suffering from the same disorder have been successfully treated with gene therapy. Their white blood cells were genetically engineered and transfused back into their bodies, bringing with them a trait they didn't have before: the ability to fight infection!

## Description of Activity

This one-hour laboratory provides children in grades 5-8 with the opportunity to simulate an enzymatic reaction often used in food production. Using the enzyme pectinase, students will be breaking down the pectin in the cell wall of fruit and releasing the juice inside. After experimentation, conclusions can be drawn about how enzymes can be used in the production of foods.

## Learning Outcomes

Students will:

- discuss the relationship between gene, proteins and enzymes.
- observe an enzymatically catalyzed chemical reaction.
- define the terms chemical reaction and catalyze.
- compare experimental results with and without using enzymes.
- understand what an experimental "control" is.

## Assumptions of Prior Knowledge

Students should be familiar with the structure and function of DNA and understand the relationship between genes and proteins.

## Misconceptions

Students often associate enzymes primarily with the digestion of food, but do not realize that enzymes play a much larger role in the world around them.

## Lesson

### Materials and Equipment

1 large jar of applesauce
1 package of clear plastic disposable cups
1 package of small disposable plastic plates
2 large graduated cylinders
40 popsicle sticks
40 coffee filters
20 droppers
1 bottle of Pectinase enzyme (100ml)

**Before Class**
**The day before:**

- Aliquot 1ml of enzyme for each team. Keep refrigerated.

**The day of the lab:**

- Place 2 tablespoons of applesauce onto plastic plates for each student.
- Prepare set-ups for each team consisting of 2 plastic cups, 2 coffee filters, 2 popsicle sticks and 1 dropper.

**During Class**

- Review the relationship between DNA, RNA, and proteins . Discuss the connection between proteins and traits. There are several classes of proteins, for example:
  Structural: make up physical structures (keratin in hair and nails)
  Hormones: Insulin (controls metabolism of glucose)
  Enzymes: Amylase (digests starch)
  Not all enzymes are proteins, but most are.
  Mention that the names of most enzymes end in "ase".
- Enzymes are the workhorses of the cell, and they catalyze chemical reactions.
- Define "catalyze" and "chemical reaction". A chemical reaction is when molecules are changed (chemical bonds are broken or built). In a chemical reaction there are reactants and products. The products are chemically different than the reactants. This can be demonstrated with a piece of paper. Ripping it or dissolving it in water can physically change the paper, but to change it chemically it would need to be burned, soaked in acid or maybe even eaten!
- Enzymes can catalyze chemical reactions. To catalyze is to speed up the rate of a reaction. For example, if you eat one apple and leave another out on a table, which will break down first? It could take weeks to months before the apple on the table fully decomposes. The enzymes in your digestive tract can fully digest the apple in just hours.
- Pectinase is an enzyme that breaks up pectin in the cell wall of plants. During the ripening process of some fruit, pectinase is produced to soften the cell wall. In food production this enzyme can be used to extract more juice from fruit (such as apples). Like all enzymes, pectinase has a specific substrate, or substance, with which it will react (pectin).
- Pectin is a sugar derived from the cell wall of plants. It aids in the retention of nutrients within the plant skin. Apples, cranberries and concord grapes are some examples of fruit that are high in pectin. When under acidic conditions, pectin forms a gel. This gel can be used in food processing as a thickening agent (as in jellies and jams).
- Pass out the Apples to Applesauce protocol and review the diagram exhibiting the chemical reaction. Pectinase is the enzyme and pectin is the substrate.

- Ask a student to read steps 1-3 out loud, and demonstrate each step. Discuss what an experimental control is, and emphasize that one partner will be the "control" and the other will be the "experiment". Finish demonstrating steps 4 through 6.
- It can be fun to have "experiment" students compete against "control" students to see which group collects the most juice!
- Collect all "experimental" juice in one graduated cylinder and all the "control" in the other.

**Analysis and Discussion**

Discuss the results and determine whether or not it was successful. Ask the students to determine how the volume of the two cylinders can relate to the function of the enzyme. Discuss how can this enzyme be used in the production of apple juice? Have the students determine why a "control" was necessary to understand the results of the experiment? Review enzyme facts discussed in the lesson by asking a series of questions.

## Further Explorations

**Group Presentations**

Are there other foods that can use enzymes in their production? Ask the students to research forms of food production that utilize enzymes. In a classroom setting have the students present their findings.

