Enzymes: the little molecules that could

When I first began making bread, the science involved was always in the back of my mind. I had an idea of what was occurring—my diagram for the chemical reactions in dough looked something like this:

It was when I started preparing a manual for a bread-making class, however, that I really began to wonder about the details. Is the sugar for fermentation part of flour? How exactly do the yeast process this sugar? Do all the complex flavors of bread really come from one organic molecule, ethanol? Numerous trips to the university libraries helped me understand many of the relevant topics; the topic presented in this article is the enzymes involved in dough.

When I realized that flour contains a very small amount of sugar, only one to two percent, I thought, "Wait a minute, how is that possible? That's not enough to make dough rise." Then I figured out that it is the starch in flour that provides most of the sugar for fermentation, and

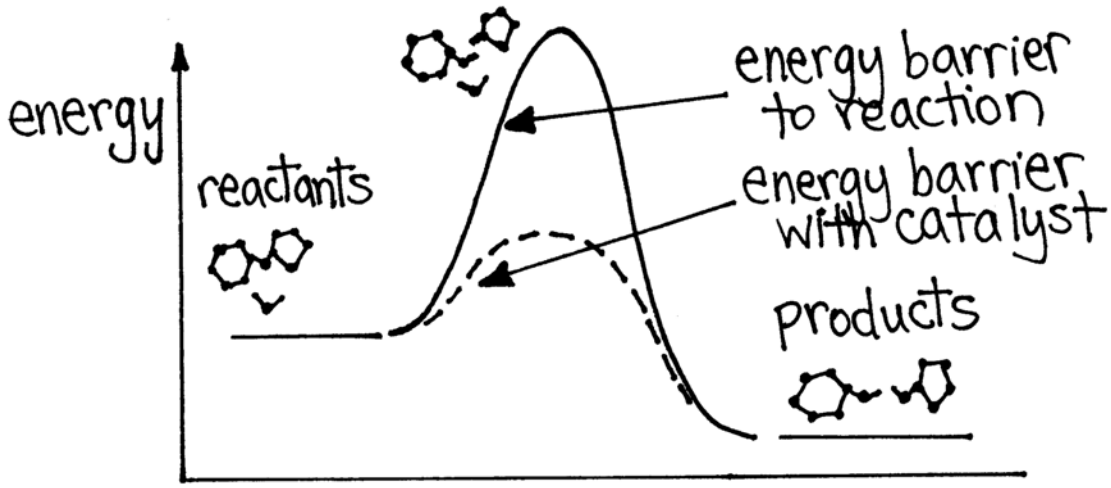
the starch must be broken down into sugar before it can be fermented. This breakdown is the work of enzymes.

An *enzyme* is defined as a large molecule, usually a protein, that catalyzes a biological reaction. This means that the enzyme speeds up the reaction by reducing whatever energy barrier is preventing the reaction from happening quickly and easily.

When two molecules bump into each other, there is a chance they will react to form new molecules. Sometimes this happens easily—the two molecules each have an unstable site, for example, and when they bump, a bond forms between the sites, creating a stable molecule. In other cases, however, bonds in the reacting molecules must break (which requires energy) before new bonds can form. The amount of energy needed to break the old bonds is the energy barrier to the reaction. This is represented by the solid line in the diagram below.

One way to increase the speed of a reaction is to heat it up. Hotter molecules move faster; they possess more energy. When two of them collide, there is a greater chance that the necessary bonds will break and reaction will occur. More molecules possess the energy needed to get over the barrier, so more reaction occurs.

The other way to speed up a reaction is to reduce the barrier, as shown by the dashed line in the diagram. When less energy is needed for the reaction, more molecules will possess enough energy to get over the barrier. Reducing the barrier is the job of ***catalysts***. They alter the situation to reduce the barrier to reaction. Enzymes are a subset of catalysts; they work on biological reactions. About 4000 reactions are known to involve enzymes, including most of the reactions that occur in the human body and several reactions in bread dough, described next.



