Inaugural Lecture Series **147**

ON THE ROAD TO BIOTECHNOLOGY

By

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Professor of Food Science and Technology

OBAFEMI AWOLOWO UNIVERSITY PRESS LIMITED

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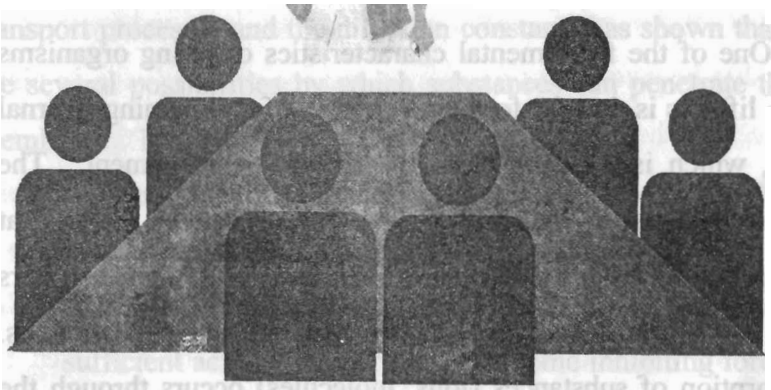
Mr. Vice Chancellor Sir, Colleagues, Ladies and Gentlemen. Sometime ago, actually on a foggy, cold November day in 1954 when I was in the second year of my studies, in the gymnasium, in one of the highly esteemed girls' schools in Budapest, Hungary, I was called to the board by my geography teacher – Zsofi neni – to answer her questions and receive her unquestionable judgment of my knowledge of Nigeria as a land, its resources and people, (Plate 1). Little did I know at that time that in 1968 I shall land in Lagos on a foggy, rainy and in very humid, hot weather to experience what I learned from books, then to teach and do research for decades in this country. Now I am again in front of the **"board"** to give account of my activities in the past 39 years. One can not avoid one's fate.

It was quite clear from the early years of my studies in the secondary school that I have to work hard to establish my position among the very best students in my class and be the pride of my parents, particularly of my father who worked extremely hard to keep his small family well above the standard of living that prevailed after the second world war in the politically and economically destroyed Hungary. His iron will as well as his iron-handed conduct in following my progress in education and my mother's unfailing diligence to give support in every aspect of our

life was rewarded by my admission into the University in 1957, for the newly established discipline in Biology and Chemistry, a combined course which allowed for full qualification in both disciplines and promised wide employment opportunities, and satisfied my wish from early childhood to learn Chemistry. It was also an excellent compromise with my father's original design on my further career as a chemical engineer. I was to spend the next five years at the now 500 years old Eotvos Lorand University and graduated in 1962 with first class degree. Those years are like a dream now and I wonder if I could cope again with the rigors of compulsory 48 hours of lectures and practicals per week, the extra late hours to complete assignments in time in acceptable quality and pass well semester, colloquia (combined 2-3 semester exams) and start exams in required subjects. Miraculously still, there was a time for occasional get-togethers, or to take part of the lively cultural life of the capital, or take a few days of holiday after exams. I was lucky enough to get summer jobs, tutored weak students to support my stipend, then from the third year of my studies



became paid demonstrator at the Dept. of Plant Physiology. All these extras provided experience, some income and paved my way towards the real life of a young degree holder. By the time my degree was ratified (it takes only 2 weeks after the last exam!) and my influential future employer got the exemption permit for me from youth service. I was a job holder as assistant research fellow in the Institute of Animal Husbandry in Godollo, located some 40km

outside Budapest. I was given the opportunity to continue the research work I have been involved in as a student with my supervisors Drs Boszormenyi and Cseh. The work aimed at studies of biological transport in plant and microbial cells.

Biological Transport

One of the fundamental characteristics of living organisms from the lifeless is that the former is capable of maintaining internal integrity, which is different from the external environment. The maintenance of the internal milieu of living organisms means that the composition of cells and tissues must be retained, while others must be excreted in order to maintain its physiological functions. The absorption of substances (ions, molecules) occurs through the membranes attached to the cell wall which are composed of lipids and proteins. The movement of dissolved molecules is termed penetration, while the property of the membrane to allow certain dissolved molecules to pass through it and block the movements of others is called permeability and expressed in terms of the amount of substance passing through $1cm^2$ of the membrane surface within 1 sec (1 flux). The flux is proportional to the concentration difference existing between the two sides of the membrane and is bi-directional. The flux into the cell is the function of the extracellular concentration, while the movement out of the cell is determined by the intracellular concentration. In case of the movement of ions the situation is somewhat more complex. The concentration difference is insufficient to determine the fluxes, the electric potential difference across the membrane must also be taken into account. The comparison between the permeability constant of different transport processes and the diffusion constants has shown that there are several possibilities by which substances can penetrate the cell membrane. These are:

- Penetration of molecules through the pores of the cell membrane (diffusion).
- Penetrating molecules migrate across the cell membrane with sufficient activation energy to overcome inhibiting forces and are transported by activated diffusion.
- Molecules are attached to carriers and transported with unchanged or decreasing free energy, while in the case of active transport with increased free energy (Boszormenyi *et al*, 1972).