**Discussion**

It is very easy to move from enzymes to genetically modified foods. Discuss other ways that enzymes and genetics can be used to make food bigger, better and more abundant.

**Note: This lab can be used in conjunction with or followed up with the "From Milk to Cheese" lesson.**

**Enzymes in Molecular Biology**

Restriction enzymes are used to cut the DNA molecule, and ligase enzymes are used to glue DNA fragments together. Using the *DNA Interactive* website, investigate the use of enzymes in recombinant DNA technology, genetic engineering and DNA fingerprinting.
Go to:
www.dnai.org < Manipulation < Techniques < Cutting and Pasting to learn more about restriction enzymes.
Go to:
www.dnai.org < Manipulation < Techniques < Amplifying to learn about amplifying DNA for techniques such as DNA fingerprinting.

## Resources

**Books:**

Discovering Enzymes. Dressler, David, and Potter, Huntington. Scientific American Library, NY. 1991.
A great resource on the discovery, anatomy, physiology and evolution of enzymes.

DNA Science. Micklos, David A., Freyer, Greg A., and Crotty, David A. Cold Spring Harbor Press, NY. 2003.
A laboratory manual on the history of recombinant DNA technology.

Have a Nice DNA. Balkwill, Fran and Rolph, Mic. Cold Spring Harbor Laboratory Press, NY. 2002.
An elementary level introduction to basic cell processes such as DNA replication and protein production.

**Internet Sites:**

http://www.dnai.org/index.htm
*DNA Interactive:* a commemorative Internet site devoted to the discovery of the double helix.
A Dolan DNA Learning Center Internet site

http://www.ygyh.org/
*Your Genes, Your Health:* A multimedia guide to genetic disorders.
A Dolan DNA Learning Center Internet site

http://www.rcsb.org/pdb/
The Protein Data Bank, by the Research Collaboratory for Structural Bioinformatics.
This site contains a database of the molecular structures of proteins, including enzymes.

http://www.cyberlearn.com/ebio/messages/1787.htm
For further information on pectin and pectinase.

http://www.geo-pie.cornell.edu/crops/enzymes.html
For further information on genetic engineering and its usage in making enzymes for food production.

## Correlations

**New York State**
NYS Standard 4: Science
The Living Environment

- Organisms inherit genetic information in a variety of ways that result in continuity of structure and function between parents and offspring.

- Organisms maintain a dynamic equilibrium that sustains life.

**National**
Content Standard C: Life Sciences
Structure and Function in Living Systems

- Living systems at all levels of organization demonstrate the complementary nature of structure and function. Important levels of organization for structure and functions include cells, organs, tissues, organ systems, whole organisms and ecosystems.
- All organisms are composed of cells—the fundamental unity of life. Most organisms are single cells; other organisms, including humans, are multi-cellular.
- Cells carry on the many functions needed to sustain life. They grow and divide, thereby producing more cells. This requires that they take in nutrients, which they use to provide energy for work that cells do and to make the materials that a cell or an organism needs.
- Specialized cells perform specialized functions in a multicellular organism. Groups of specialized cells cooperate to form a tissue, such as a muscle. Different tissues are in turn grouped together to form larger functional units, called organs. Each type of cell, tissue, and organ has a distinct structure and set of functions that serve the organism as whole.
- The human organism has systems for digestion, respiration, reproduction, circulation, excretion, movement, control and coordination and for protection from disease. These systems interact with each other.

Reproduction and Heredity

- Every organism requires a set of instructions for specifying traits. Heredity is the passage of these instructions from one generation to another.