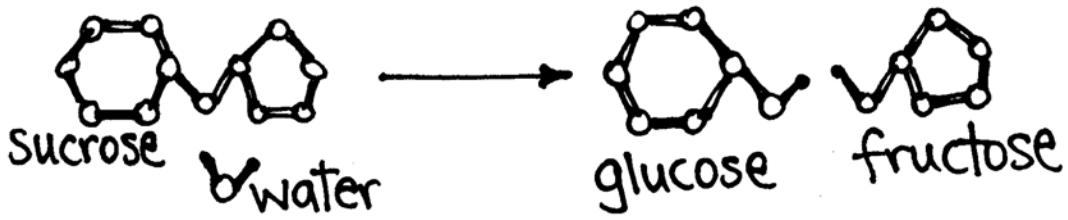
Enzymes catalyze three reactions in bread-making: breaking starch into maltose, a complex sugar; breaking complex sugars into simple sugars; and breaking protein chains. The breakages could happen without the enzymes, but the energy barrier is so large that it is very

unlikely. Essentially, the enzymes are necessary for the reaction to occur.

It is easy to start seeing enzymes as little critters that come in, recognize the site where they can work, and begin to chew on bonds or snap them in half. While this is a convenient picture, it does a disservice to the marvels of biology. Enzymes do not think or act, but still manage to arrive at the sites where they are needed. Each enzyme has a very specific job to do and only interacts with the appropriate molecules for which it is designed, ignoring all others. Enzymes work efficiently and are not used up by the process; after the reaction occurs, the original enzyme molecule is left intact and can proceed to a new site.

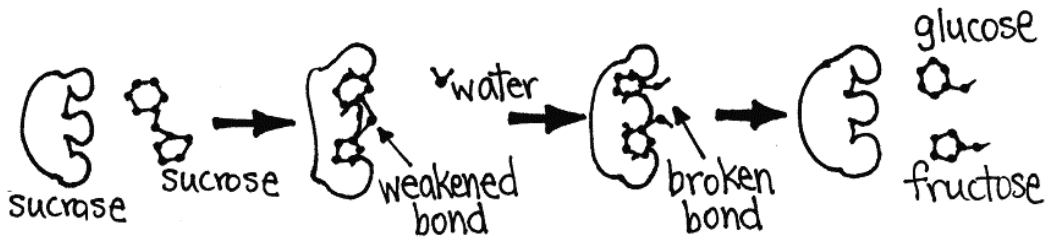
If the enzyme does not think, how does it manage to perform its specific task? The simplified picture presented in general chemistry textbooks is called the "lock and key model." An enzyme has a specific shape that fits together with the ***substrate***, the molecule on which it will be working. The enzyme bonds to the substrate with a weaker chemical bond, a hydrogen bond or hydrophobic bond, for example. It alters the substrate in a way that makes reaction favorable. Once reaction occurs, the enzyme releases the products and moves on.

For example, the substrate ***sucrose*** is a complex sugar that can react with a water molecule to form two simple sugar molecules, ***glucose*** and ***fructose***.



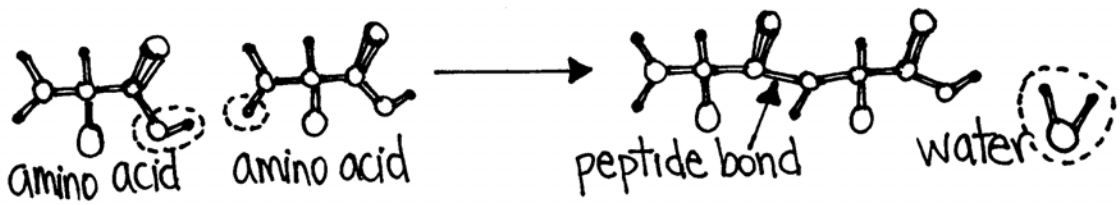
There is an energy barrier to the reaction because it takes a lot of energy to break the middle bond of the sucrose.

The enzyme ***sucrase*** fits together with the sucrose (below). In order to bond to the enzyme, the sucrose must stretch. This stretching weakens the sucrose's middle bond, which becomes susceptible to attack by water molecules. The energy barrier has been lowered. When a water molecule comes along, the middle bond easily breaks and reacts with the water molecule. The enzyme is now holding the product molecules, which it releases. Sucrose has been broken into glucose and fructose.



Another example emphasizes the bonding nature of the enzymes; they are not simply fitting into substrates like puzzle pieces, snapping into place. Bonds must form. Once bonded, the active site of the enzyme is positioned near the reaction site of the substrate, which it alters to reduce the energy barrier.

In this example, the substrate is a protein. Proteins are chains of amino acids linked by peptide bonds. When a peptide bond forms, a water molecule is released.