In the 1960's the molecular biological approach of understanding active transport and its possible practical implication led to the comparative studies on amino acid transport using various plant cells, brewer's yeast and animal (chicken intestine) cells by

this author in collaboration with senior colleagues, and formed the basis of my 1967 Ph.D. thesis in biochemistry and microbiology. The findings of these investigations were reported in a series of papers: Balogh *et al*, 1961, 1963a, 1963b, 1965, 1966, 1967, 1968, 1969, using radio-isotope labeled compounds. It was established that inhibitors (i.e. chloramphenicol) had only a slight inhibiting effect on the accumulation of organic and inorganic ions and there is a relationship between protein turnover and ion accumulation in plant cells, i.e. in wheat roots. Experiments carried out with brewer's yeast (*Saccharomyces carlsbergensis)* proved that the amino acid (methionine) uptake is a complex that mediated process at low concentration and permeation is in higher dependence on the concentration. The process is regulated and can be repressed by different amino acids and the inhibition is a definite competitive type at low concentration. At relatively high concentrations there has been a derivative from the normally saturating concentration and the operation of a second mediated system was supposed to be responsible for it. Inhibition of the uptake could be affected by ammonium ions and in pre-treatment with methionine and other amino acids but the inhibition of transport could be repressed by puromycin a situation considered similar to enzyme repression. Since puromycin is a known inhibitor in protein synthesis at ribosomal level, the conclusion was drawn that the pre-loading effect is connected with protein synthesis. Concerning the function of the synthesized protein it was assumed that the protein is a specific transport inhibitor protein, whose synthesis has been induced by the pre-loading which reacts directly with a transport protein (permease) or, the protein is a repressor inhibiting the transcription of a specific transport protein at the nuclear level.

The experiments carried out to compare S-containing amino acid uptake by 2 weeks old New Hampshire chicken indicated that the dual uptake mechanism of methionine was the same. However, the effect of competition for the different amino acids – from the feed plays an important role in the formation of amino acid balance, methionine metabolism and opens a wide possibility for inheritable differences. The production of S^{35}-labeled fodder plants and labeled feed premixes in feeding experiments proved the variable availability of amino acids and their utilization in the various organs (Balogh, Meszes and Boszormenyi, 1963b; Toth, Balogh and Boszormenyi, 1966), which was important industrially in the design of animal feed formulas supplemented with amino acids – fairly expensive at that time.

While these experiments were in progress, I changed jobs and became the head of the microbiological control laboratory

within the large quality control conglomerate of the National Hungarian Breweries producing 4.5 million-hectoliters of beer yearly. This change was necessary to remove the burden of daily travels to my working place and stay closer to the University where most of my research work for the Ph.D. thesis was carried out. The 4 years spent in this position allowed for wide experience in brewing practice and shifted my interest firmly towards microorganisms as far as research was concerned. The topics covered were on the ion-binding characteristics of brewer's yeast from which conclusions were drawn on its flocculation properties (Balogh, Boszormenyi and Jambor, 1967) and the depletion of amino acids, sugars from wort by yeast with the aim of finding biological explanation for defaults and possibly correct it. It should be noted that the barley used for malting was very high in protein content (the good quality for economic reasons were sold for hard currency!) Therefore, the wort for fermentation contained lots of undesirable components which adversely affected yeast activity and often manifested in the occurrence of high level of spoilage microorganisms and irregular fermentation pattern (Balogh and Kulcsar, 1966; Balogh *et al.* 1969).

Research in Nigeria

My research activities in Nigeria began shortly after assumption of duty as research fellow at the Department of Animal Science, University of Ife in September 1968 I was scared – not of the work but of the people around me who spoke English fast- what I could not understand or just a few words enough to call my husband to find out further details. Luckily this period passed and within a short time I had not only organised my assigned laboratory but started to converse with colleagues and fearlessly attended meetings.

The first assignments given by Prof. A. Adegbola, the Head of the Department concerned the microbial aspects of ruminant nutrition, a methodically very difficult project, and parallel to this, the studies on the possible utilization of Gamba grass as fodder plant in silage preparation. The results obtained from these works have shown that there was no substantial difference between the microbial flora and its activities by the tropical dwarf goat and temperate ruminants, therefore other means of nutritional improvement, i.e. nitrogen or carbohydrate rich feed ingredients use needed to be employed (Hungate, 1966; Balogh and Adegbola, 1971). Similarly, we found that the Gamba grass, due to its variable low level of carbohydrate (sugar and starch) (Fig.1 and 2) content is

not suitable for silage preparation unless supplemented with cheap industrial by-product such as molasses (Adegbola and Balogh, 1974). At that time molasses was widely spread in Nigeria, actually wasted as binder in dirt road making as we saw in Jebba!

Fermentation Studies

While this works were in progress and after organizing the first **International Radiation Microbiology Meeting** in Ife in 1971, we obtained a grant from **IAEA** for an inter-departmental research for the nutritional improvement of cassava and its fermented form – gari – through microbial protein production as obtained from the fermented food processing practices of the Orient (*oncom, tempeh, puto, red rice, etc.*) and further strengthened by the overall interest in cassava utilization with increased protein and decreased cyanide content. In our experiments (Balogh, 1972; Adegbola and Balogh, 1972; Balogh, 1974) we used microorganism known to possess high amylolytic activity for decomposition of starch to obtain fermentable sugars to provide fast growth and protein synthesis. Radiation with Co^{60} source was used to delay sporulation in the filamentous fungi (*Rhizopus oligosporus*) and to obtain mutants with high level protein synthesizing ability, while the other fungi (*Saccharaomyces cerevisiae, Geotrichum candidum* and

