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History and Introduction of ENZYMES

Have you ever wondered how does the biochemical reactions work in our body? These biochemical processes are of paramount importance to sustain our lives and they involve the works of different enzymes. Enzymes are intriguing protein molecules with unique three dimensional shapes.

Enzymes were first discovered in 1833 by a French chemist. Yeast fermentation was the pre-discovery of enzymes. Until 1900s, more scientists proposed the ideas and theories about enzymes. Acting as a biological catalyst, enzyme reacts specifically on substrate and produces end-products that aid our bodily functions.

We need energy to carry out daily activities, and same goes for biochemical reactions. In order to allow a chemical reaction to happen, activation energy has to be achieved. Enzymes can increase the rate of reaction by lowering the activation energy of different biochemical reactions in our body. To further speed up the reaction rates, cofactors are the ideal aids for enzymes, while inhibitors do the opposite. Seemingly easy yet complicated, only two types of metabolisms are available, as indicated in Figure 1. However, an estimation of thousand different reactions are involved in these metabolisms.

Figure 1. Two types of metabolisms in enzymatic reactions.

To ease the understanding about enzymes, let’s discuss about the most common enzymes first, which are digestive enzymes. We do not swallow a whole food when we know we would choke on it. Chewing foods into smaller pieces makes them easier to swallow. Enzymes do the same thing too! For example, when the food travels all the way down to the stomach, more digestive enzymes would catalyze on the substrates (nutrients in food), easing the gut absorption of these nutrients. This is one of the role enzymes play in our body.

As technologies advance, enzymes do not only work for biochemical functions in our body. They are also extracted from different sources (e.g. plants, microorganisms) for the utilizations in many industries, like biological, chemical, and food industries. To brief up, enzyme is a boon to inhabitants from every corner of this world.
1.1 Lock & key model

Before enzymes catalyze any chemical reaction, they begin by binding with the specific substrates. The active site is the region of an enzyme where substrate binds and undergo catalyze reaction. To describe this catalytic reaction, different mechanisms such as “Lock & Key model” and “Induced Fit model” have been proposed to describe the mechanisms of enzymes. Among these models, Lock & Key model is relatively famous and well known to the public.

Lock & Key hypothesis is a model proposed by Emil Fischer in 1894. In our daily lives, door locks have their specifically designed keys. We cannot unlock the locks, unless we use the specific key to open. Enzymes work the same way.

As shown in Figure 2, enzymes are like locks, their active sites are specific and distinctive. The right substrates are combined to form a temporary “enzyme-substrate complex”. Only the correct key fits into the designated key hole of the lock. Smaller keys, larger keys or keys with different positioned blade will not fit into the lock properly or open the lock. After the catalyzed reaction, the products are released from the active sites, so other substrates with complementary shape can repeat the same process.

![Figure 2. Lock & Key hypothesis.](image)

Take sucrose (one of our daily food ingredients) as an example, sucrose (substrate) binds to sucrase (a common digestive enzyme) to form sucrase-sucrose complex. Sucrase catalyzes sucrose to produce glucose and fructose. Then, sucrase is now free again, and ready to hydrolyze other sucrose.
Types of **ENZYMES**

In general, enzymes are protein molecules found in living organisms. There are so many types of enzymes that exist in this world and each of them has its specific functions. Before purchasing or consuming enzymes, let’s understand where can they be found and how is it classified.

2.1 Sources of enzymes

Enzymes are found everywhere in our daily life. However, we take them for granted and rarely notice their existence. For instance, glycogen synthase (enzyme) helps control the synthesis and degradation of glycogen in a human, allowing cells to respond to changes in blood sugar; pectinase (enzyme) softens banana by breaking down the cell wall materials or pectin during ripening process; polyphenol oxidase (enzyme) reacts with oxygen on the surface of apple slices, hence the apple turns brown in colour through enzymatic browning reaction.

Hundreds and thousands of enzymes can be also found in human body. Each enzyme plays their own role in the maintenance of the whole system. Growth, digestion, metabolism, energy, healing are some representative examples that enzymes are needed in our body.

