Chapter 1

INTRODUCTION

1.1 INTRODUCTION

Enzymes are proteins whose biological function is the catalysis of chemical reactions in living systems. Enzymes are composed of two parts, a protein portion called as apoenzyme and a nonprotein portion, either a coenzyme (organic) or cofactor (inorganic). Enzymes combine with the substances on which they act called as substrate to form intermediate enzyme-substrate complex, which is then converted to a reaction product, and liberated enzyme, which continues its catalytic function. Enzymes are highly specific; a few exhibit absolute specificity and catalyze only one particular reaction, while others are specific for a particular type of chemical bond, functional group or stereoisomeric structure [1].

The Commission of Enzymes (EC) of the International Union of Biochemistry has classified the enzymes into six general groups:

1. Hydrolases,
2. Isomerases,
3. Ligases,
4. Lyases,
5. Oxidoreductases
6. Transferases

The enzymes involved in food decomposition and in the digestive process are hydrolases. When raw food is ingested, enzymes present within the food are released, thereby assisting the body's digestive processes in breaking down the food into its simplest components for utilization within the body.
There are several categories of food enzymes present such as:

1) Lipase which breaks down fats into free fatty acids and glycerol
2) Protease which breaks down long protein chain into smaller amino acid chains
3) Amylase, which reduces large carbohydrates to disaccharide including sucrose, lactose and maltose.
4) Lactase which breaks down lactose

Enzymatic activity begins in the mouth where salivary amylase, lingual lipase, and ptyalin initiate starch and fat digestion. In the stomach, hydrochloric acid

activates pepsinogen to secrete pepsin which breaks down protein, and gastric lipase begins the hydrolysis of fats. Most of digestion and absorption takes place in the small intestine and is mediated by pancreatic amylase, protease, lipase, and bile. Without proper enzyme production, the body has a harder time digesting food which may lead to a variety of chronic disorders [2].

Today millions of people are suffering from various digestive disorders. Most food enzymes are essentially destroyed under the conditions used to cook and process food, leaving foods devoid of enzyme activity. Placing the full digestive burden on the body, the body's digestive process can become over stressed. In addition, problems with digestion can occur simply as a result of aging. It is fairly common for elderly individuals to experience both a decrease in hydrochloric acid production as well as a general decline in digestive enzyme secretion [3].

With an increasing aging population burdened with unhealthful diets and stressful life styles, it is likely that healthcare professionals will see more and more patients developing digestive problems. Currently, it is estimated that fifty-eight percent of the population suffers from some type of digestive disorder [4].

Digestive problems can result, causing improper digestion and malabsorption of nutrients. Consequences of malabsorption can include impaired immunity, allergic reaction, poor wound healing, skin problems and mood swings. Supplemental enzymes can improve the level of digestion and help assure that the maximum level of nutrient absorption is attained.

Supplemental enzymes of microbial and plant origin work at the pH found in the upper stomach. Food sits in the upper portion of the stomach for as long as an hour before gastric secretions begins their action. Several studies have shown that the enzymes in saliva continue their digestive activity in the upper stomach and can digest partially protein, starch and fat during the 30 to 60 minutes after consumption. Although salivary enzymes accomplish a significant amount of digestion, their activity is limited to a pH level above 5. Supplemental microbial enzymes, and some plant enzymes, are active in the

pH range of 3 to 9 and can facilitate the hydrolysis of a much larger amount of protein, carbohydrates and fat before hydrochloric acid is secreted in sufficient amounts to neutralize their activity. Obviously, these enzymes can contribute significantly in improving food nutrient utilization [5-8].

The alpha amylase (or diastase) is an enzyme that catalyzes the hydrolysis of starch into smaller molecules like maltose and glucose. Carbohydrate digestion occurs primarily in the small intestine. Most carbohydrates are easily digested and 90% to 98% are absorbed. Bile acids in the stomach aid in acid hydrolysis to break down sucrose into glucose and fructose. Before the stomach pH falls below 6.5, up to 50 % of starches may be partially broken down but digestion of most carbohydrates is completed in the small intestine [9].

Starch is composed of approximately 20 % amylose and 80 % amylopectin. Amylose is the straight chain made up of glucose monomers with alpha-1, 4 glucosidic bonds. The long chain forms a spiral with each loop composed of six glucose molecules. It imparts gelatinization. Starch amylose forms a blue colour in the presence of iodine. Amylopectin is the branched section and is also made up of glucose monomers with apha-1, 6 glucosidic linkages.

