Review

A review of the biochemical, biotechnological and other applications of enzymes

Soetan, K. O.[1]*, Aiyelaagbe, O. O.[2] and Olaiya, C. O.[3]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.
[2]Department of Chemistry, University of Ibadan, Nigeria.
[3]Department of Biochemistry, University of Ibadan, Nigeria.

Enzymes are proteins evolved by the cells of living organisms. Their specific function is to catalyse chemical reactions. Enzymes increase the rate at which reactions approach equilibrium. Enzymes have found wide and diverse applications in different disciplines and fields of human endeavours. Enzymes play critical role in the metabolic activities of all living organisms, whether humans, animals, plants or microorganisms and are widely applied in agriculture, biochemistry, biotechnology, chemistry, genetics, industries, human and veterinary medicine, pharmacy, research, etc. The different applications of enzymes abound in the literature. The aim of this review is to discuss the diverse applications of enzymes for the purpose of teaching, education and research.

Key words: Biochemical, biotechnological, applications, enzymes.

INTRODUCTION

Enzymes are biological catalysts that increase the rate of chemical reactions taking place within living cells without themselves undergoing any overall change (Palmer, 2001). Enzymes play vital roles in biological systems by acting as catalysts. A catalyst is necessary in such systems because, at the temperature and pH of the human and animal body, reactions would not occur at a rate sufficient to support rapid muscular activity, nerve impulse generation and all the other processes required to support life (Devlin, 1986). The investigation and interpretation of changes in serum enzymes in diseases is one of the most rapidly expanding fields in clinical biochemistry (Chatterjea and Shinde, 2002).

Enzymes are unchanged by their action on their substrates; they remain as they are and active until denatured by heat or other factors or until the substrate is exhausted. Depending on the process, enzymes may be removed from the final product, or denatured and left in, or may even be potentially active. How they are labelled in final product formulations should be dependent on the specific outcome for the product in question.

Enzymes have wide or extensive applications in diffe-rent fields of research or areas of life. In human and veterinary medicine, the assay of plasma enzymes can be very useful in helping to confirm the diagnosis of diseases in humans and animals. In recombinant DNA technology (sometimes called genetic engineering), varieties of enzymes are used, e.g. restriction endo-nucleases (from bacteria) and DNA ligases (from bacteria or bacteriophages), to insert extra genes into cells with the help of vehicles termed vectors. The diagnosis of an inborn error of metabolism can be established by demon-strating the reduced activity of a particular enzyme in a tissue, or by revealing a gene abnormality by recom-binant DNA technology (Palmer, 2001). Enzymes are also applied in the management and treatment of some diseases. Enzymes are used as reagents in clinical biochemistry and in the industry. Enzyme assay is very important in the food and drink industries in monitoring the processing and storage of foods and drinks and as a means of detecting microbial contamination. All these applications will be discussed in details.

Due to the importance of enzymes and its wide applica-tions in biochemistry, biotechnology, chemistry, genetics, industries, human and veterinary medicine etc., this review is an attempt to gather together the different uses and applications of enzymes for the purposes of teaching and research.

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*Corresponding author. E -mail: soetangboye@yahoo.com.

BIOMEDICAL IMPORTANCE OF ENZYMES

Enzymes are biologic polymers that catalyze the multiple dynamic processes which makes life processes possible. Enzymes play central role in health and disease because they are determinants of the rates at which physiologic events take place. The breakdown of foods to supply energy and chemical building blocks, the assembly of these building blocks into proteins, membranes and the DNA that encodes genetic information and the harnessing of energy to produce cell movement are all made possible by the carefully coordinated actions of enzymes (Murray et al., 2000).

While in health, all biochemical and physiological processes occur in an ordered, regulated manner and homeostasis is maintained, homeostasis can be profoundly disturbed in pathologic states. For example, the severe tissue injury that catalyses liver cirrhosis can profoundly impair the ability of cells to form the enzymes which catalyze a key metabolic process such as urea synthesis. The resultant inability to convert toxic ammonia to non toxic urea is then followed by ammonia intoxication and ultimately hepatic coma (Murray et al., 2000). A series of rare but frequently debilitating and often fatal genetic diseases are additional examples of the drastic physiologic consequences that can follow impairment of the activity of a single enzyme.

Following severe tissue injury (e.g. cardiac or lung infarct, crushed limb, liver damage or trauma to the liver) or uncontrolled cell growth (e.g. prostatic carcinoma), enzymes that may be unique to specific tissues are released into the blood. Measurement of these intracellular enzymes in blood serum therefore provides medical and veterinary doctors with invaluable information on the diagnosis and prognosis of diseases in the human and animal body/system.

Enzyme Commission (E.C.)

This is a special commission of the International Union of Biochemistry (IUB) that made recommendations for the classification and naming of enzymes and for the definitions of the mathematical constants used in enzymology. The recommendations were first published in 1964 and were published in revised form in 1972, 1978 and 1984 (Stenesh, 1989).

Enzyme classification

This is the systematic arrangement and the naming of enzymes that is based on the 1972 recommendations of the Enzyme Commission of the International Union of Biochemistry (Stenesh, 1989). Reactions and the enzymes that catalyze them form 6 classes, each having 4-13 subclasses. The enzyme name has 2 parts. The first names the substrate or substrates. The second, ending in-ase, indicates the type of reaction catalysed (Murray et al., 2000). Each enzyme is denoted by a number composed of four figures. The first figure denotes one of the six main divisions: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. The second figure denotes the subclass and the third figure denotes the sub-subclass. The last figure denotes the serial number of the enzyme in its sub-subclass. The enzyme number is preceded by the abbreviation E.C. (Stenesh, 1989). For example, E.C.2.7.1.1 denotes class 2 (a transferase), subclass 7 (transfer of phosphate), sub-subclass 1 (an alcohol is the phosphate acceptor). The final digit denotes hexokinase, or ATP: D-hexose 6 - phos-photransferase, an enzyme catalyzing phosphate transfer from ATP to the hydroxyl group on carbon 6 of glucose (Murray et al., 2000). There are 6 classes of enzymes. They are:

(i) Oxidoreductases: These enzymes are involved in oxidations and reductions of their substrates e.g. alcohol dehydrogenase, lactate dehydrogenase, xanthine oxidase, glutathione reductase, glucose-6-phosphate dehydrogenase.
(ii) Transferases: These enzymes catalyze the transfer of a particular group from one substrate to another e.g. aspartate amino transferase (AST), alanine aminotransferase (ALT), hexokinase, phosphoglucomutase, hexose-1-phosphate uridyltransferase, ornithine carbamoyl transferase etc.
(iii) Hydrolases: These enzymes bring about hydrolysis e.g. glucose- 6 -phosphatase, pepsin, trypsin, esterases, glycoside hydrolases etc.
(iv) Lyases: These are enzymes that facilitate the removal of small molecule from a large substrate e.g. fumarase, argino succinase, histidine decarboxylase.
(v) Isomerases: These enzymes are involved in isomerization of substrate e.g. UDP-glucose, epimerase, retinal isomerase, racemases, triose phosphate isomerase.
(vi) Ligases: These enzymes are involved in joining together 2 substrates e.g. alanyl-t-RNA synthetase, glutamine synthetase, DNA ligases (Chatterjea and Shinde, 2002).

Enzyme specificity

Specificity is an important characteristic of enzymes. The 3 different types of enzyme specificity/specificities are:

(i) Stereochemical specificity.
(ii) Reaction specificity.
(iii) Substrate specificity, which can be absolute or relative substrate specificity (Chatterjea and Shinde, 2002).

Enzyme kinetics

This is the kinetics of enzyme-catalyzed reactions. It in-

cludes derivations of rate equations and graphical analysis of experimental data for all types of enzyme reactions such as single or multiple substrate reactions, uninhibited or inhibited reactions, equilibrium or steady-state systems (Stenesh, 1989).

Immobilized enzyme

This is an enzyme that is physically confined while it carries out its catalytic function. This may occur naturally, as in the case of particulate enzymes, or it may be produced artificially by chemical or by physical methods (Stenesh, 1989). In the chemical methods, the enzyme is linked covalently to a support. These methods include attachment of the enzyme to a water-insoluble support, incorporation of the enzyme into a growing polymer chain, or cross linking of the enzyme with a multifunctional low molecular weight reagent. In the physical methods, the enzyme is not linked covalently to a support. These methods include adsorption of the enzyme to a water-insoluble matrix, entrapment of the enzyme within either a water-insoluble gel or a microcapsule, or containment of the enzyme within special devices equipped with semipermeable membrane (Stenesh, 1989). Enzyme immobilization technique has 2 advantages:

(i) Expensive enzymes can be recovered and used again.
(ii) The enzyme can also be used in a variety of configurations of bioreactors that permit continous operation (Coombs, 1992).

COMMERCIAL APPLICATIONS OF IMMOBILIZED ENZYMES

Immobilised enzymes are mainly used in the production of high fructose syrups (also known as HFCS or isoglucose). Other commercial uses include production of semi-synthetic penicillins, the hydrolysis of starch using amyloglucosidase and the resolution of DL-mixtures of amino acids (Coombs, 1992). Immobilized enzymes also have applications in the medical sciences.

Enzymes and coenzymes

Many enzymes require a coenzyme which functions as group transfer reagents. Many enzymes that catalyze group transfer and other reactions require, in addition to their substrate, a second organic molecule known as a coenzyme, without which they are inactive. Coenzymes expand the repertoire of the catalytic capabilities of an enzyme far beyond those offered by the functional groups alone of the amino acids that constitute the bulk of the enzyme. Coenzymes that are tightly associated with an enzyme through either covalent bonding or non covalent forces are often referred to as prosthetic groups (Murray et al., 2000).

Coenzymes that are freely diffusible generally serve as continually recycled carriers of hydrogen, flavin adenine dinucleotide (reduced) (FADH), hydride nicotinamide adenine dinucleotide (reduced) (NADH) and nicotinamide adenine dinucleotide phosphate (reduced) (NADPH), or chemical units such as acyl groups (coenzyme A) or methyl groups (folates), shuttling them between their points of generation and consumption. These latter coenzymes can thus be considered as second substrates (Murray et al., 2000).

Enzymes that require coenzymes include those which catalyze oxidoreductions, group transfer and isomerization reactions and reactions that form covalent bonds (IUB classes 1, 2, 5 and 6). Lytic reactions, including the hydrolytic reactions catalyzed by the digestive enzymes, do not require coenzymes.

Classification of coenzymes

Coenzymes can be classified according to the group whose transfer they facilitate. Based on the above concept, coenzymes may be classified as follows:

(i) Coenzymes involved in transfer of groups other than hydrogen: Biotin, CoA-SH, cobamide (B_{12}) coenzymes, folate coenzymes, pyridoxal phosphate, lipoic acid, sugar phosphates, thiamine pyrophosphate.
(ii) Coenzymes involved in transfer of hydrogen.
Nicotinamide adenine dinucleotide (oxidized) (NAD^+), Nicotinamide adenine dinucleotide phosphate (oxidized) ($NADP^+$).
Flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD), lipoic acid, coenzyme Q (Murray et al., 2000).

METHODOLOGY OF ENZYME PRODUCTION

Enzymes are produced by cellular anabolism, the naturally occurring biological process of making more complex molecules from simpler ones. Source organisms include bacteria, fungi, higher plants and animals (White and White, 1997). Enzymes may be extracted from a given source organism by a number of different methods (Nielsen et al., 1991). Most of the organisms that produce commercial enzymes are fungi. These organisms are molds *Rhizopus oryzae*, *Aspergillus niger*, *Rhizomucor meihei*, blights such as *Endothia parasitica* and yeasts such as *Saccharomyces* spp. and *Candida* spp.

A considerable amount of research has been conducted on genetically modifying fungi or other organisms to increase the yields and consistencies of enzymes.

Many of the prospective donor organisms are reported to be pathogenic and are being screened for genetic sequences to be inserted into non-pathogenic hosts (Surgey et al., 1996). Classical methods of hybrid-dization can also be used to improve enzyme-producing organisms (Solis et al., 1997).

