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IN PRAISE OF ENZYMES

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DEDICATION

DEDICATION TO THE MEMORY OF MY LATE FATHER

MR. ANOSIKE UGOJI

AND

TO UDODIRIM, ENYI, CHINEDUM, CHIDI, OKECHUKWU AND EMEKA

WITH LOVE FROM

EMMANUEL OKOGBUO ANOSIKE.

IN PRAISE OF ENZYMES

This is the second inaugural lecture to come from the Faculty of Science in the relatively short life history of this University; the first being from F.A Onogeghara, Professor of Botany. That distinguish academic in discussing the topic “Botany in Human Affairs”, went to great lengths to get his audience acquainted with Botany as a scientific discipline. He did this admirably drawing from numerous examples from plant materials around us. For me to discuss the role of enzymes in such a way that the person without scientific background will follow is not a mean task.

The lecture shall be handled in two parts. In the first part in which I shall speak in praise of enzymes, I intend to show the central role enzymes studies occupy in the field of Biochemistry. The second part will deal with my personal association with some enzymes during the past sixteen years of my academic career.

The story enzymes are the study of the subject “Biochemistry”, for it is impossible to talk about biochemical reactions without the enzymes that catalyze or speed them up. I therefore think it appropriate for me to go a little bit into the history of the subject of Biochemistry before focusing on the “wonder” molecules that are called enzymes.

Biochemistry is very young branch of science compared, for instance, to Biology, chemistry, physics or Mathematics, in the first few decades of this century, it was rightly seen to offer the best hopes of understanding biological phenomena at fundamental level beyond mere description. Thus, it is a subject that attempts to explain the molecular basis of life using the tools of the Chemist, Biologist, Physicist, and the mathematician. This fact places the subject of biochemistry in a central position among the life sciences. The truth of this statement is brought out most vividly if one considers the number of Noble prizes in the life sciences since the end of the Second World War. A large proportion of them has been for work of a predominantly biochemical nature, whether they were awarded for Chemistry, physiology or Medicine. At this stage of the development of the subject therefore, a biochemist is the most qualified among other life scientists to discuss the chemical basis of “life”. Indeed, in our present state of scientific development, Biochemistry has been described as “not only the very stuff or language of Cell Biology, Immunology, Microbiology, Physiology, and Pharmacology. Pathology and Genetics but also the rational language for discuss in Ecology, Clinical Medicine and Agriculture.

ORIGIN OF BIOCHEMISTRY.

The history of the development of Biochemistry as a distinct scientific discipline started with attempts associated with an understanding of processes induced by

enzymes activity. Such processes include fermentation and decay. These processes had been observed by man from time immemorial without an understanding of the bases of the changes manifested. One biochemistry author has in fact wondered whether Noah could be considered the world's first biochemist since his acquaintance with fermentation is described in the book of Genesis! It is of course hardly conceivable that Noah was equipped or even bothered to understand the process involved. If he had made and effort in that direction, he would probably have become the world's first enzymologist or indeed the first biochemist.

On a more serious note, it is believed that the earliest origins of biochemistry began with speculations on the role of air in the utilization of food on the nature of fermentation. Leonardo da Vinci who lived between 1452 and 1519 was the first to compare animal nutrition to the burning of a candle. This line of reasoning was further developed by a gentleman called Van Healmont in 1648. Later study of fermentation and the enzyme processes involved was the origin of Biochemistry.

It is necessary at this stage to explain what enzymes are. They have been defined as biological catalysts which start and direct in their course, virtually all chemical reactions in living nature.