Three main sources of enzymes are animals, microorganisms and plants. Enzymes from microorganisms and plants are the common sources of enzymes being used in the food industry. Table 1 below shows some examples of enzymes derived from animals, microorganisms and plants.
Table 1 Examples of enzymes derived from animals, microorganisms and plants

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Sources</th>
<th>Industrial use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>liver</td>
<td>Animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>food, milk, beverage, salad</td>
</tr>
<tr>
<td>Lipase</td>
<td>pancreas</td>
<td>food, fats and oil</td>
</tr>
<tr>
<td>Pepsin</td>
<td>animal stomach</td>
<td>digestive aid, meat tenderizer</td>
</tr>
<tr>
<td>Rennet</td>
<td>abomasum</td>
<td>cheese</td>
</tr>
<tr>
<td>Trypsin</td>
<td>animal pancreas</td>
<td>medical uses, meat tenderizer, beer haze removal</td>
</tr>
<tr>
<td>Glucose isomerase</td>
<td>Bacillus</td>
<td>fructose syrup, cereal, starch, fruit and vegetable, beverage, sugar, honey</td>
</tr>
<tr>
<td>Glucoamylase</td>
<td>Aspergillus</td>
<td>starch, fruit and vegetable, beverage, sugar, honey, confectionery</td>
</tr>
<tr>
<td>Lactase</td>
<td>Kluyveromyces</td>
<td>dairy, milk, cheese, dietary foods</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Aspergillus</td>
<td>drinks, fat and oil, fruit and vegetable</td>
</tr>
<tr>
<td>Protease</td>
<td>Bacillus</td>
<td>fish meat, fish, soup, broth</td>
</tr>
<tr>
<td>α- Amylase</td>
<td>malted barley</td>
<td>brewing, medical use</td>
</tr>
<tr>
<td>β- Amylase</td>
<td>malted barley</td>
<td>brewing</td>
</tr>
<tr>
<td>Bromelain</td>
<td>pineapple latex</td>
<td>brewing, medical use</td>
</tr>
<tr>
<td>Ficin</td>
<td>fig latex</td>
<td>food</td>
</tr>
<tr>
<td>Papain</td>
<td>pawpaw latex</td>
<td>tenderizer</td>
</tr>
</tbody>
</table>

Classification of enzymes from the 2.2 academic point of view

In 1956, experts from the Enzyme Commission (EC) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) classified enzymes with EC numbers. This classification system is categorized by their overall chemical reaction catalysed.

This classification system considers few factors before giving them their proper EC numbers. The types of reactions being catalysed by the enzyme are factors to be considered. As indicated in Table 2, one out of the six classes of enzymatic reactions is generally the first category to look at. Other factors like chemical bond changes, reaction center, transferred chemical group, metabolites, and cofactors are then taken into considerations while classifying the EC number of the enzyme. In terms of scientific aspects, this type of classification is used universally.
Table 2  Enzymes classified by the types of chemical reactions

<table>
<thead>
<tr>
<th>Classes</th>
<th>Types of reactions</th>
<th>Example of enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductases</td>
<td>$\text{Ard} + \text{Box} \rightleftharpoons \text{Aox} + \text{Bred}$</td>
<td>redox reaction (reduction and oxidation)</td>
</tr>
<tr>
<td>Transferases</td>
<td>$\text{A} - \text{B} + \text{C} \rightleftharpoons \text{A} + \text{B} - \text{C}$</td>
<td>transfer a chemical group from one molecule to another</td>
</tr>
<tr>
<td>Hydrolases</td>
<td>$\text{A} - \text{B} + \text{H}_2\text{O} \rightleftharpoons \text{A} - \text{H} + \text{B} - \text{OH}$</td>
<td>hydrolysis reaction</td>
</tr>
<tr>
<td>Lyases</td>
<td>$\text{A} + \text{B} \rightleftharpoons \text{A} - \text{B}$</td>
<td>join two or more compounds</td>
</tr>
<tr>
<td>Isomerases</td>
<td>$\text{A} - \text{B} - \text{C} \rightleftharpoons \text{A} - \text{C} - \text{B}$</td>
<td>isomerization</td>
</tr>
<tr>
<td>Ligases</td>
<td>$\text{A} + \text{B} + \text{ATP} \rightleftharpoons \text{A} - \text{B} + \text{ADP} + \text{Pi}$</td>
<td>formation of high energy bonds with hydrolysis of nucleoside triphosphate</td>
</tr>
</tbody>
</table>

In other circumstances, though enzymes can be produced naturally by our body, enzyme production may not be sufficient. For instance, many Asians are having a problem of lactose intolerance. By taking lactase supplements, it could help alleviate the symptoms of abdominal cramps, diarrhea, bloating, nausea, flatulence when taking lactose containing products. Moreover, if someone is suffering from sickness, supplementation of digestive enzymes may also be needed to maintain healthy digestion.