Properties

Enzyme preparations may consist of whole cells, parts of cells or cell-free extracts from the source used. Enzymes may be in liquid, semi-liquid or dry form. Enzymes in general are readily soluble in water. They are practically insoluble in alcohol, in chloroform and in ether. The colour of preparations may vary from virtually colourless to dark brown. Individual preparations are generally cha-racterized by functionality and activity rather than the properties of the product (NAS, 1981).

APPLICATIONS OF ENZYMES

Enzymes have a wide variety of uses (ETA, 1999). They are used in the industries (food and drinks, pharma-ceutical etc), they are used for research purposes, medical applications, commercial applications etc.

Enzymes with biochemical applications

Many enzymes are used in biochemical systems. Some of these are:

a) aa-tRNA synthetase: It is used for charging a specific tRNA molecule with the appropriate amino acid.
b) Adenylate cyclase: It produces cyclic Adenosine monophosphate (cAMP).
c) Aspartokinase: This enzyme catalyses the initial step in the pathway for the biosynthesis of several amino acids. It's a key regulatory step.
d) ß-galactosidase: This enzyme splits lactose to the constituent monosaccharides glucose and galactose. It is the first step in lactose fermentation.
e) ß-lactamase: This enzyme hydrolyses the ß-lactam bond in the nucleus of penicillins and cephalosporins. It is responsible for penicillin resistance.
f) Chloramphenicol acetyltransferase (CAT): This enzyme causes chloramphenicol resistance, by inactivating the antibiotic and is used as a selective marker and as a reporter gene.
g) Glutamate dehydrogenase: It is important in ammonia assimilation.
h) Luciferase: This enzyme catalyses a light-emitting reaction. It is used as a reporter.

i) Resolvase: It catalyses site-specific recombination to resolve the co-integrate intermediate in transposition into two separate molecules.

Enzymes with biotechnological applications

There are many enzymes employed in biotechnological operations. These include:

1) Alkaline phosphatase: In gene cloning, this enzyme is used to remove phosphate groups from the 5^I end of DNA molecules and also used as a reporter gene for identification of secretion signals.
2) Exonuclease: This is an enzyme that removes nucleotides from the ends of DNA fragments. A 5^I----3^I exonuclease removes nucleotides from the 5^I end, while a 3^I----5^I exonuclease removes nucleotides from the 3^I end.
3) DNA polymerase 1: It is primarily known as a 'repair' polymerase, which fills in single-stranded gaps. It is also involved in repair of the gaps formed on the lagging strand during replication. It also possesses both 5^I --- 3^I and 3^I --- 5^I exonuclease activity.
4) Klenow fragment of *E. coli* DNA polymerase 1: This enzyme is used for sequencing DNA using the Sanger Dideoxy System, filling the 3 recessed termini of restriction enzyme treated DNA and also used for labeling the termini of DNA fragments (Daini, 2000). The enzyme is also used for second strand cDNA synthesis in the cDNA procedures. 'Klenow fragment' is a proteolytic cleavage of DNA polymerase I. It leaves a fragment that is devoid of 5^I---3^I exonuclease activity.
5) DNA polymerase III: This is the main 'replication' polymerase.
6) Helicase: It unwinds DNA, for example in conjugal plasmid transfer.
7) T4 DNA polymerase: This enzyme is used in the labeling of DNA fragments for use as hybridization probes.
8) Terminal deoxynucleotidyl transferase: This enzyme is isolated from calf thymus and catalyses the addition of dNTP to the 3^I-OH of DNA molecules. One of the primary uses of terminal transferase is the tailing of vectors and cDNA with complementary bases, thus permitting the cloning of the cDNA fragments (Daini, 2000). It can also be used for labeling of 3^I ends of DNA fragments.
9) T4 Polynucleotide kinase: This enzyme is isolated from T4 infected *E. coli* and catalyzes the transfer of α phosphate of ATP to a 5^I-OH end in DNA or RNA. It is also used for the labeling of 5^I termini of DNA for Maxam and Gilbert DNA sequencing and the phosphorylation of DNA lacking 5^I-P termini.
10) Reverse transcriptase: This is an enzyme coded for by avian myeloblastosis virus. It catalyses the synthesis of cDNA from an RNA template. It can also be used for the labelling of termini of DNA with extended 5^I ends.
11) Restriction endonucleases: Restriction endonuclea-

ses are enzymes that cut DNA molecules at specific positions. It recognizes 'foreign' (unmodified) DNA at a specific site, and degrades it by internal cleavage. Most commonly used are the type II restriction enzymes, which cut within the recognition site. Some examples are EcoRI from *E. coli,* HindIII from *Haemophilus influenzae*, BamHI from *Bacillus amyloliquefaciens,* PstI from *Providencia stuartii,* SmaI from *Serratia marcescens,* Sau3A from *Staphylococcus aureus,* AluI from *Arthrobacter luteus,* TagI from *Thermus aquaticus and* HpaII from *Haemophilus parainfluenza*.

A uniform nomenclature has been developed to simplify the naming of the restriction endonucleases (Smith and Nathans, 1973). The designation of these enzymes come from the source organism, using the first letter of the genus name plus the first 2 letters of the species, with additional letters/numbers to indicate the specific enzyme (since one species may produce several different restriction enzymes) (Dale, 2001).

12) T4 DNA Ligase: This enzyme catalyses the formation of a phosphodiester bond between 3^l-OH and 5^l- phosphate ends in DNA using DNA molecules with cohesive ends as substrate (Daini, 2000).

13) Exonuclease III: This enzyme is used for generating linear template DNA for the dideoxysequencing technique and generating staggered ends on dsDNA due to its 3^l --- - 5^l exonuclease activity.

14) λ Exonuclease: This enzyme recognizes the terminal 5^l-phosphate of dsDNA for its exonuclease activity. Its primary use is the removal of protruding 5^l terminus from dsDNA which is needed for the terminal transferase tailing of DNA (Daini, 2000).

15) SI Endonuclease: This enzyme is isolated from *Aspergilus cryzae* and it acts exclusively on ssDNA or RNA. It can break supercoiled DNA because it contains ss bubbles. It can also be used to distinguish supercoiled from both non-supercoiled, covalent circles and nicked circular DNA, both of which are resistant to the enzyme.