In the early days, alcoholic fermentation was practiced without any idea that its process lies with living systems or their products. The chemical nature of the process was understood by Lavoisier at the end of the eighteenth century without his worrying about its causative agent. He was able to show that all the carbon, hydrogen and oxygen of the sugar that is broken down reappear in the form of alcohol and carbon dioxide. We of course now know that the chemical formula of the process is $C_6H_{12}O_6$---------------- $2C_2H_5OH + 2CO_2$. In 1835, the Swedish Chemist, Berzelius introduced the name and notion of catalysis and actually thought that yeast was the catalyst for the conversion of sugar to carbon dioxide and alcohol. As far as he was concerned, yeast was nothing more than a remarkable catalyst. This was three years after Payen and Persoz had a first clear recognition of an enzyme. They called the enzyme they recognized diastase which was an alcohol precipitate of malt extract that converted starch to sugar.

In 1837, it is reported that no less than three independent workers namely; Cagniard-Latour, Schwann and Kutzing made the claim that yeast is in fact a living organism. At first Berzelius was to dismiss this claim and the evidences for it. In Germany, the great Liebig, a pioneer in applying the new chemistry to biology problems ridiculed the experimental observations of a microscopic nature that led to the claim that yeasts are living organisms. Liebig rather thought that the ferment was a

labile organic substance formed by interaction of air with something in plant juices and which affects a breakdown by transforming its own instability to sugar.

The great French founder of Microbiology, Pasteur held a contrary view and mid-nineteenth century the opposing views of fermentation------- the nature of the causative agent--- had become a battle between two great giants. By 1860, Pasteur had demonstrated that yeast could grow--- with increase in dry weight—in a simple medium containing only sugar and salts. He thus concluded that fermentation brought about by living orangnisms capable of growth and multiplication. In spite of this, it still took a long time (1871) for the great Liebig to give in.

Thus, fermentation became established as a physiological act connected with the life and organization of yeast cells. For this reason, Pasteur thought that there could be no fermentation without life. Yet by 1897, Edward Buchner, another German Chemist, prepared a cell-free extract of yeast that could ferment sugar. It was therefore concluded that alcoholic fermentation does not require the complex apparatus of a Yeast Cell and that the fermenting juice is a dissolved substance that was called zymase. This has of course turned out to be a mixture of enzymes proteins and non-protein substances or coenzymes.

I have gone into this historical account to illustrate how Biochemistry, as a subject, was born; Liebig's was a chemical approach since he was a chemist while Pasteur's was biological. The work of Buchner brought the two view points together giving an insight into how chemistry and Biology could, by combine application lead to an understanding of the phenomenon of the process of fermentation. Thus, the science of Biochemistry was born. Even them it still was a fairly long time before the first enzyme protein was crystallized. This feat was achieved by J.B.Sumner in 1926 when he reported his crystallization of a pure form of the enzyme, urease from Jack beans. Between that time and now, hundreds of other enzymes have been purified and characterized. As a matter of fact, because of the important role of enzymes, as catalysts in all biochemical reaction, the study of emzymology, has continued to occupy a central position in the field of Biochemistry. For, is it not true that biological inheritance can be reduced to the ability of off-spring to acquire the necessary machinery from their parents for synthesizing enzymes? The rapidly expanding frontier in the areas of Applied Biochemistry called genetic engineering or biotechnology can be reduced to possibilities of inducing life systems of the lower type produced enzymes proteins that catalyze reaction of our choice.

We shall now take a look at a few endeavours or pursuits that affect the quality of life of man on earth in which enzymes play critical roles.

1. ENZYMES IN CLINICAL MEDICINE

Assays of some enzymes present in blood plasma or serum are routinely carried out in most clinical chemistry laboratories for diagnostic purposes. The majority of such plasma enzymes are simply those that have leaked out of blood or tissue cells. For each such enzyme there is usually a balance between its rate of arrival by leakage from the cells and its rate of removal by catabolism or excretion. Thus, for each plasma enzyme, there is a normal concentration range. Diseases cells of a tissue whose membranes are no longer intact will have their contents leak out into the blood stream at an increased rate and these enzymes associated with those cells will be found in plasma in elevated amounts. Thus, the presence of these enzymes or isoenzymes associated with particular tissues can help identify the location of damaged cells. The following are a few example of the application of plasma enzyme assay to diagnosis:

a) **LACTATE DEHYDROGENASE**: liver tissue and skeletal muscle cells contain mainly the M4 form while those of heart muscle contain mainly H4 and MH3; all of the isoenzymes are found in kidney cells and erythrocytes. Abnormal isoenzymes pattern can be of help in diagnosis.
b) **ASPARTATE AMINOTRANSFERASE (AST) FORMERLY GOT**: raised plasma activities usually indicate severe damage to the cells of the heart (myocardial infarction or liver damage (viral hepatitis or toxic liver necrosis).
c) **ALANINE AMINOTRANSFERASE (AIT)-(GPT**): marked raised activity of this enzyme indicates a severe liver disease.
d) **CREATINE KINASE**: mainly found in heart and skeletal muscle and in brain; MB type in heart, MM in muscle and BB type in the brain. Plasma activity of the MB isoenzyme is good indicator of possible myocardial infarction while high activity of MM isoenyme indicates damage skeletal muscle cells as in muscular dystrophy.
e) **ACID PHOSPHATASE**: an isoenzyme of this is found in large amounts in the prostate gland and its assay in plasma is used in the diagnosis of prostatic carcinoma.

2. 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 genetic mutation. These are relatively rare but severe conditions often cause mental retardation or even death in infancy. For diagnosis, there is an observed buildup in plasma or urine of the metabolic intermediate which is the substrate for the defective enzymes. To confirm, the enzyme is assayed for in say, the liver tissue. If the diagnosis is right, the activity will be found to be very small or non-existent. If because of previous family history, it is considered possible

that particular pregnancy might result in the birth of a baby with an unborn error of metabolism, there is the possibility of prenatal diagnosis by assaying the enzyme in the amniotic fluid cells, mainly derived from the skin. Tay-Sacs disease, a deficiency of hexosaminidase activity has been diagnosed in this way.

Phenylketonuria is an example of an autosomal recessive inborn error where the patient has a severely limited ability to convert the amino acid, phenylalanine to tyrosine because of reduced activity of phenylalanine hydroxylase. X-linked dominant inborn errors are also found. Here the matured gene forms part of the X-chromosome. So X- linked dominant disease are inherited through the mother alone who is heterozygous but not necessarily clinical abnormal. A heterozygous daughter may manifest the disease while a hemizygous son will certainly be severely affected. An example of an inborn error which appears to be transmitted by an X-linked dominant mode is carnithine transcarbamoylase deficiency also called hyperammonaemia – a defect of urea cycle metabolism.

ENZYME THERAPY:

Enzymes are peculiar therapeutic agents since in small amount they can produce very large specific effects at physiological pH and temperature. For this reason, higher purified forms have been used for the treatment of cancer, clotting disorders, genetic defects, inflammation, digestive problems, drugs toxicities and kidney failure.

Techniques to improve therapeutic properties of enzymes include:

I. Soluble chemical modifications.
II. Insoluble chemical modifications or binding to surfaces and
III. Encapsulation in biodegradable or inert materials.

We shall look at some example of enzymes therapy.

a) **Enzymes therapy in genetic disorders**:
Enzymes replacement is the logical approach for the treatment of genetic disease that are caused by defined enzyme defects.
Ideally, complete treatment would be achieved if some normal enzyme could be introduced at the appropriate site. For enzymes of the gastrointestinal tract, this could be achieve by oral administration e.g. for lactase deficiency in the cells of the intestine. The condition may be treated by removing milk products from the diet or by adding to it the enzymes B-galactosidase extracted from micro-organisms. However where the main site of action of the enzyme is not in contact with external environment e.g. liver or the CNS, the problem becomes difficult

since direct infusion could lead to its destruction by antibodies. Attachment of sugar units to enzymes prior to infusion increases their chance of reaching the target organ. It is thought that sugar triggers some specific transport system in membranes.