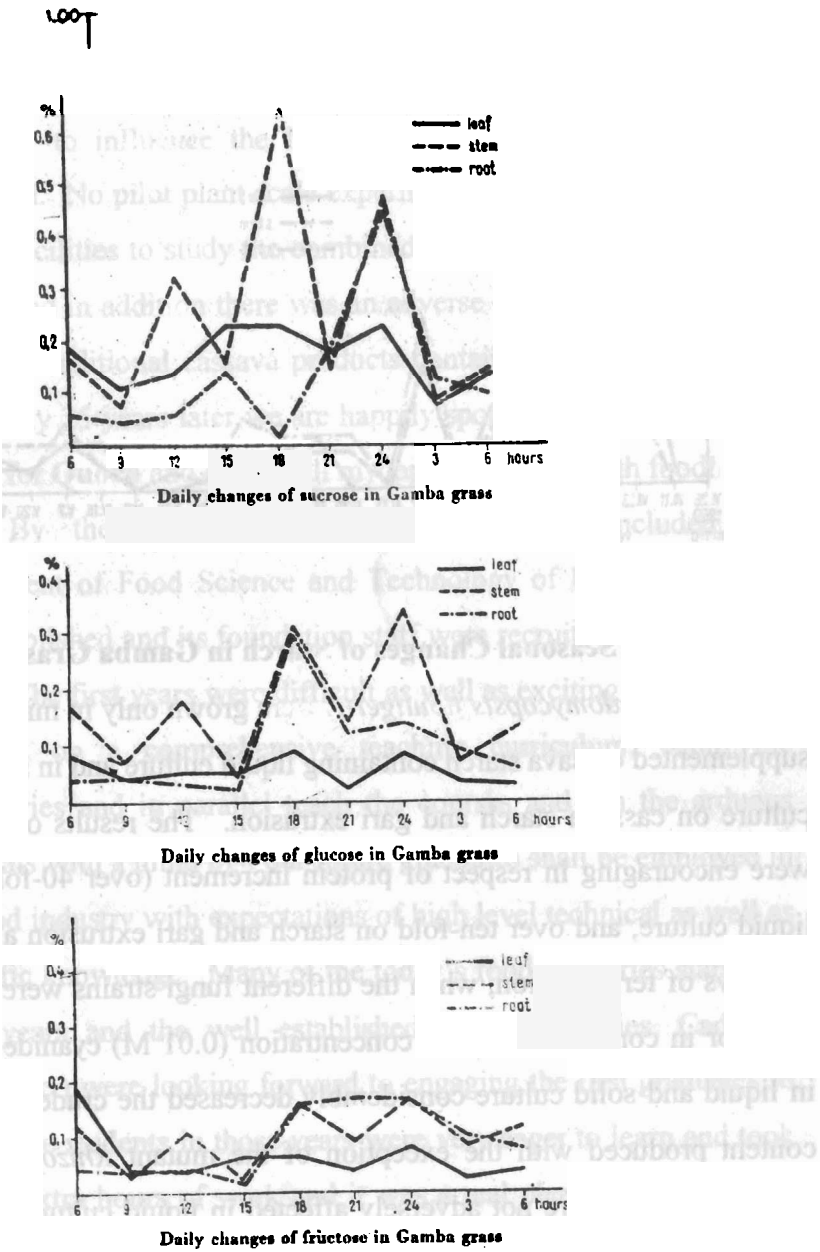


Fig. 1: Daily Changes of Sugars (Sucrose, Glucose, and Fructose) in Gamba Grass

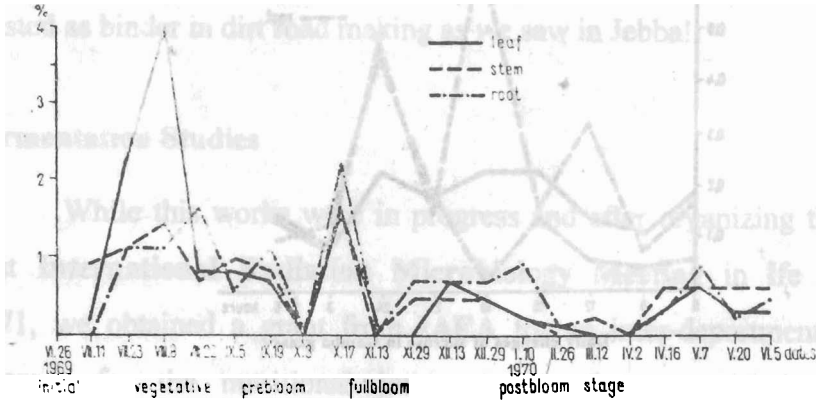


Fig. 2: Seasonal Changes of Starch in Gamba Grass

3 strains of *Endomycopsis fibuligera* were grown only in minerals - supplemented cassava starch containing liquid culture and in solid culture on cassava starch and gari extrusion. The results obtained were encouraging in respect of protein increment (over 40-fold) in liquid culture, and over ten-fold on starch and gari extrusion after 3 – 5 days of fermentation, when the different fungi strains were used alone or in combination. Low concentration (0.01 M) cyanide both in liquid and solid culture considerably decreased the crude protein content produced with the exception of the mutant *Rhizopus* and *Candida* which were not adversely affected in liquid culture. This

indicated that the processing conditions for starch or the fermentation practice for gari concerning its residual cyanide content are likely to influence the level of crude protein content if not controlled. No pilot plant scale experiments were carried out due to lack of facilities to study the combined fermentation process and its control and in addition there was an adverse opinion concerning the eating of traditional cassava products containing microbial protein. Just barely 25 years later we are happily spooning as full meals and paying for **Quorn** and others (all mycoproteins) as health food!