16) Ligase: It seals single-stranded gaps (nicks) in double-stranded DNA. It is also used for the formation of recombinant DNA molecules in gene cloning.

17) Polynucleotide kinase: It transfers a phosphate group from ATP to the 5^l OH end of DNA or RNA.

18) Primase: This is a special RNA polymerase, which makes a short primer required for DNA synthesis.

19) Replicase: This is RNA-directed RNA polymerase used in replication of some RNA viruses.

20) Reverse transcriptase: This is RNA-directed DNA polymerase. It synthesizes DNA (complementary DNA) using mRNA template.

21) Ribonuclease (RNase): This enzyme degrades RNA molecules.

22) RNA Polymerase: This enzyme synthesizes RNA, using a DNA template.

23) RNase H: This is a specific RNAse which cuts RNA---DNA hybrids. It is involved in replication of ColE1-like plasmids.

24) S1 nuclease: This enzyme degrades single-stranded DNA.

25) Terminal transferase: This adds nucleotides to the 3^l end of DNA, without requiring a template strand.

26) Topoisomerase: This is a class of enzymes that alters the conformation of DNA, for example by changing the degree of winding or supercoiling.

27) Transposase: This enzyme catalyses the initial steps in transposition.

Enzymes as markers for disease

Some enzymes are found only in specific tissues or in a limited number of such tissues. For example, lactase dehydrogenase (LDH) has 2 different forms, called isozymes, in heart and skeletal muscle (Campbell, 1999). The 2 forms differ slightly in amino acid composition and can be separated on the basis of charge as a result. Since LDH is a tetramer of four subunits, it too can exist in 5 different forms depending on the source of the subunits. An increase of any form of LDH in the blood indicates some kind of tissue damage. A heart attack can usually be diagnosed with certainty if there is an increase of LDH from heart. Also, there are different forms of creatine kinase (CK), an enzyme that occurs in the brain, heart and skeletal muscle. Appearance of the brain type can indicate a stroke or a brain tumour, whereas the heart type indicates a heart attack. After a heart attack, CK shows up more rapidly in the blood than LDH. Monitoring the presence of both enzymes extends the possibility of diagnosis, which is useful, since a very mild heart attack might be difficult to diagnose. An elevated level of the isozyme from heart in blood is a definite indication of damage to the heart tissue (Campbell, 1999).

Another useful enzyme assayed is acetyl cholinesterase (AChE), which is important in controlling certain nerve impulses. Many pesticides affect this enzyme, so farm workers are often tested to be sure that they have not received inappropriate exposure to these important agricultural toxins (Campbell, 1999). There are several enzymes that are typically used in the clinical laboratory to diagnose diseases. There are highly specific markers for enzymes active in the pancrease, red blood cells, liver, heart, brain, prostate gland and many of the endocrine glands. Since these enzymes are relatively easy to assay using automated techniques, they are part of the standard blood test veterinary and medical doctors are likely to need in the diagnosis and treatment/ management of diseases.

Enzymes utilized in clinical diagnoses of diseases

Many enzymes are involved in the clinical diagnoses of various diseases in human and veterinary medicine. These enzymes facilitate or enhance rapid diagnoses of

these diseases. These enzymes could be classified into many classes. They are:

1) Alkaline Phosphatase (ALP; EC 3.1.3.1): Alkaline phosphatases were the earliest serum enzymes to be recognized to have clinical significance, when in the 1920s, it was discovered that they increase in bone and liver diseases (Kaneko, 1989). Since then, they have been the subject of more publications than any other enzyme (Stigbrand et al., 1984). Alkaline phosphates are a group of isoforms which hydrolyse many types of phosphate esters, whose natural substrate or substrates are unknown. The term "alkaline" refers to the optimal alkaline pH of this class of phosphatases *in vitro*.

In both humans and animals, the major sources of ALPs are the liver, bone, kidney and placenta. In humans, it is involved in bone and hepatobiliary diseases. ALPs are also of diagnostic importance in animal diseases. Total serum ALP activity has diagnostic value in the hepatic and bone diseases in dogs and cats (Kaneko, 1989). It is of little value in hepatic diseases of horses and ruminants because of the broad range of reference values against which the patients' values must be compared. The range of serum ALP value in goats may be 10-fold with no evidence of hepatic damage. Values within the individual are fairly constant for sequential evaluation (Kaneko, 1989).

2) Creatine Kinase (CK; EC 2.7.3.2): Creatine kinase isozymes are the most organ-specific serum enzymes in clinical use (Kaneko, 1989). They catalyse the reversible phosphorylation of creatine by ATP to form creatine phosphate, the major storage form of high-energy phosphate required by muscle. Creatine kinases are found in many parts of the body like the heart, brain, skeletal muscle and smooth muscle (Nduka, 1999), but they have their highest specific activity in the skeletal muscle (Kaneko, 1989). In humans, CK is associated with myocardial infarction and muscle diseases (Nduka, 1999). Increase in CK in cerebrospinal fluid have been associated with a number of disorders in dogs, cats, cattle and horses (Mayhew et al., 1977; Wilson, 1977). The CKs are such sensitive indicators of muscle damage that, generally, only large increases in serum activity are of clinical significance (Lewis, 1978).

3) Alanine Aminotransferase (ALT; EC 2.6.1.2): It was formerly known as glutamic puruvate transaminase; GPT). It catalyses the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and glutamate in the cytoplasm of the cell. ALT can be found in the liver, skeletal muscle and heart (Nduka, 1999). The greatest specific activity of ALT in primates, dogs, cats, rabbits and rats is in the liver. It is a well established, sensitive liver-specific indicator of damage. However, ALT in the tissues of pigs, horses, cattle, sheep or goats is too low to be of diagnostic value (Boyd, 1983). It is used as an indicator of hepatopathy in toxicological studies which use small laboratory rodents as well as dogs (Kaneko,

1989).

