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Enzyme Reactions

THE NATURE OF ENZYMES

Enzymes, produced by living things, are compounds that catalyze chemical reactions. Reactions involving enzymes may be said to proceed in two steps. In step 1, $E + S \leftrightarrow ES$ (where E = enzyme, S = substrate, and ES = unstable intermediate complex that temporarily involves the enzyme). In step 2, $ES + R \leftrightarrow P + E$ (where R = a substance in the substrate that reacts with the complex, P = the final product of the reaction, and E = enzyme liberated from the complex). Enzymes are critical to life because they have the ability to catalyze the chemical reactions that are important to life. Chemical reactions take place when the necessary reactants are present, but usually an energy input (activation energy) is required to start a particular reaction. The analogy usually given to illustrate this concept is that of a boulder located at the top of a hill. The boulder has the potential energy for rolling down the hill, but must first be pushed over the edge (see Fig. 8.1). The potential energy of the boulder could be great, depending on its mass and altitude. It could start a rock slide or landslide involving a great amount of energy. Although the energy required to push the boulder over the edge is insignificant compared to the total energy involved in the rolling of the boulder down the hill, that initial energy (called the activation energy) is nevertheless important, for without it there would be no landslide.

It is well known that the rate at which reactions take place is dependent on temperature. Because reactions can proceed more rapidly at higher temperatures but quite often have to accelerate within a system of constant temperature, such as within our bodies, only by the action of enzymes can they occur. Enzymes are produced by living organisms from the lowest single-celled members to the highest, most complex members of the plant or animal kingdoms, including humans. All life depends on enzymes to convert foods or nutrients to a form in which they can be utilized, and to carry out cellular functions.

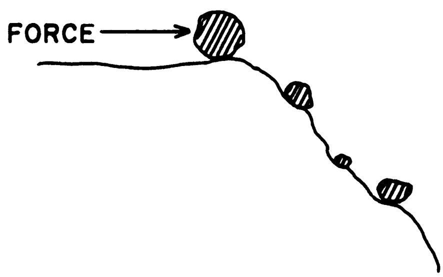

Figure 8.1. Force needed to start rock slide.

In composition, enzymes always contain a protein. They may also contain or require complex chemical compounds in order to become functional. These compounds are known as prosthetic groups (also called coenzymes) and are usually vitamins, especially those belonging to the B group. Some enzymes also require trace amounts of a metal, such as copper, to function. Enzymes therefore consist of either pure proteins, proteins with a prosthetic group, or proteins with a prosthetic group plus a metal cation.

Through their action, enzymes convert foods into less complex chemical substances that can be utilized for energy and for the building of cellular protoplasm. Proteins, fats, and carbohydrates are thus broken down to less complex compounds by enzymes in order that they may be utilized. More complex carbohydrates are broken down to glucose, a source of ready energy that can be absorbed and eventually converted to carbon dioxide and water or built up into fats that can be stored as a source of reserve energy. When reserve energy is needed, fats are first hydrolyzed (water is inserted to split the molecule), breaking down into glycerine and fatty acids. The fatty acids are then converted to acetates that can be utilized for energy. When the acetates are not completely used, those remaining may be reformed into fats and deposited as an energy reserve.

Proteins are broken down to their primary units (amino acids), in which form they may be incorporated into cellular protoplasm as proteins used for cell repair or growth. In some instances, the nitrogen portion of the amino acid may be removed and the remaining compound oxidized to provide energy.

As previously stated, enzymes always contain a protein (chain of amino acids) in which the amino acids are combined in a particular sequence, the protein itself having a particular shape or configuration.

There are approximately 20 to 22 known amino acids. Proteins consist of a large number of amino acids combined in a particular sequence. Also, chains of amino acids are crosslinked one to another and the different proteins form special configurations and shapes. Proteins are also sometimes combined with carbohydrates, lipids, or phospholipids (fatlike compounds containing phosphoric acid as part of the molecule). These are called conjugated proteins. The number and sequence of amino acids in the chain, the shape or relationship of one protein chain with another, and conjugation with carbohydrate or lipid all affect the functional properties and determine the manner win which a protein will react to physical and chemical energy.