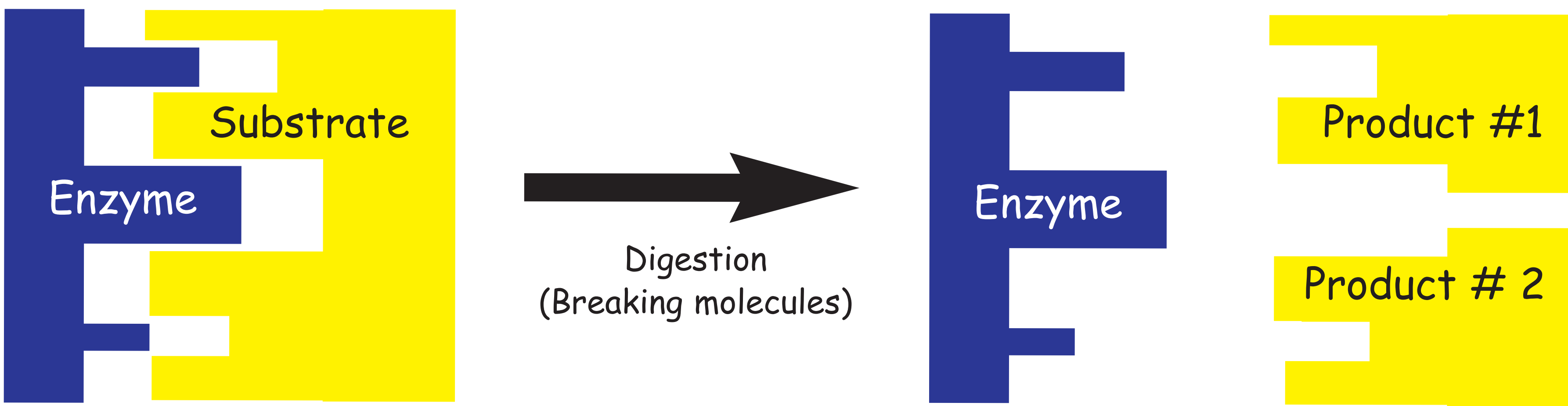
**AAAS Benchmarks**
Standard C: Cells

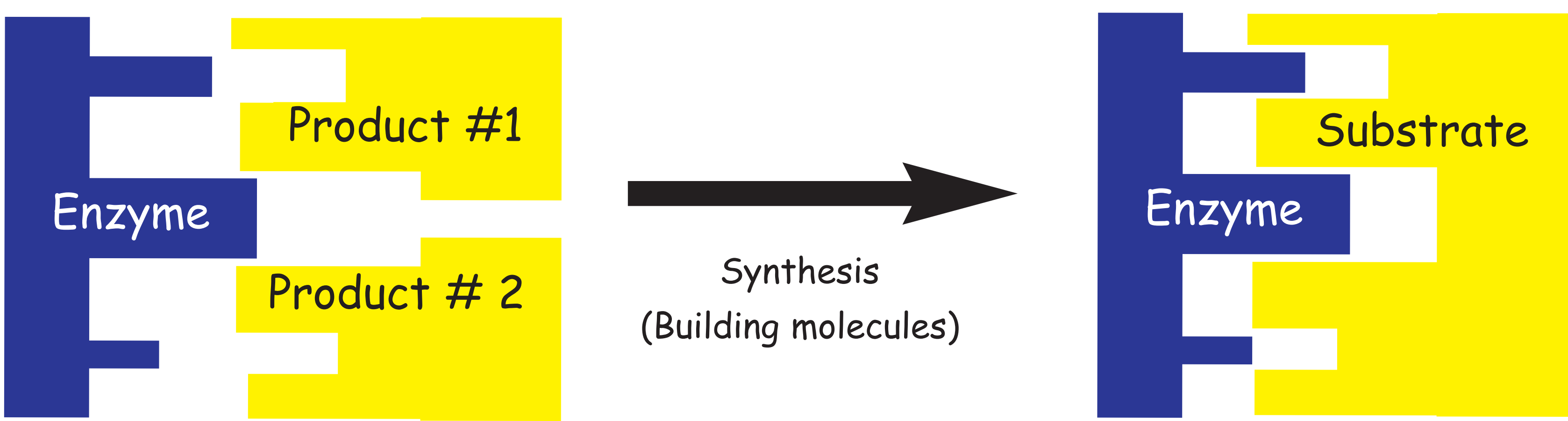
- Some living things consist of a single cell. Like familiar organisms, they need food, water and air; a way to dispose of waste; and an environment they can live in.
- Microscopes make it possible to see that living things are made mostly of cells.

Some organisms are made of a collection of similar cells that benefit from cooperating. Some organisms' cells vary greatly in appearance and perform very different roles in the organism.