4) Aspartate Aminotransferase (AST; EC 2.6.1.1): It was formerly called glutamic oxaloacetic transaminase; GOT). It catalyses the transamination of L-aspartate and 2-oxo-glutarate to oxaloacetate and glutamate. AST is found in skeletal muscle, heart, liver, kidney and erythrocytes and is associated with myocardial, hepatic parenchymal and muscle diseases in humans and animals. The pre-sence of AST in so many tissues make their serum level a good marker of soft tissue but precludes its use as an organ-specific enzyme (Boyd, 1983). Red blood cells contain a large amount of AST which leaks into plasma before haemolysis is seen (Kaneko, 1989).

5) Sorbitol Dehydrogenase (SDH; EC 1.1.1.14): It is also called L-iditol dehydrogenase; IDH. It catalyses the reversible oxidation of D-sorbitol to D-fructose with the cofactor NAD (Gerlich, 1983; Lessing and McGuiness, 1982). The plasma activity is low in dog and horse plasma but appreciably greater in cattle, sheep, and goat serum. Aside from the testes, it is found in appreciable amounts only in hepatocytes (Boyd, 1983). As a result of this, an increase in plasma SDH is consistent with hepatocyte damage. SDH is liver specific in humans (Nduka, 1999) and all species of animals (Kaneko, 1989) and hepatic injury appears to be the only source of increased SDH activity. Although SDH is liver specific in all species, the already established usage of ALT in dogs and cats has limited SDH as a diagnostic indicator of hepatocellular damage to horses, cattle, sheep and goats (Kaneko, 1989).

6) Lactate Dehydrogenases (LDH; EC 1.1.1.27): It catalyses the reversible oxidation of pyruvate to L(+) lactate with the cofactor NAD. The equilibrium favours lactate formation, but the preferred assay method is in the direction of pyruvate because pyruvate has an inhibitory effect on LDH. Lactate dehydrogenase has isoenzymes. LDH can be found in the heart, liver, erythrocyte, skeletal muscle, platelets and lymph nodes. In humans, it is involved in myocardial infarction, haemolysis and liver disease (Nduka, 1999). LDH isoenzyme profiles were the first isoenzyme profiles used in clinical veterinary medicine in an attempt to detect specific organ damage (Kaneko, 1989). The introduction of more highly organ-specific procedures has resulted in LDH no longer being in common use in veterinary medicine (Moore and Feldman, 1974).

7) Cholinesterase (ChE): Serum cholinesterase (ChE) activity is composed of two distinct cholinesterases. The major substrate is acetylcholine, the neurotransmitter found at the myoneural junction. Acetylcholinesterase (AChE; EC 3.1.1.7) found at the myoneural junction is the true ChE and is essential in hydrolyzing acetylcholine so that the junction can be reestablished and prepared for additional signals (Whittaker, 1983). The myoneural junction AChE is also found in red blood cells (RBC), mouse, pig, brain and rat liver. Only a small amount of AChE is found in plasma. The ChE of plasma is a pseudocholine-

sterase, butylcholinesterase (ButChE; EC 3.1.1.8), which hydrolyses butyrylcholine four times faster than acetyl-choline and is also located in white matter of the brain, liver, pancrease and intestinal mucosa (Kaneko, 1989). Decreases in ButChE have been reported in humans with acute infection, muscular dystrophy, chronic renal disease and pregnancy, as well as insecticide intoxification.

8) Lipase (Lip): Serum pancreatic lipases (EC 3.1.1.3; triacylglycerol lipase) catalyse the hydrolysis of trigly-cerides preferentially at the 1 and 3 positions, releasing two fatty acids and a 2'- monoglyceride. Lipase can be found in the pancrease and hepatobiliary tract and is involved in pancreatitis and hepatobiliary disease (Nduka, 1999).

9) α-Amylase (AMY; E.C. 3.2.1.1): α-amylases are calcium-dependent metalloenzymes that randomly catalyze the hydrolysis of complex carbohydrates, e.g. glycogen at the α1-4 linkages. The products of this action are maltose and limit dextrins. The enzyme is a Ca^{2+} metalloenzyme which requires one of a number of activator ions such as Cl^- or Br^- (Wahlefeld, 1983). Amylase can be found in the salivary glands, pancrease and ovaries and is used as a diagnostic aid for pancreatitis.

10) γ-Glutamyltransferase (GGT; 2.3.2.2): This is a carboxypeptidase which cleaves C-terminal glutamyl groups and transfers them to peptides and other suitable acceptors. It is speculated that GGT is associated with glutathione metabolism (Kaneko, 1989). The major sources are the liver and kidney and is involved in hepatobiliary disease and alcoholism. Cholestatic disorders of all species examined result in increased serum GGT activity (Braun et al., 1987).

11) Trypsin (EC; 3.4.21.4): Trypsins are serum proteases which hydrolyse the peptide bonds formed by lysine or arginine with other amino acids. They are secreted by the pancreas as the zymogen trypsinogen, which is converted to trypsin by intestinal enterokinase or trypsin itself. Trypsin can be found in the pancrease and is involved in pancreatitis, biliary tract and fibrocystic diseases. In humans, serum trypsin-like immunoreactivity (TLI) increases in conditions which also result in increases in serum amylase, including renal failure (Kaneko, 1989). Serum TLI decreases in canine exocrine pancreatic insufficiency (Williams and Batt, 1983, 1988).

12) Glutathione Peroxidases (GPx; EC 1.11.1.9): These are metalloenzymes containing four atoms of selenium per molecule of enzyme. They catalyze the oxidation of reduced glutathione by peroxide to form water and oxidized glutathione (Kaneko, 1989). Because of the high concentration of selenium in glutathione peroxidases, there is a good direct correlation between the amount of red blood cell GPx activity and the selenium concentration of other organs (Anderson et al., 1976; Ammerman et al., 1980).

Other enzymes with disease diagnosis applications are acid phosphatase (ACP), found in prostate and erythro-cytes and is used in diagnosis of prostate carcinoma. Aldolase (ALD), found in skeletal muscle and heart and involved in muscle disease. Glutamate dehydrogenase (GLDH), found in the liver is used to diagnose hepatic parenchymal disease. Hydroxybutyrate dehydrogenase (HBD), which is the heart form of lactate dehydrogenase is involved in myocardial infarction.

