Paper No.: 02

Paper Title: The Principles of the Food Processing & Preservation

Module No. : 28

Module Title: Enzymes As Preservation Aids

28.0 Introduction

Enzymes are proteins that act as catalysts in all living organisms - microorganisms, plants, animals, and humans. Catalysts are compounds that increase the rate of chemical reactions in biological systems. Very small quantities of enzymes can increase the rate of reactions up to ten million times. Enzymes operate within a narrow set of conditions, such as temperature and pH (acidity), and are subject to inhibition by various means.

Enzymes have been used unknowingly in food production, e.g. dough making, for centuries. They can be obtained by extraction from plants or animals or by fermentation from micro-organisms. They are usually purified but may contain varying traces of the other naturally occurring constituents of these three sources. They are normally added to perform a technological function in the manufacture, processing, preparation and treatment of foods. Examples include enzymes used to break down the structure of fruit so that manufacturers can extract more juice, or to convert starch into sugar in alcohol production.

Historically enzymes are considered to be non-toxic and not of safety concern for consumers since they are naturally present in ingredients used to make food. However the development of more efficient production methods and use of new sources such as genetically-modified micro-organisms used by the food industry have resulted in more complex enzymes.

Enzymes are classified by the type of reaction they catalyse and the substance (called a substrate) they act upon. It is customary to attach the suffix "ase" to the name of the principle substrate upon which the enzyme acts. For example, lactose is acted upon by lactase, proteins by proteases, and lipids by lipases. Additionally, many long-used enzymes have common names, such as papain, from papaya, which is used to tenderize meat.

28.1 History of enzymes use in food production

Enzymes extracted from edible plants and the tissues of food animals, as well as those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in food manufacturing.

- 2000 BC: The Egyptians and Sumerians developed fermentation for use in brewing, bread-baking and cheese-making.
- 800 BC: Calves' stomachs and the enzyme, chymosin, were used for cheese-making.
- 1878: The components of yeast cells which cause fermentation were identified and the term "enzyme" was first used, derived from the Greek term meaning "in yeast".
- 1926: Enzymes were first shown to be proteins.
- 1980: Enzyme preparations were developed to improve the digestibility and nutrient-availability of certain animal feeds.
- 1982: The first food application of a product of gene technology, alpha-amylase, took place.
- 1988: Recombinant chymosin was approved and introduced in Switzerland, marking an early approval of a product of gene technology for a food use.
- 1990: Two food processing aids obtained using gene technology: an enzyme for use in cheese-making in the US, and a yeast used in baking in the UK.

28.2 Structure & Mechanisms of enzymes

Enzymes are in general globular proteins and range from just 62 amino acid residues in size, for the monomer of 4-oxalocrotonate tautomerase, to over 2,500 residues in the animal fatty acid synthase. A small number of RNA-based biological catalysts exist, with the most common being the ribosome; these are referred to as either RNA-enzymes or ribozymes. The activities of enzymes are determined by their three-dimensional structure. However, although structure does determine function, predicting a novel enzyme's activity just from its structure is a very difficult problem that has not yet been solved.

Most enzymes are much larger than the substrates they act on, and only a small portion of the enzyme (around 2ï 4 amino acids) is directly involved in catalysis. The region that contains these catalytic residues, binds the substrate, and then carries out the reaction is known as the active site. Enzymes can also contain sites that bind cofactors, which are needed for catalysis. Some enzymes also have binding sites for small molecules, which are often direct or indirect products or substrates of the reaction catalyzed. This binding can serve to increase or decrease the enzyme's activity, providing a means for feedback regulation.

Like all proteins, enzymes are long, linear chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a specific structure, which has unique properties. Individual protein chains may sometimes group together to form a protein complex. Most enzymes can be denaturedð that is, unfolded and inactivatedð by heating or chemical denaturants, which disrupt the three-dimensional structure of the protein. Depending on the enzyme, denaturation may be reversible or irreversible. Structures of enzymes with substrates or substrate analogs during a reaction may be obtained using Time resolved crystallography methods.

28.3 Specificity

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereo specificity, region selectivity and chemo selectivity. Some of the enzymes showing the highest specificity and accuracy are involved in the copying and expression of the genome. These enzymes have "proof-reading" mechanisms. An enzyme such as DNA polymerase catalyzes a reaction in a first step and then checks that the product is correct in a second step. This two-step process results in average error rates of less than 1 error in 100 million reactions in high-fidelity mammalian polymerases. Similar proof reading mechanisms are also found in RNA polymerase, aminoacyl tRNA synthetases and ribosomes. Whereas some enzymes have broad-specificity, as they can act on a relatively broad range of different physiologically relevant substrates, many enzymes possess small side activities which arose fortuitously (i.e. neutrally), which may be the starting point for the evolutionary selection of a new function; this phenomenon is known as enzyme promiscuity.

28.4 "Lock and key" model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve.