The other category of enzyme is “(B) enzymes that cannot be produced naturally by our body”. This type of enzyme is commonly termed as “functional enzymes” in the market due to the fact that many studies have reported various advantageous effects after taking these functional enzymes. Fibrinolytic enzyme is a good case in point, as many studies have shown that it can possibly assist in lowering the risk of cardiovascular disease.

2.3 Classification of enzymes from the marketing point of view

In the market, enzyme products can be generally classified into two big categories (Figure 4), namely enzymes produced by our body and enzymes that cannot be produced by our body.

In the first category "(A) enzymes that are produced in human body", it can further be classified into “(A1) needless to be supplemented” and “(A2) can be retrieved from foods or supplements”. For some cases, healthy individuals need not worry about enzymes insufficiency, the body would produce sufficient enzymes on its own.
3.1 Enzyme activity

Alas, what should we do when we are lacking enzymes? Enzymes are indispensable in the way that they sustain our life by maintaining normal biological functions. However, some people may have enzymatic disorders, they are either inborn or acquired disorders. Fortunately, enzymes supplements have been available for decades to help those in need.

When choosing enzyme supplements, we have to understand the actual enzyme activity first. We should consider the activity of enzymes instead of the weight. Weight is not a right way to determine the activity of enzymes, as enzymes may retain weight even if they are destroyed and have null activity. Food Chemical Codex (FCC) introduced an internationally recognized standard measurement for different activity units for each type of enzymes.

Factors affecting enzyme activity

Factors can be two-sided, either good or bad. Like emotional influences, we could sometimes be happy or sad, depending on different external factors. Enzymes face the same situations. Their activities are basically affected by several factors such as temperature, pH, enzyme concentration, and substrate concentration. Two important factors are described as Table 3 below:

<table>
<thead>
<tr>
<th>Factors</th>
<th>Descriptions</th>
</tr>
</thead>
</table>
| Temperature | • When the temperature goes beyond the optimum temperature (e.g., 37°C), enzymes will denature as the bonds and forces are broken.  
  | • Substrates can no longer fit in the altered active sites. This process is irreversible.  |
| pH value  | • Every enzyme has its own optimum pH values.  
  | • Enzyme activities decrease when the pH falls or rises.  
  | • At extreme pH values, enzymes start to denature irreversibly.  |
Production of **ENZYMES**

As technology improves with time, enzymes are studied more in depth. Enzymes are now produced more efficiently and higher in purity. Figure 6 shows some common examples of the enzyme production methods.

Enzyme production by biotechnology 4.1

In the biotechnology industry, enzymes can be obtained through microbial fermentation, extraction, and purification. Biotechnology-related skills, knowledge, and facilities are required to produce enzymes.

Enzymes can be extracted from living organisms like animals, microorganisms or plants. The source of enzymes has to be studied in detail in order to obtain high yield of enzymes. The enzymes are further purified and stabilized using special techniques under hygienic conditions. Finally, they are formulated by the manufacturer for different commercial purposes.

Enzymes can also be produced by fermenting raw food like soy beans, fruits and vegetables with the addition of fermentation starter microorganisms. In this case, raw food materials are used as the medium. However, the main end-products may not necessarily be enzymes, it depends on the production and starter microorganisms.

Homemade enzyme 4.2

On the other hand, homemade enzymes are very different from the biotechnologically-produced enzyme products. A way to make homemade enzymes is to add fruits, sugar and water in a jar and let it ferment for a period of time. Most consumers may have misconceptions that these products are sources of enzymes. In fact, this kind of liquid enzymes are actually fermented fruits and vegetables liquid.

In homemade enzymes, specific starter microorganisms are not added and the production is not as hygienic as the one produced using biotechnological approaches (e.g. using sanitized fermenter). It should be noted that enzymes are not the main end-product in homemade enzymes. Homemade enzymes are more like pickles that are sat for a period of time.