Enzymes cause chemical reactions to occur at their fastest rates when the temperature is at an optimum level. For most enzymes, this is in the range of 60 to 150°F (15.6 to 65.5°C), but some action may occur at temperatures above or below the optimum range. Thus, some enzymes are able to react slowly at temperatures well below that of the freezing point of water and others at temperatures above 160°F (71.1°C).

Because proteins are changed chemically and physically or are coagulated by high temperatures, especially when moisture is present, enzymes are usually inactivated at temperatures between 160 and 200°F (71.1 and 93.3°C). There are some exceptions to this, however, and at least one enzyme, which splits off fatty acids from fish phospholipids, is known to remain active even after steaming at 212°F (100°C) for 20 min.

Enzymes have an optimum pH at which they cause reactions to occur at the fastest rate. Water solutions having a pH value less than 7 are said to be acidic; those having a pH value greater than 7 are said to be alkaline; and those having a pH value of exactly 7 are said to be neutral. As in the case of temperatures, some action will occur at pHs above or below the optimum, although there are low and high limits beyond which a particular enzyme action cannot take place.

PROTEOLYTIC ENZYMES (PROTEASES)

Enzymes involved in the breakdown or splitting of proteins are called proteolytic enzymes or, more simply, proteases. Proteases comprise two general classes: proteinases and peptidases. Proteinases split the protein molecules into smaller fragments called proteases and peptones, then into polypeptides and peptides. Peptidases split polypeptides and peptides into amino acids. Because most amino acids in foods are water-soluble, food proteins are essentially liquefied by proteinases and peptidases.

In meat, such as beef, pork, or poultry, held in the eviscerated state (intestines and organs removed), the proteases present in the tissue are called cathepsins. The temperatures and times under which these products are held prior to utilization are not such that extensive proteolysis can occur. Therefore, while there may be some tenderization of the tissues during holding, which may, in fact, be due to proteolysis, there is not extensive breakdown of the tissues.

In fish, proteolytic enzymes are much more active than in meats. Even when fish is held in the eviscerated state in ice or under refrigeration, there may be sufficient proteolysis to cause softening of the tissues over a period of days. In fish held in the round (uneviscerated), proteolysis is accelerated owing to a concentrated source of enzymes present in blind tubules (the pyloric ceca) attached to the intestines. Thus, even though fish in the round are refrigerated, within a few days, sufficient proteolysis may occur to dissolve the tissues of the abdominal wall, exposing the entrails. Members of the herring and mackerel families handled in the uneviscerated state are quite subject to this type of enzyme deterioration, especially if they have been feeding when caught. Other fish such as flounders and ocean perch, handled in the uneviscerated state, seem not to be especially subject to this type of deterioration.

Lobsters provide an especially good example of a deteriorative change that may take place through the action of proteolytic enzymes. As long as the lobster is alive, autolytic proteolysis does not occur. However, if the lobster dies and then is held for some hours, even under refrigeration, but especially at high temperatures, proteolysis takes place to such an extent that the lower abdominal portion will be partially liquefied. When such a lobster is cooked, the flesh will be soft and crumbly (short-meated) and part of the tail portion will have dissolved, leaving only a part of this section intact. For this reason, lobsters should never be held long after death prior to cooking. The present accepted practice is to cook lobsters from the live state. Other crustaceans (shrimp and crabs) are also subject to enzyme proteolysis, although with shrimp, this usually is not extensive, especially if the head portion (cephalothorax) is removed shortly after the shrimp are caught. The relatively high activity of enzymes in marine species is attributed to the low-temperature conditions in the marine environment. That is, in order to make reactions proceed at low temperatures, the activation energy system must be more efficient.