The fungal alpha amylase attacks the second linkage from the nonreducing terminals (i.e. C4 end) of the straight segment, resulting in the splitting off of two glucose units at a time and the product is a disaccharide called maltose [10].

Digestive enzymes are used for indication of indigestion, flatulence, bloating, anorexia, dyspepsia, hepatic and pancreatic insufficiency, post operative digestive upsets and convalescence. In several conditions that cause malabsorption, such as pancreatic insufficiency and cystic fibrosis, doctors sometimes prescribe digestive enzymes to improve absorption of food.

Doctors often tell people to try using pancreatic enzymes with meals when they have symptoms of indigestion that cannot be attributed to a specific

cause. In a double-blind study, microencapsulated pancreatic enzymes were shown to reduce gas, bloating, and fullness after a high-fat meal [11].

People with pancreatic insufficiency and cystic fibrosis frequently require supplemental pancreatic enzymes (which include proteolytic enzymes, lipases, and amylases). In addition, those with celiac disease or Crohn's disease and perhaps some people suffering from indigestion may be deficient in pancreatic enzymes [12, 13].

In addition to consuming adequate levels of raw foods, a common approach to supporting the patient with digestive enzyme inadequacy is oral enzyme replacement. This entails providing enzyme supplements, whether they be animal or nonanimal derived, in the quantity necessary to maintain adequate digestive capacity and facilitate absorption of essential nutrients.

Oral enzyme replacement has proven to be helpful over many years of use [14-18]. In addition to the well known benefits associated with enzyme replacement, it has been suggested that oral supplementation with enzymes may have a sparing effect on the body's own digestive enzymes [19, 20].

Enzyme therapy involves using an array of enzymes, or complex proteins from plants and animals, in supplement form to aid digestion and treat a wide variety of maladies thought to stem from nutritional problems. These enzyme supplements are believed to bolster the thousands of natural enzymes produced by the human body and also obtained from foods, which not only fuel digestion but also aid hundreds of other processes essential for life.

Indigestion is the term used to describe pain and discomfort in the upper abdomen or chest that can develop after a meal. The medical term for it is dyspepsia. Sometimes a burning feeling is felt in the chest, and this is known as heartburn. People of all ages including children and both sexes are affected by indigestion. Most people have suffered from indigestion after a large meal or after excessive alcoholic consumption at some time, especially after a holiday meal or party. Every year about 6.5 million people in USA [21], 0.7

million people in Canada and 23.5 million people in India suffer from indigestion [22].

Alpha amylase is produced from the pancreas of higher animals such as swine and cattle, higher plants such as barley and by microorganisms including bacteria and fungi. Among these sources, fungi produce in general, highly active alpha amylase and gradually replacing amylase preparations produced by higher plants and animal. The first commercial enzyme from a fungus *Aspergillus oryzae* was Taka-diastase produced by Dr. Jokichi Takamine in 1894. Since then, major enzyme manufacturers continued production of fungal diastase by a unique tray culture fermentation process using *Aspergillus oryzae* which is cultured on wheat bran and the produced enzyme is extracted with water, purified with alcohol and diluted with suitable diluents and made them available for agriculture, food, beverages, animal feed and pharmaceutical industries.

Enzymes are proteins and proteins are constructed from chain of amino acids. As an unfolded chain, the enzyme has no activity. Only the folded structure forms the catalytic or active site. However this folded structure will generally be held together by noncovalent interactions such as ionic bridges, hydrogen bonds, hydrophobic and hydrophilic interactions and so on. As the temperature increases to optimum, the rate at which an enzyme catalyses the breakdown of starch increases. The amylase has an optimum shape or flexibility and will hold this ideal shape at the optimum temperature.

As temperature increases over the optimum, chemically chances of starch breakdown increases, also increases the chances of breakdown of three-dimensional structure of the enzyme. As the heat in the system increases, the vibrational energy of the entire alpha amylase molecule also increases. This puts a strain on the weak interactions that hold the enzyme together. At higher temperature these bonds get apart and the three dimensional structure of proteins destabilizes, this is called denaturation. Other forces that also disrupt these bonds will have same effect: extremes of pH, extreme concentrations of salt and so on [23].

The stabilization of enzymatic activity is a standing problem in all areas of technology where enzymes are likely to be used. Stability in this sense stands for resistance to decrease in enzymatic activity prior to usage e.g. under storage conditions. Enzyme stability problems are most important when the enzyme containing composition or additive formulated with water or is used in aqueous solutions [24].

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