By the time these experiments were concluded, the Department of Food Science and Technology of University of Ife was established and its foundation staff were recruited. I was one of them. The first years were difficult as well as exciting since we had to work on a comprehensive teaching curriculum, equip the laboratories and in parallel teach the courses and run the arduous practicals with a mind that our future graduates shall be employed in the food industry with expectations of high level technical as well as scientific knowledge. Many of the today's food industries started in those years and the well established ones (Breweries, Cadbury, Nestle, etc.) were looking forward to engaging the first graduates in 1976. The students in those years were very eager to learn and took happily extra hours of work and it was a real pleasure to teach and

guide projects. The relationship with food industries started to develop and was further strengthened by the establishment of the Nigerian Institute of Food Science and Technology, the professional body of food scientists in 1975, and a few years later by the launching of the *Nigerian Food Journal* in 1983, whose editor I became in 1990 for 4 years, and fellow of the Institute in 1993. I have actively participated in the events organized by the Institute and outside Nigeria in International Conferences and involved the best students working under my supervision on projects concerning fermentation studies, microbial enzymes, microbiological quality and preservation of foods and pursued my personal interest in food product development from tropical raw materials and history of foods (Balogh 1981, 1987, 1988a, 1988b, 1989a, 1989b, 1992, 1996, 1997a, 1997b; Emiowei and Balogh, 1996; Folaranmi *et al*, 1990; Enujiugha and Balogh, 1992; Enujiugha *et al*., 1994)

As early as 1965 Hesseltine (Hesseltine, 1965) the best known indigenous food fermentation expert marked the 20th century the "millennia of food fermentations" probably more in tropical countries than in temperate zones. The sophisticated techniques developed and practiced by civilizations – often involving large populations – in this century became a wealth of techniques for the food industry and provided bases for elegant and seemingly simple

processes, and allowed for exploitation of conventional and unconventional raw materials as sources of food. The short fermentation time, the essence of tropical fermentations, at naturally optimal temperature, may be regarded biotechnologically as very modern in relation to high reaction rates demanded by the bio-process industry to achieve the much desired high level fermentation economics. So, several indigenous fermentations originally carried out on cottage industry level became the focus for industrial development to emphasize the virtues conferred on potential food materials by fermentation, (Table 1). In no time we learned that the "small is beautiful" concept originated by Schumacher in the 1960's is "small is difficult" when it applies to conversion of indigenous fermentations to industrial ones. This is along this line and advanced understanding of the role of microorganisms, their complexity yet adaptability based on their genetical characteristics led scientists to the detailed study of fermentations in terms of raw material preparation, preservation of inoculum and controlled optimized conduct of fermentation (Stanton. 1998).

Table 1: Virtues conferred on a potential food material by fermentative processing

Contribution to the economy of food-winning
Preserves perishable raw material at low cost, aids dehydration. Salvages waste otherwise not usable as food.
▪ Reduces cooking time, hence reducing demand on the food-winning time by reducing fuel demand.
▪ Enhances nutritional value by improving digestability, protein value, vitamin content.
Protective value
Destroys toxic, undesirable or anti-digestive components of the raw material; this action increases the range of raw materials available as food. Adds positive antibiotic components, destroys harmful biota and protects against infection or infestation.
Psychological and social value
▪ Improves appearance and flavor, often imparting a meat-like flavor.
▪ Functions as appetite stimulant and condiment.
Imparts texture, fibre and bite/chewiness, makes the product enjoyable.

Source: ***Stanton (1998)***

Vinegar

In the 1980's in Nigeria, strong interest prevailed concerning the utilization of local raw materials to produce almost anything that could be labeled as "value added".

Agricultural by-products, under-utilized, abundantly available plant materials became the focus of interest as potential raw materials for fermentation. Further driving force evolved from the fact that many essential foods and ingredients were imported (vinegar, spices, wines, alcohol, protein supplements, etc.) and were costly, therefore any efforts to replace or substitute for them seemed viable. With this consideration in mind, we started investigation into the production of vinegar (acidulant, preservative) by fermentation from the abundantly available breadfruit (*Arthrocarpus commumis*). The designed fermentation process was in two stages: first the conversion of the starchy breadfruit pulp to fermentable sugars was effected by using a cocktail of amylolytic, pectolytic and cellulotic enzymes which was subsequently fermented to alcohol (7 – 8%), then in the second stage of fermentation the alcohol was converted to vinegar in the manner of **Orleans method** traditionally used to obtain fine quality vinegar in semi-continuous process. The vinegar obtained contained 4 – 5% acetic acid, kept its pleasant aroma which remained unchanged after aging and pasteurization. However, when

distilled only the sharp acid taste persisted as expected and was similar to distilled white vinegar. The economic production was monitored through the GK (Gesammte Konzentration) value, which was just slightly lower at the end of fermentation, indicating that the conversion was complete and only minimal evaporative loss occurred, (Table 2) (Balogh and Mbajunwa, 1987).

Single Cell Protein (SCP) Production

As early as 1978 the problem of energy shortage was anticipated and the need for alternative energy sources became of considerable interest (Lipinski, 1978; Balogh, 1978). Actually the interest was dual, in the sense that the bioconversion process through microbial fermentation were versatile and suitable for the conversion of cellulosic agricultural waste often abundantly available, not utilized (burnt on the field with undesirable consequences) and generally posed nuisance. Taking advantage of the availability of large quantities of corn straw we set out to study its potential for fuel alcohol and single cell protein production (Omobuwajo, Balogh and Layokun, 1987). The results have shown that the acid hydrolyzed corn starch substrate with locally isolated yeasts (*Candida intermedia* and *Saccharomyces cerevisiae*) yielded 89% and 54% biomass respectively. based on

Table 2: Determination of the G.K. (Gesammte Konzentration) value in the Acetification of Breadfruit wine