Just as enzyme assay is used to diagnose diseases in humans and animals, it may also be applied to the investigation of diseases in plants. For example, it has been found that an injury (either mechanical or pathogenic) results in a marked, localized increase in the activity of glucose-6-phosphate dehydrogenase, but not of glucose phosphate isomerase, indicating diversion of glucose breakdown from glycolysis to the pentose phosphate pathway.

Enzymes as antioxidants

Various radicals can contribute to lipid oxidation in biological systems (Hundson, 1990). Superoxide radicals O_2^-, which may be produced by the enzyme xanthine oxidase and hydrogen peroxide (Kellogg and Fridovich, 1975) may be removed by the enzyme superoxide dismutase according to (Endo et al., 1985).

$$2O_2^- + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} H_2O_2 + 3O_2 \text{ (Endo et al., 1985).}$$
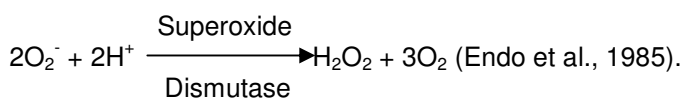
The enzyme catalase may also play an important role in converting hydrogen peroxide to water and oxygen (Foote and Denny, 1968).

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + 3O_2 \text{ (Foote and Denny, 1968).}$$
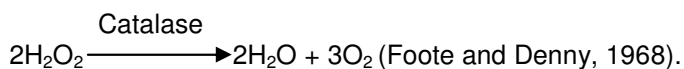
Since milk contains xanthine oxidase and superoxide dismutase, Korycka-Dahl and Richardson, (1980) concluded that these reactions may contribute to the stability of milk.

Enzymes used in immunoassays

Enzymes may also be used as an alternative to radioisotopes as markers in immunoassays (Palmer, 2001). Such procedures, known as enzyme-immunoassays, have been used for the determination of a variety of proteins and hormones. The role of enzymes in immunoassay procedures is a secondary role, they are used to replace radioisotopes as markers, since they are not hazardous to health and can be detected by techniques which are more generally available. Any enzyme with a sensitive and convenient assay procedure can be used

for this purpose. Two common examples of enzyme-immunoassay (EIA) procedures are enzyme-linked immunosorbent assay (ELISA) and enzyme-multiplied immunoassay test (EMIT). ELISA is a highly sensitive assay that can be used to detect either antigen or antibody (Engvall and Carlson, 1976).

Applications of ELISA include diagnostics for noninfectious diseases involving hormones, drugs, serum components, oncofetal proteins, or autoimmune diseases, as well as diagnostics for infectious diseases caused by bacterial, viral, mycotic or parasitic organisms (Voller et al., 1979). The enzymes frequently used in ELISA are Horseradish peroxidase, alkaline phosphatase and ß-galactosidase. In EMIT, the activity of malate dehydrogenase is assayed by standard enzyme methodology for the detection of thyroxine by Enzyme-labelled immunoassay.

Enzymes as therapeutic agents

In a few cases enzymes have been used as drugs in the therapy of specific medical problems (Devlin, 1986). Streptokinase is an enzyme mixture prepared from streptococcus. It is usefull in clearing blood clots that occur in the lower extremities. Streptokinase activates the fibrinolytic proenzyme plasminogen that is normally present in plasma. The activated enzyme is plasmin. Plasmin is a serine protease like trypsin that attacks fibrin, cleaving it into several soluble components (Devlin, 1986).

Another enzyme of therapeutic importance is asparaginase. Asparaginase therapy is used for some types of adult leukemia. Tumor cells have a nutritional requirement for asparagine and must scavenge it from the host's plasma. By administering asparaginase i.v., the host's plasma level of asparagine is markedly depressed, which results in depressing the viability of the tumor (Devlin, 1986). Enzyme replacement in individuals that are genetically deficient in a particular enzyme are also applications of enzymes as therapeutic agents. Also, enzymes such as u-plasminogen activator, formerly known as urokinase, extracted from human urine, can be infused into the blood stream of patients at risk from a pulmonary embolism (a fragment of a blood-clot lodging in the pulmonary artery): these enzymes stimulate a cascade system responsible for the production of active plasmin, a proteolytic enzyme which digests fibrin, the main structural component of blood-clots. Some enzymes may also be used to restrict the growth of cancer cells by depriving them of essential nutrients: for example, L-asaparaginase may be used in the treatment of several types of leukaemia, since the tumour cells, in contrast to normal cells, have a requirement for exogenous L-asaparagine.

Another example of therapeutic application of enzymes is the use of immobilized enzymes as components of arti-ficial kidney machines, which are used to remove urea and other waste products from the body, where kidney disease prevents this being done by natural processes (Palmer, 2001). Urea enters the machine from the blood, by dialysis (termed haemodialysis) and is converted to CO_2 and NH_4^+ by immobilized urease; toxic NH_4^+ is then either trapped on ion exchange resins or incorporated into glutamate by the action of immobilized glutamate dehydrogenase linked to alcohol dehydrogenase to ensure coenzyme recycling, before the fluid is returned to the blood stream.

Enzymes and Inborn errors of metabolism

An inborn error of metabolism is characterized by the loss of activity of a specific enzyme as a result of a genetic mutation. Inborn errors are rare but possibly severe conditions, often causing mental retardation or even death in infancy (Palmer, 2001).

Enzymes are also used in the treatment of some inborn errors of metabolism. Ideally, complete treatment would be achieved if some normal enzyme could be introduced at the appropriate site. In the case of the enzymes of the gastrointestinal tract, this may easily be achieved by oral administration. For instance, where there is a lactase (ß-galactosidase) deficiency in the cells of the intestine, which may occur as an inborn error or as a secondary feature of other diseases, ingested lactose (in milk) cannot be broken down to galactose and glucose and severe discomfort results. This condition may be treated by removing milk products from the diet, or more conveniently by adding to it ß-galactosidase extracted from micro-organisms to perform the task normally done by the body's lactase.