# Enzymes and Substrates

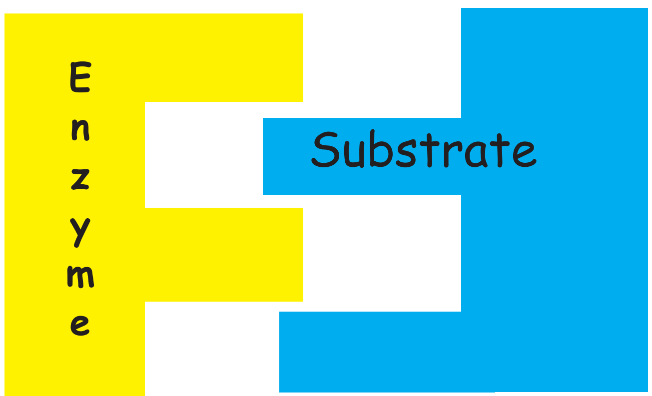
Enzymes, whether they break or build molecules, have specific substrates with which they can react. An enzyme and its substrate fit together like a lock and a key.



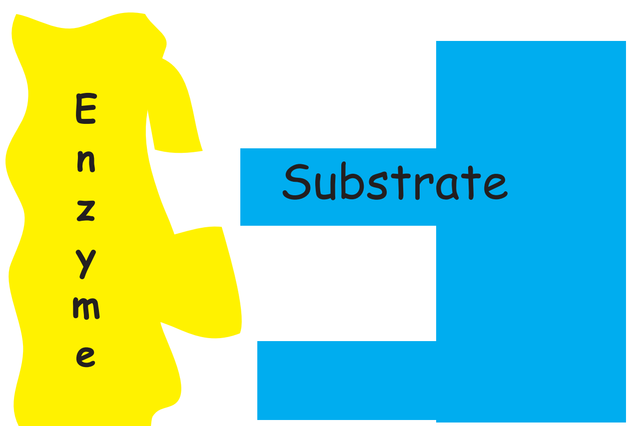



# Enzymes and Substrates

If the shape of an enzyme is altered by a change in heat or pH, it can no longer bind with it's substrate. This change in shape is called denaturation. A denatured enzyme is like a melted key, it cannot fit with its substrate, and is non-functional.




The fit of an enzyme and its specific substrate.

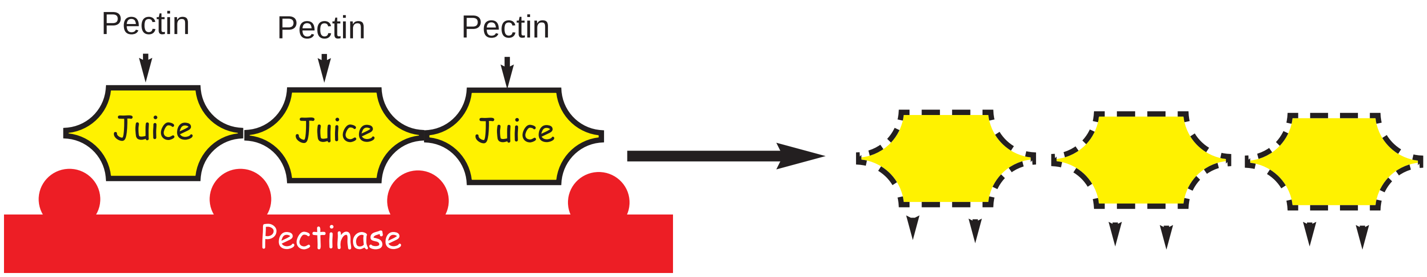



A denatured enzyme does not fit with it's substrate.

# Applesauce to Apple Juice

Millions of apples are used to make juice every year in countries around the world. In the flesh of an apple, apple juice is stored in tiny particles surrounded by walls of pectin. Pectin is a sugar molecule, and must be destroyed to release the juice trapped within. The enzyme **pectinase** is used to perform this chemical reaction in juice factories today. Years ago, apples would be pressed, or crushed to extract their juices. Which method yields more juice? In this experiment, we will find out!

**Pectinase performs the following chemical reaction:**




Apple juice is released from pectin particles.

**Procedure:**

1. Label 2 plastic plates: one **experiment** and the other **control**.

2. Place an equal amount of applesauce in each plate.

3. Add 1ml of pectinase to the experiment plate and stir. Let stand for 5 minutes. **What do you observe?**

4. Place separate coffee filters over two plastic cups. Label one cup experiment, and the other control.

5. Pour the applesauce from each plate onto the filter of the appropriate cup. Juice will pass through the filter and collect in the cup, and the fruit pulp will remain on top of the filter.

6. Measure and compare the amount of juice collected from each plate.