TIME DAYS	ACETIFIC-ATION PERIOD/ DAYS	CHARACTE-RIZATION DURING ACETIFICA-TION ACIDITY %w/v ACETIC ACID	ETHANOL (% v/v)	GK VALUE	PROCEDURE
0	0	1.98	4.08	6.06	200ml of vinegar +
4	4	0.68	0.68	6.08	200ml of stock alcohol
	0	3.06	3.01	6.07	400ml of vinegar + 200ml of stock alcohol
7	3	4.92	0.77	5.69	
	0	3.42	2.68	6.10	600ml of vinegar + 200ml of stock alcohol
11	4	5.10	0.44	5.54	200ml of vinegar withdrawn
	0	4.26	1.52	5.78	600ml of vinegar + 200ml of stock alcohol
13	2	4.94	0.86	5.80	400ml of vinegar + 200ml of stock alcohol
	0	3.60	1.92	5.52	400ml of vinegar + 200ml of stock alcohol
15	2	5.20	0.35	5.55	600ml of vinegar withdrawn

Source: *Balogh and Mbajunwa (1987)*

the 50% substrate carbon conversion in aerobic fermentation. The calculated economics of fermentation and the processing/treatment of straw have shown favorable figures (1000kg SCP from 5000kg corn straw) at about 10% of the cost of production in the US on the prevailing prices of that time. On the other hand, the alcohol production proved to be not feasible due to the difficulties arising during fermentation which evolved from the presence of inhibitors, long acclimatization time, and presence of unfermentable sugars by the yeast strains used.

Formulated and Extended Palm Wine

The studies on indigenous food fermentations and their development into at least medium level industrial process focused mostly on gari and palm wine. My interest in palm wine evolved from the fact that palm wine is not really a wine in a strict oenological sense since its alcohol content is far below that expected in grape (*Vitis vinifera*) wine, it has short shelf life and it is cloudy white in appearance. Its microbiology and biochemistry was studied and reviewed by Okafor (1978) extensively. The series of studies carried out by Mmegwa, Balogh and Ngoddy (1985, 1988) first tackled the possibility to augment for the palm wine quantities required by the palm wine bottling units which by this time began to

proliferate in the country. Formulated and extended palm wine was prepared, then pure culture inoculum was made from natural palm sap for formulated palm sap fermentation (Uzochukwu, Balogh and Ngoddy, 1991). In further studies, the volatile constituents and flavor profile of palm wine and sap, (Uzochukwu, Balogh Tucknot, Lewis and Ngoddy, 1994a and 1997), the role of microbial gums in color consistency (Uzochukwu, Balogh and Ngoddy, 1994b) and the role of yeast and bacteria in palm wine aroma development was studied and reported (Uzochukwu, Balogh, Tucknot, Lewis and Ngoddy, 1999). The results obtained have shown that the formulated palm wine (composed of sugar solution, minerals and natural palm wine microorganism flora) and its various blends with natural palm wine (termed extended palm wine) passed the sensory evaluation test for taste, aroma and overall acceptability, but the viscosity and color slightly differed from that of natural palm wine. (Fig. 3) From studies on other traditionally fermented plant saps (agave) similar to palm sap, it was observed that the white turbidity was due to a lactic acid bacteria (*Leuconostoc*) capable of forming dextrans which were found to be responsible for the viscosity.

Our studies also proved that in natural palm wine these organisms were present and produced gums which could be used to restore viscosity and by increasing the inoculum concentration the

colour restoration is achieved by natural additives. The composition of gums were found to be 80% glucans and 20% fructans produced by *Leuconostroc* and two other bacteria not fully identified (due to peculiar morphology) and a homofermentative *Lactobacillus*, respectively in individual pure culture fermentation of palm sap. (Table 3) Thus it was established that the viscosity and color of palm wine is due to bacterial activities and the yeasts do not play any role in this. However, as it has been shown by Mmegwa (1987) and confirmed by GC and GC-MS studies later (Uzochukwu et al, 1994, 1997 and 1999) that yeasts are important in the aroma and taste of palm wine. Distinct differences were found between the palm sap and palm wine volatiles. Some 82 compounds were identified: 47 esters, 9 alcohols, 5 acids, 6 carbonyls, 2 acetals, 4 terpens and 9 hydrocarbons. The odor evaluation suggested that no one compound is responsible for the characteristic palm wine odor, from which only a few (7) are truly native to palm sap and the acetates of higher alcohols and the ethyl esters of straight chain aliphatic $C_6 - C_{10}$ acids seem to play important role in imparting the characteristic fruity nuances in palm wine odor. The qualitative differences between sap and wine were further promoted by the presence of low-boiling esters in the sap and their apparent absence in wine. The individual role of yeasts and bacteria (*Lactobacillus*

and others) in palm sap fermentation indicated 2.8 and 2.5 fold increase in total volatiles when *Saccharomyces cerevisiae* and *S. chevalieri* was used. Additional 27 compounds were detected in the headspace, volatiles comprised of 17 esters, 4 alcohols, 4 terpenes and 2 hydrocarbons. In the fermentations carried out by bacteria the total volatiles decreased, but sulphur compounds were detected. Based on sensory evaluation results, it was concluded that the yeast are mainly responsible for the palm wine's characteristic odor. It does not, however, preclude the role by the volatiles produced by the LAB bacteria in the subtle nuances of the beverage's aroma. I believe these findings and further details patented by Uzochukwu (1996) give enough background for entrepreneurs to establish and operate palm vineries to allow us to buy standard, good quality, bottled palm wine.