Enzyme assays are used to confirm the diagnosis of inborn errors of metabolism. Examples are Tay-Sachs disease, caused by a deficiency of B-N-acetylhexosaminidase (also known as hexosaminidase A) activity. An example of an autosomal recessive inborn error of metabolism is phenylketonuria, where the patient has a severely limited ability to convert phenylalanine to tyrosine, because of a reduced activity of phenylalanine-4-monooxygenase, better known as phenylalanine hydroxylase. An example of an inborn error which appears to be transmitted by an X-linked dominant mode is ornithine carbamoyl transferase (OCT) deficiency, also known as hyperammonaemia, a defect of urea cycle metabolism (Palmer, 2001). Another X-linked, but recessive, disease is the Lesch-Nyhan syndrome, a disorder of purine metabolism resulting from a deficiency of hypoxanthine phosphoribosyl transferase activity.

Enzymes as sources of biofuels

Frances Arnold is reported to be designing better enzy-

mes used for making biofuels from cellulose (Goho, 2008). Cellulolytic enzymes break down the cellulose found in biomass so that it can be used as a feedstock for biofuels. The increased use of cellulosic biofuels could cut green house-gas emissions and reduce reliance on soil. Sources of cellulosic biofuels are agricultural waste, wood chips and prairie grasses. Advantages of cellulosic biofuels over both gasoline and corn ethanol are (i). Burning cellulosic ethanol rather than glucose could cut greenhouse gas emissions by 87% and (ii). Cellulose is the most abundant organic material on earth. However, converting corn starch into sugar requires a single enzyme while breaking down cellulose involves a complex array of enzymes called cellulases that work together (Goho, 2008). The general aim of the research work is that when fully exploited, cellulose ethanol will significantly reduse the use of gasoline.

Enzymes in research

Enzyme assay is used for research into processes as the browning of plant products, which prevents problems during the conversion of fruits and vegetables into drinks and preservatives. Enzymes are also important for researches into diseases of humans and animals, biotechnology research and other research areas, that is, enzymes have been used in research for better and alternative treatment of diseases. For example, Billigmann (1995) reported enzyme therapy as an alternative in treatments of herpes zoster. Bartsch (1974) reported the use of proteolytic enzymes in the treatment of herpes zoster. Scheef (1987) also reported the use of enzymes for therapeutic purposes. Pathney and Pachori (1986) reported a study of serum glycolytic enzymes and serum B hepatitis in relation to LIV.52 therapy. Also, (Stauder and Kabil, 1997; Kabil and Stauder, 1997) reported oral enzyme therapy in hepatitis C patients. Nikolaev et al. (1998) reported clinical use of Belosorb and Wobenzyme in the treatment of viral hepatitis B. Korpan et al. (1997) reported the mechanism of therapeutic efficacy of Wobenzyme in the treatment of toxic hepatitis. Jager (1990) reported hydrolytic enzymes in the treatment of HIV infections. Mikazans (1997) reported the possibility to treat Herpes Zoster using enzymes. Mudrak et al. (1997) reported adjuvant therapy with hydrolytic enzymes in recurrent laryngeal papillomatosis. The determination of enzyme activity is of importance in ascertaining the metabolic state of fruit tissues and their keeping quality on storage. For instance, the activity of lipoxygenase, peroxidase and acid phosphatase were enhanced in tomato fruits following treatments with Indole-acetic acid, Indolebutyric acid and Naphthalene acetic acid (Olaiya, 2006).

Enzymes as reagents in clinical chemistry

Enzymes are used as reagents in clinical chemistry. D-glucose in blood and other physiological fluids is commonly analysed by means of procedures involving glucose oxidase (Palmer, 2001). The reaction catalysed by glucose oxidase is utilized in a test-strip (Clinistix) for the screening of urine specimens. Glucose oxidase and the reagents for a colour-producing indicator reaction are impregnated into a plastic strip, so that the intensity of the blue colour obtained when it is dipped into a specimen gives an indication of the D-glucose content. This can be used in the diagnosis of diabetes mellitus. D-glucose may also be analysed by a hexokinase-catalysed reaction, although this enzyme can utilize any D-hexose as substrate, or by use of glucose dehydrogenase (Palmer, 2001).

Blood lactate and pyruvate are usually determined by means of lactate dehydrogenase-catalysed methods and blood urea is sometimes analysed by procedures involving urease (Palmer, 2001). Also, blood cholesterol may be determined by a reaction catalysed by cholesterol oxidase. Triglycerides and urate may also be analysed by enzymatic methods.

Luciferase is a Mg^{2+}- activated enzyme. Luciferase-catalysed procedures are used for the analysis of adenosine triphosphate (ATP) (e.g. from blood platelets) and may also be used to determine the Mg^{2+} concentration or the pO_2.

Enzymes as analytical reagents

Enzymes display several characteristics which makes them suitable for analytical applications. Enzyme catalyses a reaction which is both substrate-specific and product-specific. As a result of this, enzymes are very valuable as analytical reagents. They can be used for the estimation of specific substances, possibly present at very low concentrations, in the presence of other, chemically similar, substances (Palmer, 2001). Enzyme-based analytical methods are both specific and sensitive, which means little or no sample preparation is required in many cases.

Another characteristic of enzyme-catalysed reactions which makes them suitable for analytical applications is that they proceed under relatively mild conditions (e.g. at near neutral pH and around room temperature). As a result of this, they are simple to set up and can be used for the analysis of substances which would be unstable under more extreme conditions. Enzyme-based analytical procedures may be designed to determine the concentration of substrates, coenzymes, activators and inhibitors (Palmer, 2001). However, all solutions used in enzymatic analysis procedures must be free from contamination by micro-organisms and metal ions.

Enzymes with industrial applications

Enzymes have wide applications in different industries particularly in the food and pharmaceutical industries.

The activity of certain enzymes may be determined before and after pasteurization and sterilization procedures to acertain whether these have been properly done. For example, alkaline phosphatase and invertase present in milk are inactivated within the same temperature range as is required for pasteurization. The activeties of these two enzymes at the end of the whole process could give an indication of its effectiveness.