Plants also contain proteolytic enzymes, but these enzymes usually contribute little to deterioration, especially as long as the tissues of fruits and vegetables are not cut or damaged. Some plants provide an excellent source of proteolytic enzymes. Bromelin is found in unpasteurized pineapple juice and is so active that people who handle cut pineapple that has not been heated must wear rubber gloves; otherwise the skin of the fingers will become eroded, and could result in the exudation of some blood. Papain is a proteolytic enzyme obtained from the latex (milky liquid) of the green papaya fruit. Ficin is a proteolytic enzyme obtained from the latex of certain fig trees. Proteolytic enzymes from plants may be extracted and purified and these enzymes may be employed, for instance, to tenderize meats.

OXIDIZING ENZYMES (OXIDASES)

Oxidation has been defined as a loss of electrons in an atom or simply a chemical combination with oxygen. An example of complete oxidation is a wick of a candle burning, where the cellulose wick is converted to carbon dioxide and water. There are a number of oxidizing enzymes that bring about changes in foods that result in deterioration due to oxidation. In plants, peroxidases, ascorbic acid oxidase, tyrosinase, and polyphenolases may cause undesirable chemical reactions to occur. Peroxidases may oxidize certain phenollike compounds in root vegetables, such as horseradish, causing the prepared product to become darker in color. This does not happen while the tissues are intact but only when the vegetable has been cut up or comminuted. Ascorbic acid oxidase, present in certain vegetables, oxidizes ascorbic acid (vitamin C) to a form that is readily further oxidized by atmospheric oxygen. The resulting oxidation product is not utilized by humans as a vitamin. Therefore, the action of this enzyme may cause loss of the vitamin C content of foods. Peroxidases may also, indirectly, cause a loss of vitamin C in vegetables. In this case, the compounds formed by the action of peroxidase react with vitamin C. Phenolases are present in some fruits and vegetables. These enzymes oxidize some phenollike compounds, also present in plant product, causing brown or dark-colored compounds to be formed when the tissues are cut.

Tyrosinase oxidizes the amino acid tyrosine to form dark-colored compounds. The molecule rearranges and further oxidizes to form a red compound. Polymerization (combination of these compounds) results in the formation of dark-colored melanin compounds.

The enzyme tyrosinase, which is present in many fruits and vegetables, may cause discoloration of the cut tissue and will also oxidize compounds related to tyrosine. This enzyme is also present in shrimp and some spiny lobsters and may cause a discoloration called black spot. In shrimp, this often occurs as a black stripe on the flesh along the edges of the segments of the tail or as a pronounced band where the shell segments overlap. It is not generally recognized, but tyrosinases are also present in clams. Hence, shucked (deshelled) clams will darken at the surface if oxygen is present and if the enzymes have not been inactivated by heat.

These reactions occur only after the shrimp or clams die. In general, oxidizing enzymes do not cause deteriorative changes in tissues that are intact. In fruits and vegetables, the tissues must be cut or bruised or there must be a breakdown of cells by their enzymes before the action of oxidizing enzymes results in discoloration.

FAT-SPLITTING ENZYMES (LIPASES)

Fats are composed of glycerine (glycerol) and fatty acids. Glycerine is a polyhydric alcohol (three alcohol groups), and fatty acids are short or long chains of carbon atoms to which hydrogen is attached, either to the fullest possible extent (saturated) or to a lesser extent (unsaturated), the latter resulting in reactive groups in the chain. At one end of the fatty acid chain there is an acid group.

In the formation of fats, each one of three fatty acids combines with one of the three alcohol groups of glycerine, splitting off water in each case. On the other hand, in the breakdown of fats, water and the enzyme lipase are present, and fats are split into their original component parts, glycerine and fatty acids.