Another approach that is being used is to trap the enzyme in artificial membrane –enclosed structures called liposome. Cells of a particular tissue might then be able to take these up from the blood stream provided their membranes were of suitable composition. A major advance has been the immobilization of all the four enzymes of the urea cycle and inorganic pyrophosphatase in a fibrin fibre formed from fibrinogen by the action of thrombin and transglutaminase. This multi enzyme system is able to carry out the urea cycle with much greater efficiency than the soluble enzymes. It could be used for the treatment of any of the genetic defects in the urea cycle.

b) CANCER CHEMOTHERAPHY:

Aminohydrolases have been used in cancer chemotherapy to deplete specific amino acids required for tumor cell growth. Such therapy requires prolong depletion of the target amino acid in the fluids bathing the tumor cells. Such enzymes are injected and these must have prolong activity in circulation and elicit little immune response. Soluble abducts with polyethylene glycols, dextran, amino acids and succinic anhydride have been shown to improve these properties with several antitumor enzymes. Thus L-asparaginase may be use to restrict the growth of cancer cells by depriving them of L-asparagine, an essential nutrient. Several types of leukemia have been treated in this way.

c) ENZYME THERAPY IN CLOTTING DISORDERS:

Both the absence of clotting factors and excessive clotting can be treated with enzymes. Most clotting factors are highly specific proteolytic enzymes. Absence of an individual factor can be treated by infusion of crude or purified enzyme preparations. Chemically modified preparations are needed to decrease antigenicity and prolong the activity of some clotting factors. Excessive clot formation is vital organs can be treated by activation or addition of fibrinolytic enzymes. In recent advances human urokinase from human tissue culture cells is used. This is infused into the blood stream of patients at risk from pulmonary embolism. This stimulates a cascade system responsible for the production of active plasmin, a proteolytic enzyme that digests fibrin.

d) ENZYME THERAPY IN TOXIC REACTIONS:

Removal of circulating levels of endogenous and exogenous toxic materials may be facilitated by specific enzymes. For this use, enzyme must be incorporated into a biocompatible device that can be rapidly attached to the circulation or implanted in an appropriate site. For example, uricase is currently in use in Europe to treat high levels of uric acid in patients with leukemia. Many toxins are candidates for enzyme degradation. These include poorly dialyzed drugs like digitalis, glycosides; benzodiazepam etc. specific enzymes may degrade some of the usual peptide and protein toxins from mushrooms, insects, and snakes.

3. ENZYMES IN INDUSTRY

This section of the lecture will deal with the applications of enzymes in industry. Immobilization of enzymes by fixing them in some way on to an inert and usually insoluble polymer matrix has widened the industrial applications of enzymes. Thus, we have enzyme reactors whose purpose is to allow enzyme and substrate to come into contact for a sufficient period of time for the reaction to take place and then to be able to separate easily the product and enzyme. Both batch and continuous flow reactors are in use today. The following are examples where these processes have been used industrially:

(a) RESOLUTION OF DL-AMINO ACIDES:

In this process, the enzyme aminoacylase is used to resolve DL-amino acid produced by chemical synthesis or by fermentation. The chemically synthesized acylated DL-amino acid is selective hydrolysed by the enzyme aminocylase to the L-amino acid and the D-acylamino acid. These can be separated by differing solubilities. Actually the industrial production of L-methionine by aminoacylase immobilized on to DEAE sephadex in a packed bed reactor is being practiced in Japan.

(b) PRODUCTION OF HIGH FRUCTOSE SYRUPS:

the controversy over the safety of artificial sweetners leading to the ban on cyclamates and reduced use of saccharin in the food industrial has place more emphasis on sucrose, the traditional sweetner. Fructose is about $1_{1/2}$ times sweeter than sucrose and so invert syrups (hydrolysed sucrose) are produced commercially. Glucose isomerase immobilized on to cellulose ion-exchange polymer in a flat bed reactor is used to fructose. The glucose from corn starch has in fact been used to produced high fructose syrups.