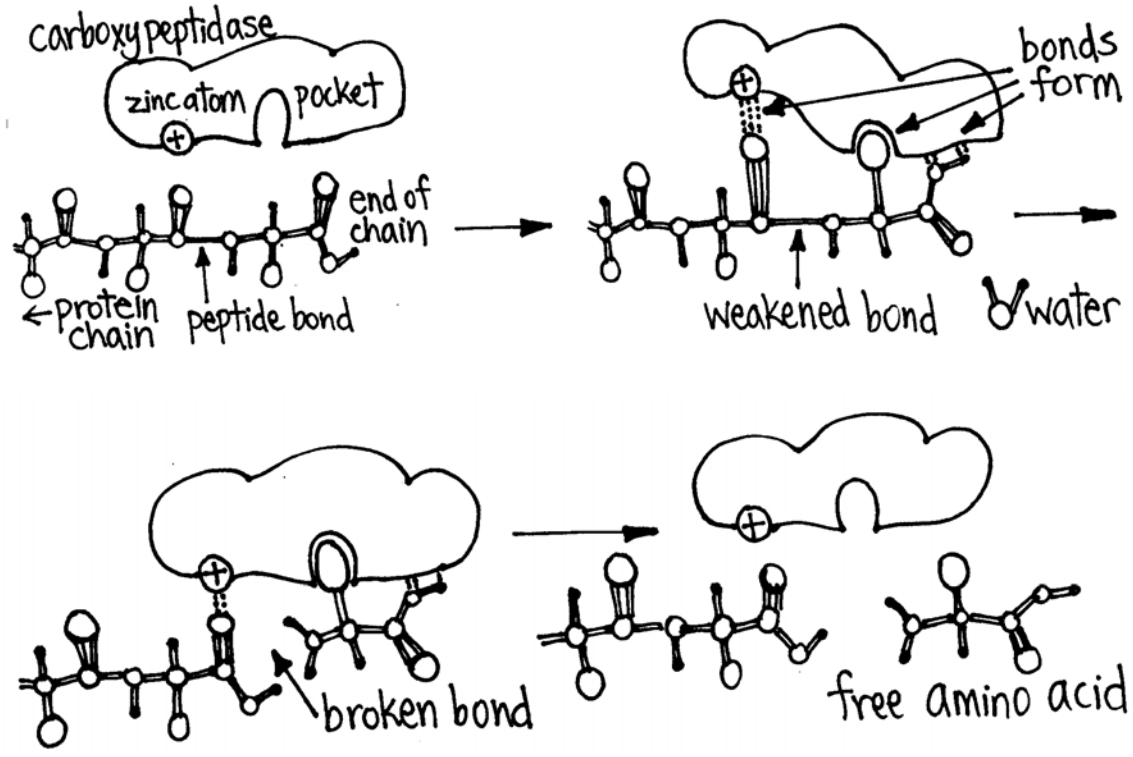


A water molecule can come back and break a peptide bond, but it usually does not have enough energy.

The enzyme ***carboxypeptidase*** catalyzes the breaking of the last peptide bond in the protein chain, releasing the end amino acid. Carboxypeptidase contains a zinc atom with a positive charge. This zinc atom bonds with the protein near the last peptide bond, pulling the electrons of the bond away from it and, thus, weakening it (below). The enzyme also has a pocket area composed of hydrophobic atoms; if the terminal amino acid has a hydrophobic group on it, the group is attracted to this pocket and held by

it. In addition, carboxypeptidase can form hydrogen bonds with the terminal amino acid, further securing it in place.

When a water molecule encounters the weakened peptide bond, it likely now has enough energy to break it, recombining with the broken ends to reform the loose amino acid. The various bonds holding the enzyme to the protein substrate are weakened, and the enzyme is released.



The first enzyme to take action in bread dough is ***amylase***. Amylase acts on starch (either amylose or amylopectin), breaking the starch chain between adjacent sugar rings. There are two kinds of amylase: α-amylase (alpha-amylase) randomly breaks the chain into smaller

pieces while β-amylase (beta-amylase) breaks maltose units off the end of the chain.

Amylase is found in flour. Wheat kernels contain amylase because they need to break starch down into sugar to use for energy when the kernels germinate. The amount of amylase varies with the weather and harvesting conditions of the wheat, so mills generally test for it and add extra or blend flours to get an appropriate amount.