Table 3: **Palm sap characteristic after 24h fermentation by pure cultures of microorganisms isolated from palm wine**

Sample/organism in palm sap	Viscosity (cps)	Color at 720nm	Color on settlement of microorganisms
Fresh Palm Sap (0-h)	1.088 ± 0.05	0.01 ± 0.005	Transparent with brownish tinge
Pasteurized palm sap (0-h)	1.021 ± 0.02	0.01 ± 0.003	- do -
Naturally fermented palm sap (Palm Wine)	1.377 ± 0.06	0.11 ± 0.030	Creamy White
Saccharomyces cerevisiae	0.960 ± 0.03	0.01 ± 0.003	Transparent
Saccharomyces chevalieri	0.960 ± 0.04	0.01 ± 0.003	Transparent
Leuconostoc mesenteroides	151.8 ± 12.11	0.80 ± 0.15	Creamy White
Leuconostoc dextranicum	1.47 ± 0.03	0.16 ± 0.02	White
Lactobacillus casei	1.024 ± 0.02	0.01 ± 0.003	Transparent
Lactobacillus case SS casei	1.024 ± 0.01	0.01 ± 0.003	Transparent
Organism No. 2	1.281 ± 0.04	0.25 ± 0.005	Faint White
Organism No. 3	1.088 ± 0.02	0.03 ± 0.005	Transparent but slimy
Unidentified *Lactobacillus* (AW)	136 ± 9.21	1.25 ± 0.10	Creamy White

Source: ***Uzochukwu et al. (1994)***

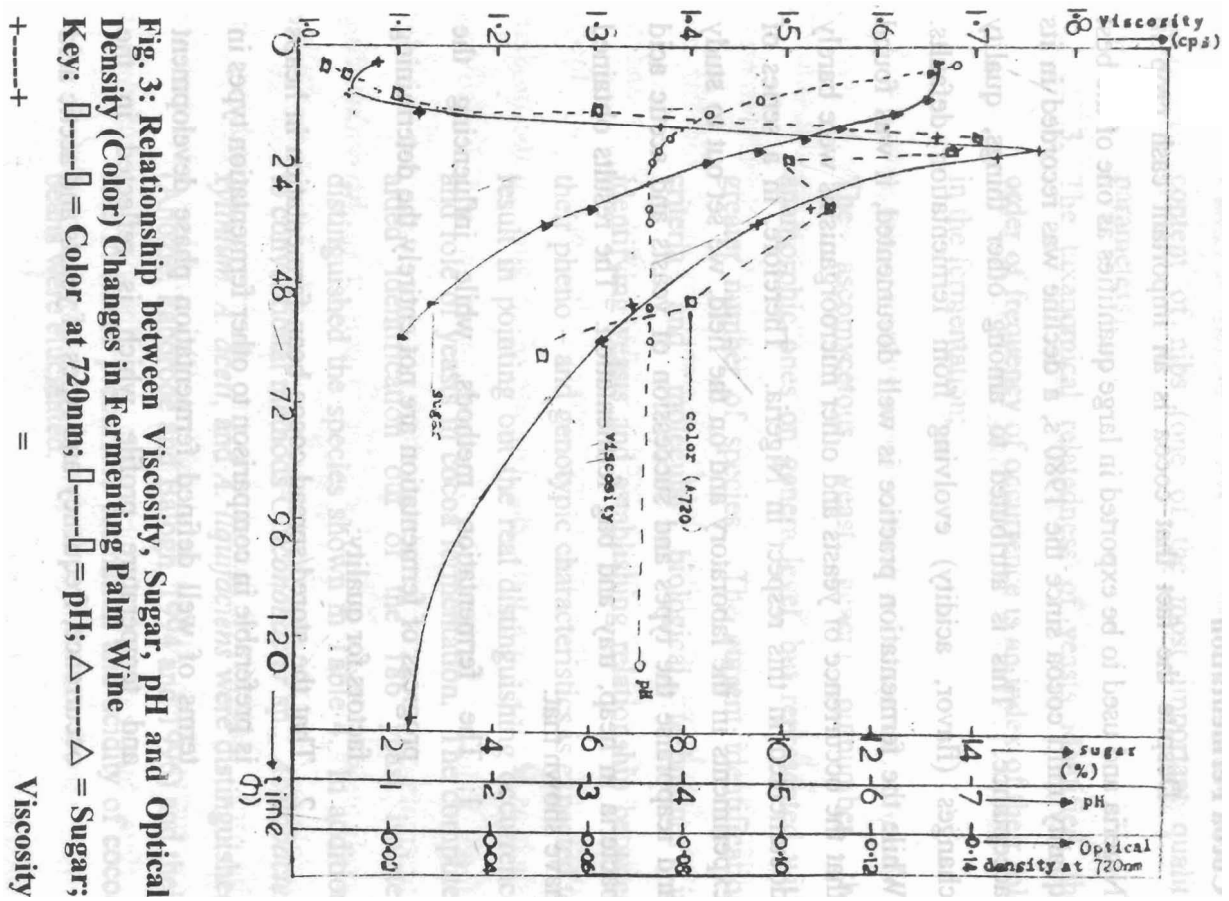


Fig. 3: Relationship between Viscosity, Sugar, pH and Optical Density (Color) Changes in Fermenting Palm Wine

Key: □----□ = Color at 720nm; □----□ = pH; △----△ = Sugar; +----+ = Viscosity

Cocoa Fermentation

Despite the fact that cocoa is an important cash crop in Nigeria and used to be exported in large quantities as one of the best quality mild cocoa since the 1980's, a decline was recorded in its acceptance. This is attributed to among other things, quality changes (flavor, acidity) evolving from fermentation defaults. While the fermentation practice is well documented, it was found that the occurrence of yeasts and other microorganisms were barely documented in this respect in Nigeria. Therefore, in a series of experiments in the laboratory and on the field, we set out to study and reappraise the types and succession of yeasts and acetic acid bacteria in heap, tray and bag fermentation. The results obtained have shown that:

1. The fermentation methods, while influencing the progress of fermentation are not entirely the determining factors for quality;
2. That the stored/spread cocoa bean fermentation in heaps is preferable in comparison to other fermentation types in terms of well defined fermentation phase development and temperature profile which is reflected in the occurrence, frequency and types of yeasts and acetic acid

bacteria and their subsequent activities, i.e. residual acid content of nibs (one of the most important quality parameters);