The surface of a fruit contains many unknown microorganisms and every batch may vary. More importantly, it may also contain unwanted microorganisms that might be harmful to us. Although beneficial microorganisms may be present, there is no guarantee and the composition of microorganisms may be too complicated to control the quality and safety. Therefore, it is not recommended to make your own so-called “enzymes” at home.
COFACTORS

No man is an island. Occasionally, we rely on certain people for help, like visiting doctors for disease recovery. In the midst of going on with our lives, we need motivations and supports to realize our aspirations. It would not be a surprise when it applies the same way on our biological processes in the body.

Enzymes are protein catalysts, but they may require non-protein molecules (partners) as assistants to carry out reactions (Figure 7). This partner is basically called cofactor. Majority of the cofactors are metal ions (e.g. iron, magnesium, or zinc) and coenzymes (e.g. vitamins or other organic compounds).

As shown in Figure 7, before an appropriate cofactor binds to the enzyme, the enzyme molecule is in its inactive form (called apoenzyme). Only after the attachment of a correct cofactor, an active form of enzyme (called holoenzyme) is formed. At this time, the enzyme can then possess the catalytic ability and display its activity.

Figure 7. Some enzymes need to bind with a cofactor to become an active form.
6.1 Improvement on gastrointestinal health

Gastrointestinal disorders are a group of diseases involving the gastrointestinal tract which is responsible for digestion in human’s body. Symptoms such as heartburn, indigestion, bloating, and constipation are commonly seen in this disorder.

There are several factors to this disorder, including gastroenteritis, colitis, ulcers in stomach or intestine, diabetes and many other causes. Diet, stress, lifestyles, allergy, or food intolerance are also associated with gastrointestinal disorders. Studies revealed that an appropriate enzyme supplementation could play a vital role in these situations.

“Zyactinase is an extract of green kiwifruit, formulated into the consumer healthcare products marketed as Phloe and Kivia, used to assist in the relief of the symptoms associated with a range of digestive system dysfunction, including constipation and Irritable Bowel Syndrome (IBS).”

“The green kiwifruit extract significantly induced normal bowel movements with no adverse effects. The kiwifruit extract relieved constipation and the symptoms of IBS such as bloating, flatulence and abdominal pain.”

Reducing the risk of cardiovascular diseases

Cardiovascular diseases are a broad term for heart and blood vessels disorders. It is one of the most common death causes and it is estimated that 17.9 million people died from cardiovascular diseases in 2016, representing 31% of the total global deaths.

Arteries are blood vessels that carry oxygen-rich blood to our heart and all over our body. Atherosclerosis is a condition when fat, cholesterol or other substances build up in artery walls, making arteries to be narrowed or even blocked. This could lead to heart attack, heart failure, high blood pressure, peripheral artery disease, stroke and many other cardiovascular diseases.

Studies showed that some enzymes could help clear the substances that clog the arteries, allowing better blood flow and reducing the risk of cardiovascular diseases.

“Various animal and human trials have demonstrated that nattokinase improves blood circulation and helps decrease the risk of a variety of cardiovascular diseases without producing any adverse side effects.”


Enzyme supplementation plays an integral role in the management of various digestive disorders, particularly with regard to exocrine pancreatic insufficiency. However, application of enzymes may also be beneficial for other conditions associated with poor digestion including lactose intolerance.


“Our results showed that taking bromelain apparently increased fecal moisture by 18% and declined fecal mucinase activity.”

“The feeding of same amounts of bromelain and inulin could lead to significant increases in the growth of total Bifidobacterium and Lactobacilli as well as the concentrations of various fecal SCFAs.”

6.3 Anti-inflammatory potential

Inflammation is a defense mechanism in the body. Warmth, pain, redness, swelling, and loss of function are some classical symptoms of inflammation. However, not all inflammation has all the above mentioned symptoms. Some inflammation may be asymptomatic.

You must have an experience of cutting your skin accidentally with a sharp object, causing your skin to turn red or slightly swollen surrounding the cut area. This is one typical example of inflammation. Furthermore, autoimmune diseases (e.g., rheumatoid arthritis, psoriasis) lead to inflammatory response of body tissue. This inflammatory problem could last for years or even a lifetime in varying degrees of severity. Studies have revealed that some types of enzymes could be able to relieve inflammation effectively.