Amylases are mobilized when water is added to the flour. This is one reason why dough's with a higher hydration often ferment faster—the amylases (and other enzymes) can move about more effectively. To reach the starch molecules, amylases must penetrate the starch's granules; thus, most of the action in bread dough happens at broken granules, where the starch is available for reaction. Fortunately, a percentage of starch granules are damaged during milling and accessible by the amylases.

An amylase is a big molecule, with hundreds of amino acids linked together. Many different groups contribute to the bonding between the amylase and the starch substrate. In addition, there are several different amylase molecules, and each functions differently. The examples of enzyme action presented above give the general idea.

Because of amylase, some of the starch in bread dough is broken into ***maltose***, a double-ring sugar composed of two glucose molecules; but fermentation reactions require single glucose rings. Simple sugars like glucose also provide flavor to the bread and participate in browning reactions that occur at the crust during baking.

Fortunately, the yeast used in bread-making contains the enzyme ***maltase***, which breaks maltose into glucose. When the yeast cell encounters a maltose molecule, it absorbs it. Maltase then bonds to the maltose and breaks it in two. Yeast cells also contain ***invertase***, another enzyme that can break sucrose, like the sucrase described above. This enzyme works on the small percentage of sucrose found in the flour. These two enzymes are responsible for producing much of the glucose needed by the yeast for fermentation.

The other major enzyme at work in bread dough is ***protease***. Protease acts on protein chains, breaking the peptide bonds between amino acids. Carboxypeptidase, described above, is an example of a protease. There are hundreds of proteases, but only a few are found in bread dough, where they chop the gluten into pieces. Proteases occur naturally in flour, yeast cells, and malt. Their

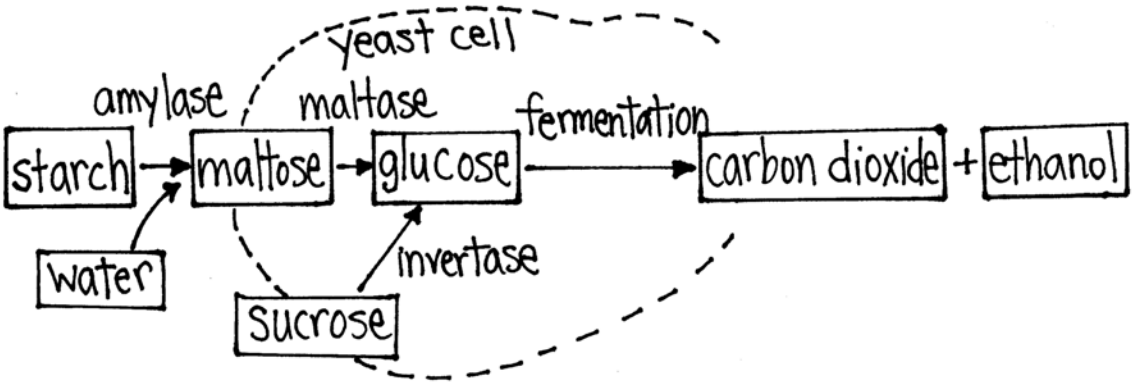
levels are measured at the mill and adjusted in the same way that amylase levels are adjusted.

Proteases in bread dough have been the subject of scientific research for the past hundred years. There has been much debate about their importance. In the early years, scientists were trying to prove their existence and measure relative activity in different brands of flour. They amplified the protease activity by adding non-gluten substrates to the mix. These substrates were ones that protease readily attacks. Eventually someone thought to look at protease activity in normal bread dough and found very little activity.

It seems, however, that this very small activity might be just what is needed in bread dough. Too much protease activity would break up the gluten, destroying the network that forms during kneading. A little bit, however, softens the dough and makes it more workable. If the dough is allowed to autolyse (i.e., rest) or if preferments are used, proteases have time to work before kneading, making the dough easier to knead. (This may be the origin of the word “autolyse,” from “autolysis,” which means “self-breaking” and could refer to the protein proteases at work on the protein chains.)

In addition to affecting the dough's consistency, proteases affect its flavor. Proteases result in single amino acids when they break the last peptide bond of the protein chain. These amino acids can participate in the flavor and browning reactions that occur at the crust during baking.

So now, my simplified diagram of the chemical reactions in bread dough looks more like this:



This diagram includes the presence of enzymes. Without enzymes, bread-making would not be possible. Then again, neither would we.