(C) CONVERSION OF STARCH TO GLUCOSE: here the enzyme amyloglucosidase is immobilized in a continuous stirred tank reactor or a packed bed reactor.

(D)TREATMENT OF MILK: the use of immobilized enzymes in the treatment of milk falls into three major areas: production of cheese, enzymic stabilization and the removal of lactose from milk products. In the first, immobilized bacterial rennin is used. In the second, milk is treated with trypsin to make it less susceptible to oxidation and loss of flavor. Immobilized lactase is used for the removal of milk sugar lactose.

(E)ANTIBIOTIC MODIFICATION: penicillin amidase immobilized with cyanogen bromide to sephadex is used to affect the deacylation of 6-aminopenicillanic acid which is semisynthetic to form benzyl penicillin.

(F)PROTEASES: some immobilized proteases are used to render food proteins soluble, to texturixed proteins or to increase their digestibility.

In the beer industry, proteins present in untreated beer will cause haze to develop as the beer whereby papain, immobilized on to chitin, has been used to remove the chill haze from beer.

These are only a few instances in which enzymes technology has been used for solutions of industrial problems. The possibilities are enormous and the Japanese are making strides in the exploitation of this area of research.

Ladies and gentlemen, I had at the start of this section of the lecture indicated that the study of Biochemistry started with the discovery of the involvement of enzymes in fermentation processes. I have also ended it by pointing out the vast potentials there are for the use of immobilized enzymes in industrial processes. Even the field of genetic engineering that has made Biochemistry the glamour science of the latter part of the twentieth century involves manipulation of a cell's ability to produce enzymes of choice. There can thus be no doubt whatsoever that enzymes have continued to occupy their prominent position in man's search to understand life processed and in his bid to use this understanding to improve the quality of his life on earth.

PERSONAL ASSOCIATION WITH SOME ENZYMES

Most people, when faced with situations in which they are forced to speak of their work and their motivations may begin with the words made famous by that great black American civil right leader, Rev. Martin Luther King. "I have a dream……" some would go on to describe dreams they had at some moment in their careers that got translated in concrete realities. Others would dwell on dreams that are still to be fulfilled or that indeed may never be translated into reality. In any case, they are dreams that are meant to give hope and inspiration to the living. Over the past many years, I, like many other Nigerian of my generation have had not just a "dream", but "dreams". These

include dreams of the day when our country would become a technologically developed country. Technology development that would be sustained and fed by a sound, pure, scientific base. Alas, the oil boom that has turned into a glut has changed all that in a way that has had its own usefulness.

It has meant that we could not hope to set up the sort of laboratories with facilities we used for our studies in the advance countries. At first the situation led to lots of frustrations which, luckily, later on gave way to improvisations in a bid to survive. Thus, the belief among many biochemists that there is usually am monogamous relationship with enzymes – "one man one enzyme" has not applied to me. Lack of such facilities like uninterrupted water supply, electricity, necessary equipment etc. which are normally taken for granted in other countries have made me "flirt" about with nothing less than five different enzymes in the past ten years. These include arginine kinase (arginine: ATP phosphotransferase); aryl sulphatase (arylsulphate: sulphohydrolase); lactate dehydrogenase (lactate:NAD oxidoreductase); rhodanese (thiosulphate:cyanide sulphurtransferase) and polyphenol oxidase (odiphenol: 02 oxidoreductase). My recent infatuation with polyphenol oxidase has been dictated, in part by its relative stability and its abundance in root tuber, like yams, that I use as its source. I shall therefore deal with our modest contributions to the understanding of the enzymic browning phenomenon in the yam tuber as well as some of the inhibitors of the enzymes-catalyed reaction.

THE BROWING PHENOMENON.

Browning of tubers, fruit and vegetables is a common observable phenomenon by anybody who has had anything to do with cooking. Whenever a yam tuber is cut and exposed to the atmosphere a number of colour changes takes place. For the white yam, *Dioscorea rotundata*, the colour changes from white to pinkish brown to blue. The complicated processed that lead to these colour changes are referred to as browning reactions and the final colours formed vary from pink to bluish black to brown(1).