3. The taxanomical relatedness of yeasts identified, the order of frequency of occurrence is an index of their role in the fermentation;

4. The ascomycetous yeast genera outnumber the anamorphic ones on genera level, but represented with greater number of species. The current identification criteria and molecular biological techniques in identifying yeasts and establishing relationship between their pheno – and genotypic characteristics is expected to result in pointing out the real distinguishing occurrence and role of yeasts in cocoa fermentation. The computer aided identification out of the 186 yeast isolates distinguished the species shown in Table 4. In addition to conventional methods *Gluconobacter* sp., *Acetobacter xylinium*, *A. aceti*, and *A. liquifaciens* were distinguished (Anozie, Faborode and Balogh, 1994 and 1996) and their role in flavor precursors formation and acidity of cocoa beans were elucidated.

Similar considerations governed studies on coffee fermentation in respect to the role of fungi in the degradation of mucilage by dry fermentation method and in storage in mycotoxin formation (Onyemachi and Balogh, 1997) and the processing and storage of onion cultivars (Wilcox and Balogh, 1998).

Microbial Enzyme Production for Legume Treatment

While the focus of the above studies was on the role of microorganisms in fermentation of various food materials, interest was developed to utilize locally isolated organism for enzyme production with the aim to promote processing of foods and to overcome difficulties often encountered when energy-rich foods such as legumes are utilized. The use of enzymes in the modern food industry is inevitable in raw materials and ingredients pretreatment to make them suitable for further processing or wider application in various products, or to correct quality-influencing attributes. In Nigeria, the largest enzyme utilizing industry is the brewing industry, followed by the confectionery industry in sweetener (corn syrup) production. The limitless opportunities offered by the use of enzymes, particularly the microbial enzymes is often barred due to high importation cost not affordable by most food producers in this country.

Table 4: Summary of Yeast Genera and Species in Nigerian Cocoa Fermentation

YEAST GENERA		
ASCOMYCETOUS	***ANAMORPHIC (Imperfect)***	***BASIDIOMYCETOUS***
Saccharomyces cerevisiae	*Candida catenulata*	*Tremella foliacae*
S. kluyeri	*C. dendrica*	
Torulaspora delbruckii	*C. glucosophila*	
Dekkera custersiana	*C. sake*	
D. bruxellensis	*C. melibiosica*	
D. anomala	*C. fennica*	
Debaryomyces hansenii	*C. tropicalis*	
Deb. Vanrijiaw	*C. viswanathii*	
Pichia farinosa	*C. tanzawaensis*	
P. silvicola	*C. halonitratophila*	
Lodderomyces elongisporus	*C. austromarina*	
Schizosaccharomyces pombe	*Kloechera lindneri*	
Saccharomyces sinensis	*Rhodotorula aurentiaca*	
Octosporomycodes octosporus	*Rhodotorula mucilaginosa*	
Zygoascus hellenicus	*Torulopsis delbruckii*	
Arxiozyma telluris		
Kluyveromyces delphensis		

Source: ***Anozie et al. (1996)***

In the studies carried out on cowpea (*Vigna unguiculata*) the enzymic decomposition of galacto-oligosaccharides (raffinose, stachyose) was tested with the aim of using it as protein supplement wihtout side effects manifesting in disorders such as flatulence. This is due to the lack of α-galactosidase enzyme in the human intestine and consequent inability to hydrolyze the α-*D*-galactosyl linkages in the oligosaccharides; Using microbial α-galactosidase in pre-treatment of beans or bean flours used in traditional food preparation seemed and proved to be the elegant solution to this problem.

Out of the four filamentous fungi obtained from soy-bean enriched garden soil surface and screened for α-galactosidase activity a strain of *Aspergillus niger* had shown the highest activity when grown on pure culture in solid and in liquid media as well. The crude enzyme extract with an activity of 5.1×10^{-2} μM/mg protein activity when used in the treatment (2h at 50°C) of cowpea flour resulted in 83 and 95% decrease in the stachyose and raffinose content respectively. (Fig. 4) With conventional treatment (dehulling, soaking, cooking) the reduction of oligosaccharides was far less (29 and 44% respectively) even after prolonged treatment time (16h for soaking, 50 min., cooking). The physico-chemical properties of cowpea flours have not been effected when treated

with crude enzymes extract in comparison to cooked cowpeas which lacked the color, texture and aroma commonly associated with commercially available cowpea flours (Somiari and Balogh, 1991, 1992, and 1993). The characteristics of α-galactosidase produced by *A. niger* in solid state fermentation on wheat and rice-bran in 5 days fermentation had shown that the cell free extract differed in pH (6.5 and 5.4 respectively) and enzyme activity ($0.125 Uml^{-1}$ and $0.08 Uml^{-1}$); however both had the same activity at pH 5 at 50°C but the enzyme extracted from rice-bran had greater thermal stability. These marked differences indicate the production of iso-enzymes with different pH and thermal stability and the α-galactosidase activity had three peaks. The crude extract differed in composition which in the case of rice-bran produced enzyme protected the proteins with enzyme activity from denaturation by heat and influenced its binding sites through its lower pH dependence. The purified concentrated enzyme contained 3 peaks of activity indicating multi-molecular forms and had a specific activity of $1566 U/\mu g\ protein^{-1}$, but had different M_r, temperature and pH for optimal activity (Fig. 5). This is considered to be an advantage in the treatment of legume slurries with this enzyme, since the pH of slurries is not expected to be adjusted to avoid change in functional properties which could consequently influence its use in traditional