“Bromelain, a mixture of proteases derived from pineapple stem, has been reported to have therapeutic benefits in a variety of inflammatory diseases, including murine inflammatory bowel disease.”

“Some studies demonstrated that bromelain had efficacy similar to standard anti-inflammatory drugs such as dexamethasone or non-steroidal anti-inflammatory agents (NSAIDs).”


“Natural compounds in the kiwifruit including protein-dissolving enzymes (Actinidin) improved different aspects of the wound healing process. Based on these benefits and safety aspects, we conclude that using kiwifruit is a simple, applicable and effective way for treatment of neuropathic diabetic foot ulcer.”

6.4 Other physiological benefits

“Our results indicate that protease supplementation may attenuate muscle soreness after downhill running. Protease supplementation may also facilitate muscle healing and allow for faster restoration of contractile function after intense exercise.”


“Better collagen deposition and presence of skin organelles in rats treated with papain-based wound cleanser demonstrated its efficacy in promoting wound healing. In addition to its wound healing effect, papain-based wound cleanser is also integrated with antibacterial properties which make it a complete package for wound management.”

Technology for the Enhancement of ENZYMES ACTIVITY

Plants naturally possess a range of anti-nutritional factors as their weapons to protect themselves against the attack from herbivores, insects, pathogens, or as a mean to survive in severe conditions. These anti-nutritional factors or natural toxicants are considered to be the factors limiting the plants’ enzyme activities as well as nutritive values. Furthermore, the presence of these anti-nutritional factors may also affect the enzyme activity along our digestive tract.

The removal of anti-nutritional factors (e.g., tannin, phytate, saponin, hemagglutinin, protease inhibitor, amylase inhibitor) is an important strategy to improve enzyme activity. In the food industry, many different approaches have been attempted to enhance the enzyme activity in plant materials or enzyme products. Until recently in the literatures, a new technology which has been developed and patented by the CNI has been found to be capable of doing so. The patented process is called “Metabolic Enzyme Nutrient Exchange Process” (MENEP). It is a biotechnological procedure that can activate metabolic enzymes in plants to overcome their anti-nutritional factor, while ensuring food integrity.

In the MENEP reactor chamber, incubation conditions such as temperature and humidity are precisely and timely controlled to activate the intrinsic metabolic enzymes in plants. At the same time, a considerable amount of anti-nutritional factors are eliminated.

As a consequence, MENEP can increase the enzymatic activity as well as enhance the overall nutritional quality and physiological functions of the plant’s elements (Figure 6).

![Graph showing enzymatic activity increase over time](source: CNI, Malaysia)

_Figure 6. Enzymatic activity can be significantly increased by the MENEP process (patented by CNI, Malaysia)._
1) Can we use hot water to serve enzyme powder?
It depends on the particular type of enzyme. Different enzymes and
different production methods have different temperature limits for
enzyme activity and stability. For example, bromelain can withstand
80°C for 8 minutes, but some other enzymes may be easily deactivated
at high temperature. In general, warm or cold water are therefore
suggested.

2) What are the important criteria in choosing enzyme products?
While purchasing or choosing an appropriate enzyme product for
health improvement or maintenance purposes, consumers are
suggested to pay attention on some important criteria including
enzyme activity, stability, and appropriate choices and combination,
rather than the number of types of enzyme.

3) What is the differences between the enzymes available in powder
and liquid forms?
Comparing the enzymes in powder form with that in liquid form,
the low moisture level allows enzyme powder to have a much
higher stability, flexibility in dosing, and a much longer shelf life. A
large amount of sugar syrup is usually added in liquid enzyme (also
known as fermented fruits and vegetables liquid) to suppress the
growth of microorganisms. In fact, no preservatives are needed in the
manufacturing of enzyme powder.

4) When is the best time to take enzyme?
There is no definite timing for taking enzyme supplements. Depending
on the quantity of enzyme product they may consume each day,
people can consider to separate the daily dosage into two to three
and serve them before or after meal.

5) Is there any age limit to take enzyme supplements?
General speaking, there is no age limit to take enzyme supplements
except those undertaking medications, infants, pregnant and lactating
women. These people are advised to consult health practitioners
before taking any supplementation.