Since the yam tuber has to be peeled or cut into slices before processing, these colour changes would invariably be permanent features of the finished product. The yam tuber is difficult to preserve due to losses attributable to rot or increased metabolic activity at the onset of sprouting (2,3). Because of this constraint, it appears more practicable to preserve the processed product such as in the form of yam flour. The industrialist engaged in yam flour production would want the finished product to retain the original colour for consumer acceptance. In order to prevent the development of undesirable colour changes in the finished product it is imperative that the browning process be clearly understood if its effect is to be countered for aesthetic purposes and

so as to avoid the reduction of the nutritional quality of the product which the browning reactions bring about. Browning of food products can be due to two different processes a non-enzymic process and an enzymic browning and the enzyme responsible of the process.

POLYPHENOL OXIDASE: this is the enzyme that is responsible for the catalytic oxidation of phenols to quinones which interact with other cell constituents to give the brown products. Below are typical reactions catalyzed by enzymes that bring about browning reactions.

In our work, we compared the activities of polyphenol oxidase in five yam (Dioscorea) species and found that D. bulbifera (aerial yam) had the highest activity. The purified enzyme from this source was characterized by us with respect to its subunit structure, stability to heat, and effect of various inhibitors (4,5). Some of the results are shown in table 1 and 2.

TABLE 1 INHIBITORS OF D. BULBIFERA POLYPHENOL OXIDASE.

Inhibitor	Catechol as substrate (3.3.mM) (% inhibition)	Pyrogallol as substrate (3.3.mM) (% inhibition)	DOPA as substrate (3.3.mM) (% inhibition)
0.05 mM B-Mercaptoethanol	41.7	35.0	85.0
0.05 mM Dithioerythritol	81.6	95.0	61.5
1.0 mM EDTA	7.8	0.0	0.0
0.5 mM Thiourea	85.4	5.0	11.5
0.1 mM Sodium metabisulphite	34.0	55.0	38.5
0.2 mM KCN	70.0	62.5	50.0
1.0 mM NaN3	30.0	7.5	15.4
0.5 mM L-Cysteine	61.0	22.5	7.7
1.0 mM p-Cresol	42.0	32.5	42.3
1.0 mM L-Phenylalanime	21.0	12.5	11.5.

Enzymes 0.1 ml containing 11.6 ug of protein was pre incubated with a mixture of the inhibitor and 0.25 M KPi buffer pH 7 for ca 2 min. at 25^0. Reaction was started by addition of the appropriate concentration of the substrate.

Table 2 effects of some inhibitors on D. bulbifera polyphenol oxidase

Inhibitor	Substrate	Type of Inhibition	K_i (mM)
p-Cresol	Catechol	Competitive	0.88
	pyrogallol	uncompetitive	0.90
L-phenylalanine	Catechol	non-competitive	4.33
	Pyrogallol	uncompetitive	28.60
p-Nitrophenol	Catechol	Competitive	0.17
	pyrogallol	Competitive	0.25
Diethyldithiocarbamate	catechol	non-competitive	0.04
	Pyrogallol	non-competitive	0.08
Thiourea	Catechol	Mixed	0.07
	Pyrogallol	non-competitive	0.50
KCN	Catechol	Non-competitive	0.16
	Pyrogallol	Mixed	0.20
NaN3	Catechol	Non-competitive	1.25
	Pyrogallol	Non-competitive	1.80
Quinoline	Catechol	competitive	4.25
	Pyrogallol	Non-competitive	4.70
Cinnamic	Catechol	Non-competitive	2.0
	Pyrogallol	Non-competitive	1.40
Orcinol	Catechol	Mixed	1.43
	Pyrogallol	Competitive	0.97
Resorcinol	Catechol	Competitive	7.90
	Pyrogallol	Non- competitive	6.40

Reaction were carried out in KPi buffer, pH 7.0. the apparent Km values for catechol and pyrogallol were 8.9 m M and 6.6 mM respectively.