The fatty acids in most fats that are found in nature consist of a chain of more than 10 carbon atoms, and these fatty acids have no particular flavor or odor. Hence, when lipase acts on most natural fats, no bad odors are generated. However, if fats or oils high in free fatty acid content (indicating deterioration) are used for deep-fat frying, the oil may smoke during heating, which is undesirable.

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\begin{array}{lcclcl}
CH_2OOC(CH_2)_2CH_3 & & CH_2OH & + & CH_2(CH_2)_2COOH \\
 & & | & & \text{butryic acid (odorous)} \\
CH_2OOC(CH_2)_8CH_3 & \xrightarrow[\text{lipase}]{\text{water}} & CHOH & + & CH_2(CH_2)_8COOH \\
 & & | & & \text{capric acid (some odor)} \\
CH_2OOC(CH_2)_{14}CH_3 & & CH_2OH & + & CH_2(CH_2)_{14}COOH \\
 & & & & \text{palmitic acid (no odor)} \\
\text{fat} & \longrightarrow & \text{glycerine} & + & \text{fatty acids}
\end{array}
$$

There are some fats that contain short-chain fatty acids, especially those fats present in the milk of cows or goats. These fats contain butyric (four carbons), caproic (six carbons), caprylic (eight carbons), and capric (ten carbons) acids. All these fatty acids have an odor and flavor. Butyric acid, especially, is pungent and considered to be distasteful. When lipase acts on butter, therefore, it splits off butyric acid, which gives the butter a strong, undesirable rancid taste. Actually, butter is an emulsion of water in oil and contains about 16% water, the water being present as fine droplets. Butter becomes rancid by the action of lipase produced by bacteria that grow in the water droplets. The lipase acts on the fat surrounding the water droplets. Lipase rancidity in butter, therefore, is really a type of deterioration caused by bacteria.

Phospholipases, enzymes similar to lipase, are split phospholipids. Most phospholipids are similar to fats in that they contain glycerine, two alcohol groups of which are combined with fatty acids. The third alcohol group in this case is combined with a molecule containing phosphoric acid, a short chain of carbons, and a nitrogen group with carbons attached to it. Lecithin is a typical phospholipid. Phospholipase splits off fatty acids from phospholipids. Such action may cause deterioration in foods in that it results in a destabilization of proteins that causes a toughening of the tissues and a loss of succulence (juiciness).

ENZYMES THAT DECOMPOSE CARBOHYDRATES (CARBOHYDRASES)

Fruits contain pectin which supports the particular structure of the product. In processed fruit juices (for instance, tomato or orange juice), if the pectin is broken down, the solids tend to settle to the bottom, leaving a clear serum on top. Pectin consists of a long chain of galacturonic acid molecules with the carboxyl groups partially esterified with methyl alcohol. It has a high water-holding capacity. There are pectic enzymes that will either break down the pectin molecule to smaller units or completely decompose the molecule to its primary unit, galacturonic acid. The emulsifying properties of pectin may be lost, causing settling in fruit juices and softening in fruit. When the pectin in whole fruit breaks down, it may result in deterioration of the fruit as a result of the action of other enzymes or invasion of the tissues by microorganisms.

In the sugar cane plant, there is an enzyme, invertase, that breaks down cane sugar (sucrose), which has 12 carbon atoms, into glucose and fructose, each having six carbon atoms. Before sugar cane is harvested, therefore, a part of the plant must be removed to eliminate the source of the enzyme. Were this not done, there would be a loss of sucrose during the processing of the cane. Many other carbohydrases, which break down cellulose or starch or break down more complex sugars to smaller units, exist.