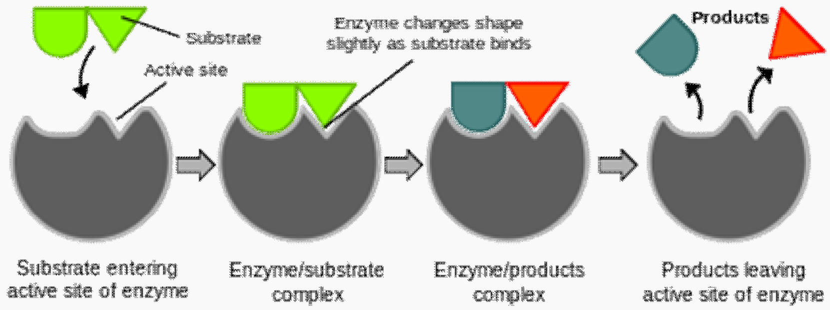

Figure 1: Induced fit hypothesis of enzyme action

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism. Based on Fischer's lock-and-key model and Koshlandôs induced fit theory, the Chouôs distorted key theory for peptide drugs was proposed to develop peptide drugs against HIV/AIDS and SARS.

28.5 Application of enzymes in food processing & preservation

Today, enzymes are used for an increasing range of applications: bakery, cheese making, starch processing and production of fruit juices and other drinks. They can improve texture, appearance and nutritional value, and may generate desirable flavours and aromas. Currently-used food enzymes sometimes originate in animals and plants (for example, a starch-digesting enzyme, amylase, can be obtained from germinating barley seeds) but most come from a range of beneficial micro-organisms. In food production, enzymes have a number of advantages:

- They are welcomed as alternatives to traditional chemical-based technology, and can replace synthetic chemicals in many processes. This can allow real advances in the environmental performance of production processes, through lower energy consumption and biodegradability.
- They are more specific in their action than synthetic chemicals. Processes which use enzymes therefore have fewer side reactions and waste by-products, giving higher quality products and reducing the likelihood of pollution.
- They allow some processes to be carried out which would otherwise be impossible. An example is the production of clear apple juice concentrate, which relies on the use of the enzyme, pectinase.

Table 1: Some uses of enzymes in food production

Market	**Enzyme**	**Purpose / function**
Dairy	Rennet (protease)	Coagulant in cheese production
	Lactase	Hydrolysis of lactose to give lactose-free milk products
	Protease	Hydrolysis of whey proteins
	Catalases	Removal of hydrogen peroxide

Brewing	Cellulases, beta-glucanases, alpha amylases, proteases, maltogenic amylases	For liquefaction, clarification and to supplement malt enzymes
Alcohol production	Amyloglucosidase	Conversion of starch to sugar
Baking	Alpha-amylases	Breakdown of starch, maltose production
	Amyloglycosidases	Saccharification
	Maltogen amylase (Novamyl)	Delays process by which bread becomes stale
	Protease	Breakdown of proteins
	Pentosanase	Breakdown of pentosan, leading to reduced gluten production
	Glucose oxidase	Stability of dough
Wine and fruit juice	Pectinase	Increase of yield and juice clarification
	Glucose oxidase	Oxygen removal
	Beta-glucanases	
Meat	Protease	Meat tenderising
	Papain, Bromelin, Ficin	
Protein	Proteases, trypsin, aminopeptidases	Breakdown of various components
Starch	Alpha amylase, glucoamylases, hemicellulases, maltogenic amylases, glucose isomerases	Modification and conversion (eg to dextrose or high fructose syrups) dextranases, beta-glucanases
Inulin	Inulinases	Production of fructose syrups

28.6 Modern production of food enzymes

In the twentieth century, enzymes began to be isolated from living cells, which led to their large-scale commercial production and wider application in the food industry. Today, microorganisms are the most important source of commercial enzymes. Although microorganisms do not contain the same enzymes as plants or animals, a microorganism can usually be found that produces a related enzyme that will catalyse the desired reaction. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques.

Direct genetic modification (biotechnology) encompasses the most precise methods for optimizing microorganisms for the production of enzymes. These methods are used to obtain high-yielding production organisms. Biotechnology also provides the tools to have a genetic sequence from a plant, animal, or a microorganism, from which commercial scale enzyme production is not adequate, to be transferred to a microorganism that has a safe history of enzyme production for food use.

Although the production organism is genetically modified the enzyme it produces is not. Enzymes produced through biotechnology are identical to those found in nature. Additionally, enzymes produced by microorganisms are extracted and purified before they are used in food manufacturing. Genetically modified microorganisms are useful from a commercial standpoint but would not survive in nature.

At present, modern biotechnology can be used to give a range of advances in enzymatic production technology:

- Improved productivity and cost-effectiveness in existing processes. By producing enzymes more efficiently, the amount of raw materials, energy and water needed to make a product can be reduced by as much as one-half by changing from a traditional strain of microbe to a genetically modified one.
- Companies can tailor their enzymes more precisely to customer demands for products with specific properties.
- Manufacturers can supply enzymes which otherwise could not be produced in large enough quantities, giving the consumer access to a wider variety of products. An example is the amylase-based product which makes bread stay fresh for longer.

28.7 The possibility of allergies

To date, there have been no reports of consumer allergies to enzyme residues in food. The levels of enzyme residues appearing in foods are so low that they are highly unlikely ever to cause allergies. Like all proteins, enzymes can cause allergic reactions when people have been sensitised through exposure to large quantities. For this reason, enzyme companies take a variety of protective measures and some enzymes are produced as liquids, granules, in capsules or as immobilised preparations to limit worker exposure.

A Gateway to All Post Gra