THE PROCESS OF ENZYME BROWNING:

Once cells are injured, discoloration known as browning starts at once, initially occurring in the injured cells but eventually a layer of cells near the wound is affected, the amount of discolouration depending on the extent of injury.

The disclouration is mainly due to the conversion of colourless phenolic compounds absorbing only ultra violet light into products which absorb visible light. From the point

of view of the plant, there must be an advantage to be derived from the browning. There are a number of such advantages that have been postulated. It is known that many phenolic compounds present in the intact organ do not have any antimicrobial activity but their oxidized products, especially the o-quinones, often have this activity associated with their inhabitation of cell wall degradation by extracellular enzymes of wound pathogens. Their coupling with fungal surface proteins and viral coat proteins leads to partial or total loss of virulence. It is also thought that polymerization of the phenolic compounds has the effect of sealing off the wound surface.

The o-quinones may also inhibit host enzymes by complex with metal ions which participate in catalysis by reacting with SH groups or by non-specific binging to the hydrophobic regions of the enzymes molecule through their aromatic rings.

It has also been suggested that quinones may interfere with the electron flow in the respiratory chain by accepting electron from reduced flavoproteins without passing them to the cytochrome system thus hampering respiration.

Thus the browning reaction can be considered as part of the plant host's defence mechanism. It makes conditions within the locality of the infection as unfavourable as possible for the invading microorganisms.

DISADVANTAGES OF THE BROWNING REACTION.

In the course of the processing of yam tubers into yam powder, the enzymic browning reactions are looked upon with disfavour on aesthetic grounds. This is because most people do not like dark-coloured yam flour, ("elubo") which is the foofoo prepared from yam powder. In any case there is even something more important than aesthetics in the whole question of browning of food products. It affects the nutritional quality of the food. Quinones, the primary oxidation products of the browning reactions, react with proteins. Of the various potentially reactive groups of the amino acid side chains,the most abundant are the primary -amino groups of lysine residues. Quinones couple in a similar fashion with the thiol group of cysteine residues in protein. Such reactions would be expected to lead to an alteration of the physical and biological properties of the proteins concerned. Most yam cultivars contain between 6-8% protein. This is fairly low and a situation in which part of this amount of protein is rendered nutritionally unavailable is undesirable for the average consumer whose additional protein supply is on the average, quite limited, proteolytic enzymes are unable to hydrolyze peptide bonds of amino acids of protein that are coupled to quinones. Even in the unlikely event of digestion taking place, the amino acid residues coupled to the quinones are nutritional unavailable. It is thus essential that the means have to the explored to reduced the reactions leading to the enzymic browning of processed yam, other tuber crops and edible fruits. This would ensure that the essential amino acids in these foods are available for utilization for growth and body repair.

CONTROL OF ENZYMIC BROWNING REACTIONS

Control of enzymic browning can be achieved by physical, chemical and biological methods. Physical methods would include the exclusion of oxygen, inactivation of the enzyme by heat, or by change in pH. On the other hand, chemical methods would include the addition of a competitive inhibitor of the substrate or the addition of substances that combine with the metal prosthetic group of the enzyme. Our interest is the chemical control of the browning process through a good understanding of the properties of the enzyme that catalyzes the reaction. Generally, chemical control includes methods involving the addition of substances that react with the quinone or other products of the reaction. This would require relatively large concentration of the additive to combine with all the intermediates formed during the whole period of storage of the cut tissue. This is usually not found practicable on medical grounds and because of the undesirable flavor that may result. Similar objections may apply to the use of substance which act as competitive inhibitors of the phenolic substrate such as p-nitrophenol or m-dihydroxyphenols. Inhibitors that inactivate the enzymes by combining with the copper prosthetic group have also been tried. Some of the limitations include the undesirable flavours introduced even when these substances are used in low concentrations.