APPLICATIONS

Whereas enzymes may cause a deterioration of foods, they may also be used in the processing of foods to produce particular products or to modify the characteristics of particular products. Proteolytic enzymes obtained from plants may be used for tenderizing meat either by injecting animals with a solution of the enzyme prior to slaughter or by sprinkling the powdered enzyme on meat surfaces and allowing it to react, prior to cooking. In the manufacture of certain kinds of milk powder (e.g., to be used in chocolate), the lipases may be allowed to act on the milk fat prior to drying to obtain a particular flavor in the finished product. Proteases may also be used to chill-proof beers, a technique used to remove proteins that would cause clouding during cool storage. Protein hydrolysates can be produced from the use of proteases from both plant and animal sources. They have many uses, for example, as flavorings, nutrients, and stabilizers to improve texture.

The characteristic flavor of certain cheeses is due to the action of lipases on the milk fat contained therein. In order to obtain the particular flavors of Roquefort, Gorgonzola, or blue cheese, the milk fat must first be broken down to fatty acids that can then be oxidized. The lipases that decompose the fats to provide fatty acids are produced by the molds allowed to grow in these cheeses. Enzymes produced by molds then oxidize the specific fatty acids that ultimately produce the unique characteristic flavors. Lipases also can be added to dried egg whites to improve the whipping quality.

There are many applications of enzyme technology that involve the use of carbohydrate-splitting enzymes. In making malt, barley is germinated to obtain an enzyme that will convert starch to a sugar (maltose), which can be converted by yeasts to ethyl alcohol and carbon dixoide. By this means, various grains can be used as the source of sugar for fermentation. Carbohydrate-splitting enzymes are also used to modify starches used in foods and to modify starches used in sizing and laundering clothes. Others are used to form corn syrup from corn starch and then isomerize glucose to fructose in the formation of high-fructose corn syrup which is used extensively in the manufacture of soft drinks. Invertase (sucrase) is used in the production of chocolate-covered cherries. The cherries are rolled in a mixture of crystalline sucrose and the enzyme before they are covered in chocolate. During a short holding period, the enzyme splits the sucrose into a mixture of glucose and fructose, which has a higher water solubility and results in the sweet taste and creamy texture that is characteristic of the product. The presence of pectins in fruit juices may cause clouding. Pectinases can be added to remove the pectin and clarify the juice.

There are many other applications of enzyme technology in the food and other industries and it is expected that the number of applications for enzymes will continue to increase. One of the factors that will serve to widen the use of enzyme technology is the development of immobilized enzymes. It has been found that enzymes can be fixed chemically to the surface of inert substances, such as glass beads. In this form, they can be packed into a column through which a solution or suspension of the material to be acted on (called the substrate) is allowed to pass. In this manner, the enzyme responsible for the conversion or change in the substrate is not lost or washed out with the substrate. Thus, the enzyme can be used for a number of substrate conversions. Moreover, in this form (immobilized enzyme), the active agent is much less subject to inactivation such as, for instance, by high temperature.

Restoration and enrichment of flavor in some processed foods can be accomplished by addition of enzymes. Examples of this include the conversion of allin in garlic into garlic oil by allinase and the addition of an enzyme preparation from mustard seeds to dehydrated cabbage which restores flavor by converting flavor precursors into the volatile sulfur-containing compounds responsible for the familiar flavors.

They may also prevent the formation of large ice crystals in frozen desserts. A significant potential for the use of gums lies in the production of certain low-calorie foods. For example, the oil in salad dressing can be replaced with gums to result in a product with the normal appearance, texture, and taste but without the calories normally associated with the product.

ENZYMES

Enzymes occur naturally in foods, and their presence may be either beneficial or detrimental, depending on the particular enzyme (see Chapter 8). When the presence of enzymes is undesirable, steps are taken to inactivate them. When their presence is desirable, either the enzymes or sources of the enzymes are intentionally added to foods. For example, the enzyme papain (from the papaya fruit) is added to steak to tenderize it. Many of the useful enzymes used in food processing are produced by microbes; consequently those microbes producing the desired enzyme may be added intentionally to food. For example, specific yeasts are intentionally added in the production of bread, beer, or cheese.