The degree of bacterial contamination of foodstuffs can be estimated by the assay of microbial enzymes not normally present in food. This principle is applied in the assay of milk to determine whether its been contaminated. Milk should normally contain only small amounts of reductases, but bacteria produces large amounts. Reductases may be easily assayed because they catalyse the reduction of methylene blue to colourless leuco-methylene blue under anaerobic conditions (Palmer, 2001). A test-strip (Bactostrip) incorporating 2,3, 4-triphenyltetrazolium salts provide a convenient way of testing for thr presence of bacteria, a red formazan dye being produced as a result of the action of reductase. Enzymes are also used to determine whether stored plant products are suitable for use as foodstuffs. For example, α-amylase should be present in relatively low amounts in stored wheat seeds. Hence, the α-amylase activity of food plants may be determined to ascertain the degree of their suitability for use for the desired purpose. The freshness of meat may be determined by the use of monoamine oxidase to detect amines formed during degradation. Also, enzyme-immunoassay is used to detect food adulteration, e.g. the addition of horse to beef products (Palmer, 2001).

The concentrations of various sugars and individual amino acids in foodstuffs may also be determined by specific methods of enzymatic analysis. In winemaking, the concentration of malic acid is sometimes determined by a method involving malate dehydrogenase. The clarification of cider, wines and fruit juices (e.g. apple) is usually achieved by treatment with fungal pectinases (Palmer, 2001). Papain is sometimes used as a meat tenderizer. Papain (and other proteases) may also be used in the brewing industry to prevent chill hazes, caused by precipitation of complexes of protein and tannin at low temperatures. Bacterial α-amylase (from *Bacillus subtilis*) which is even more heat stable than wheat α-amylase, is of importance in the brewing industry. The traditional use of yeasts (e.g. *Saccharomyces carlsbergensis*) in the baking and brewing industries arose because they contain the enzymes for alcoholic fermentation, alcohol dehydrogenase (Palmer, 2001). Invert sugar, a mixture of glucose and fructose, is produced from sucrose by the action of yeast ß-fructofuranosidase, better known as invertase (an enzyme which can be extracted by disruption of the yeast cell wall). Enzymes are also of great value in the pharmaceutical industry. For example, they are used for the conversion of naturally occurring penicillin G (benzyl

penicillin) to 6-amino penicillanic acid (6-APA). This reaction is catalysed by penicillin amidase (Palmer, 2001). Bacterial proteases are also used in the leather and textile industries to loosen hair (or wool) and enable it to be seperated from hide. Enzymes are also used in the production of biological molecules (Daini, 2000).

Enzymes were used in a variety of ways long before they were recognized as definite biochemical components in living cells. Processes for making bread, wine, cheese, vinegar, alcohol etc have been known from antiquity (Aurand et al., 1987).

Enzymes have several characteristics that make them significant for use in industrial processes. They are:

1. They accomplish and accelerate a reaction efficiently.
2. The rates of the reaction can be readily controlled by adjusting the temperature, pH and reaction time.
3. Enzyme activity may be destroyed by heating to denaturing temperatures.
4. They are natural in origin and non-toxic and therefore may remain in the product without any harmful conesquences.
5. They exhibit great specificity and can be used generally at levels of less than 1% of the commercial product batch (Aurand et al., 1987).

Enzymes and immune system

Enzymes become part of the immune system (Alpha-2-macroglobulin), working with it and facilitating its function. (Laver et al., 2001) reported the modulation of growth factor binding properties of alpha 2-macroglobulin by enzyme therapy. Ingestion of proteinases was found to trigger the formation of intermediate forms of alpha-2-macroglobulin displaying high affinity of transforming growth factor- beta (TGF-ß). They observed maximum binding of TGF- ß 1 - 2 h after bolus ingestion and steadily leveled off with time. They concluded that intestinal absorption of proteinases triggers the formation of TGF-ß binding species of alpha 2 macroglobulin in blood. Mediated by this process high concentrations of TGF- ß might be reduced via enhanced clearance of alpha 2-macroglobulin TGF- ß complexes. Thus, proteinase therapy may have beneficial effects in treatment of fibrosis and certain cancers accompanied by excessively high TGF- ß concentrations. Desser et al. (2001) reported that oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood. In the study, they stated that therapy with oral proteolytic enzymes (OET) with combination drug products containing papain, bromelain, trypsin and chymotrypsin have been shown to be beneficial in clinical settings such as radiotherapy-induced fibrosis, bleomycin pneumotoxicity and immuno-suppression in cancer, all of which are nowadays knowned to be accompanied by excessive transforming growth factor - beta (TGF- ß) production.They concluded

that their results support the concept that OET is bene-ficial in diseases characterized in part by TGF- ß1 overproduction.

Animal-derived enzymes

Enzymes derived from animals e.g. rennet, bovine liver catalase, animal lipase, pancreatin, pepsin, trypsin and lysozyme that are not genetically engineered may be used in processed foods labeled as "organic". Incidental additives, including processing aids used in the produc-tion of enzyme preparations, must be non-synthetic or be substances that appear on the National list of ingredients allowed for use in foods labelled "organic" (consensus). Animal-derived rennet and bovine rennet are direct food substances affirmed as Generally Recognized As Safe (GRAS) (FDA, 1995). Rennet and bovine rennet are commercial extracts containing the active enzyme rennin, also known as chymosin. Rennet is the aqueous extract prepared from cleaned, frozen, salted or dried fourth stomachs of calves, kids or lambs. Bovine rennet is the product from adults of the animals listed above. Both products are called rennet. Rennin is the milk coagulating enzyme of the mucosa of the fourth "true" stomach (abomasum) of young calves. The structure of rennin consists of a single polypeptide with an internal disulfide bridge (Budaveri, 1996). Animals used for the production of rennet and bovine rennet must certified free of bovine spongiform encephalopathy (BSE) (cattle) or scrapie (sheep, goats). Rennet is a coagulant used to curdle milk to be made into cheese or sour cream. The milk-clotting effect of rennin is due to a specific and limited hydrolysis of the E-casein surrounding the protein micelles in milk. As a result, the micelles lose their electrostatic charge and are able to aggregate with the help of calcium and phosphorus ions to form a network that traps the fat micelles and a gel structure is thus formed (Nelsen, 1992).

Plant and fungi-derived enzymes

There are many different plant and fungal enzymes used in processing. Among them is pectinase, poly (1,4-α-D-galacturonide) glycanohydrolase, poly (1,4-α-galacturo-nide) lyase and pectylhydrolase (NOSB, 1995).

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