Mapson (6) has reported that although a large number of chemical substances which control the activity of the phenolase system are known, no deal substance has been found. The following are the properties of such a substance:

i. It should be effective in inhibiting the enzyme system in low concentrations for a reasonable period of time.
ii. It should readily penetrate the outer layers of the tissue.
iii. It should be tasteless in the concentrations used.
iv. It should be acceptable on health grounds.
v. It should not destroy any important nutritive element of the tissue.

For the potato, ascorbic acid has been said to fulfill most of these requirements with exception that its retardation of enzyme browning is of short duration.

One of the most commonly used chemical additives which have the least disadvantage is sodium sulphite or sodium metabisulphite. The best result was obtained by the use of sulphite at pH 6.0. Under these conditions, dipping the potato slice in a $5X10^{-2}$M solution of metabisulphite for 2 minutes gave a satisfactory storage life of a least 6 days at 5^0C. Our results with the purified yam enzyme show that sodium metabisulphite is a good inhibitor of its activity with various polyphenols as substrate (Table 2). It has however been reported that the chief disadvantage of this antioxidant is that at higher levels, it has appreciable destructive effect on thiamine. (7). Also at concentrations above 10^{-2}M, it has been reported that it can be detected organoleptically in cooked potato slice by many individuals. For the yam tuber, we

have consistently stored dried yam tubers for hours in 1% sodium metabisulphite solution without any visually detectable browning even after removal of this solution. With L-ascorbate solution as inhibitor, browning was observed once the tuber slices were removed from solution this indicates that inhibition of browning with L-ascorbate is reversible while that with sodium metabisulphite is not. The latter compound is already being used to arrest browning during the processing of yam flour marketed by the National Root Crops Production Company Ltd. Under the name “Yamfufu”.

Our work has contributed towards some understanding of the Biochemistry of the yam tuber and at the same time provided information that will be of use to the industrialist interested in the preservation of the yam tuber through processing.

Summary

Mr. Vice-Chancellor, Distinguished Ladies and Gentlemen, I had at the start of this lecture indicated that the study of Biochemistry started with the discovery of the involvement of enzymes in fermentation process. I went on to point out the central role enzymes can play in health care delivery, nutrition and industrial processes. I also pointed out that the field of genetic engineering or Biochemistry that has made biochemistry the glamour science of the latter part of the twentieth century involves manipulation of a cell’s ability to produce enzymes of choice. The facilities for the use of enzymes for the purposes that aim at improving the quality of life of man on earth exist for those who desire. Our past record as a nation and the half-hearted support we give to encourage the study of science do not lend credence to our wish to be self-reliant. There is more rhetoric than concrete action aimed at making our country where policy makers expects scientists to be magicians that have the ability to make something out of nothing. My fear is that with the current trend in the University and Research Institutes, the little scientific culture we were about to copy from the advanced nations is slowly being erased. One need only to look at empty laboratories and stores in science departments to realized that we are fast approaching the “dark ages” of scientific development in Nigeria. If academics in the sciences have appeared to remain in their “ivory towers” (or is it now “mud towers) oblivious of the problems of the wider Nigerian society, the fault can only be traced to their having not been trained to be magicians. It is therefore high time we start to give positive encouragement to scientific research. When we do this, our biochemist and medical practitioners will begin to use enzymes, as the “wonder molecules”. They are, for purpose aimed at improving the quality of life of our people.

I cannot end this lecture without paying tribute to some of those who have had a profound influence on my career and what I have made of its choice. I publicly acknowledge the inspiration I derived from Prof. A.U. Ogan, my Head of Department for many years at the University of Nigeria, Nsukka. I also at this moment would like to remember Dr. D.C. Watt and Prof. G.A.D. Husslewood of the Department of

Biochemistry, Guy's Hospital Medical School, University of London who introduced me to the enzymes studies. Lastly, I wish to thank the many generations of students that have gone through my Enzymology class for their contribution in giving me a sense of fulfillment.

Thank you all for listening to me patiently.

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