The use of enzymes as food additives presents no problem from the standpoint of safety, because enzymes occur naturally, are nontoxic, and are easily inactivated when desired reactions are completed. Enzymes called amylases are used together with acids to hydrolyze starch in the production of syrups, sugars, and other products.

Invertase

Certain enzymes, such as invertase, split disaccharides, such as sucrose (table sugar), to lower sugars (glucose and levulose). Invertase has many applications, and is used, for example, to prevent crystallization of the sucrose that is used in large amounts in the production of liqueurs. Without invertase, the liqueurs would appear cloudy.

Pectinase

Pectinases are enzymes that split pectin, a polysaccharide that occurs naturally in plant tissues, especially those of fruit. Pectin holds dispersed particles in suspension, as in tomato juice. Because it is desirable to keep the thick suspension in tomato juice, pectinases that occur naturally in it are inactivated by heat. On the other hand, products such as apple juice are customarily clear, and this is accomplished by adding commercial pectinase to the product, which degrades the pectin in the apple juice, resulting in the settling out of the suspended particles, which are then separated from the clear juice. In the manufacture of clear jellies from fruits, it is first necessary to add pectinase to destroy the naturally occurring pectin in order to clarify the juice. This pectinase must now be inactivated by heat. Then more pectin must be added to the clarified juice to produce the thick consistency of jelly. If the pectinase is not inactivated after clarification, the enzyme would also break down the newly added pectin required to produce the thick consistency.

Cellulases

Cellulases are enzymes that can break down cellulose, said to be the most abundant form of carbohydrate in nature. Cellulose, the principal structural material in plants, is insoluble in water and is indigestible by humans and many animals. Ruminants are able to digest cellulose because of a cellulase (produced by microorganisms in the large stomach) contained in their gastric juice. Commercial applications of cellulases are not widespread at present. Cellulases are used for tenderizing fibrous vegetables and other indigestible plant material for the production of foods or animal feed.

Proteases

Proteases are enzymes that break down proteins, polypeptides, and peptides. Peptides are the structural units of which polypeptides consist, and polypeptides are larger structural units that make up the protein. There is a large number of specific proteases, and each attacks protein molecules at different sites, producing a variety of end products. Proteases are used to produce soy sauce from roasted soybeans, cheese from milk, and bread dough from flour. They are also used to tenderize meat and chill-proof beer which, if untreated, develops an undesirable haze when chilled.

Lipases

Lipases, the lipid (fat or oil) splitting enzymes, have limited commercial application, with oral lipases having the widest. Lipases prepared from oral glands of lambs and calves are used in a controlled way in the production of certain cheeses and other dairy products, as well as lipase-treated butter fat used in the manufacture of candles, confections, and baked products. Lipases are also used to remove fat residuals from egg whites and in drain cleaner preparations.

Glucose Oxidase

Glucose oxidase is an enzyme that specifically catalyzes the oxidation of glucose to gluconic acid. This reaction is important in preventing nonenzymatic browning, because glucose is a reactant in the undesirable browning reaction. The most important application of this enzyme is in the treatment of egg products, especially egg whites, prior to drying. Eggs treated with this enzyme before they are dried do not undergo nonenzymatic browning during storage, because the sugar has been removed. In some cases, the enzyme is added to remove traces of oxygen to prevent oxidative degradation of quality. Examples of this type of application are mayonnaise and bottled and canned beverages (especially beer and citrus drinks).

Catalase

Catalases are used to break down hydrogen peroxide (H_2O_2) to water and oxygen. Therefore, catalases are used when the presence of hydrogen peroxide is undesirable or when hydrogen peroxide is used for specific purposes, such as in bleaching, but then must be removed from the system. Examples of the latter case are the uses of hydrogen peroxide for preserving milk in areas where heat pasteurization and refrigeration are unavailable and in the manufacture of cheese from unpasteurized milk. Hydrogen peroxide is produced during the spray-drying process. Catalase is used to convert the unwanted H_2O_2 to water and oxygen.