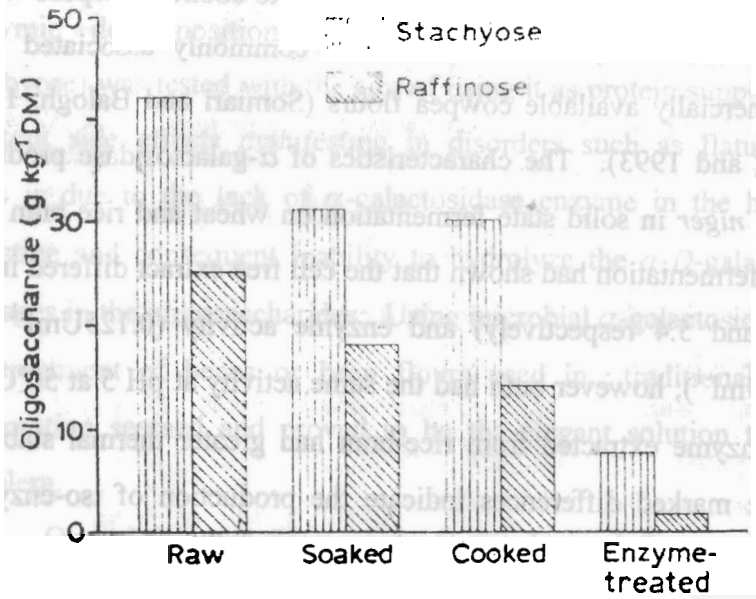


Fig. 4: Mean level of stachyose and raffinose in cowpea before and after soaking (for 16 h), cooking (for 50 min) and enzyme treatment (for 2 h at 50°C)

food preparation. Further studies indicated that the α-galactosidase activity containing protein had two polypeptide chains (78KDa and 69KDa), therefore the estimated M_r is 147KDa, which was within the range reported for fungal α-galactosidase from other species of fungi. The hydrolysis of oligosaccharides occurred simultaneously but at different rates, faster for raffinose and slower for stachyose indicating different affinity of the enzyme for these substrates. Calculations have shown that the consumption of 100g of beans

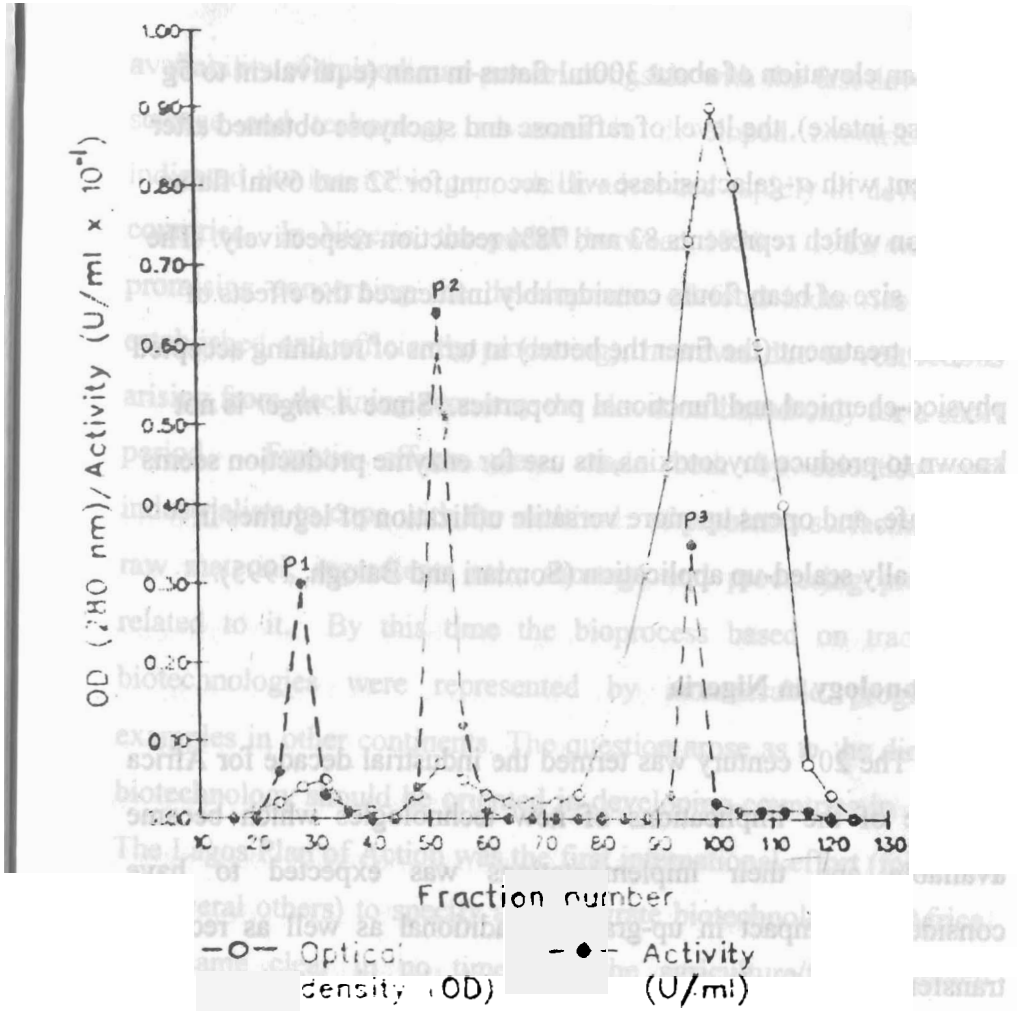


Fig. 5. Elution profile of Sephadex G-100 chromatography of α-galactosidase preparation from *Aspergillus niger*. The column was eluted at a flow rate of 16.7ml hr^{-1}, with acetate buffer (20mM, pH 5.0). enzyme activity (●---●), absorbance at 280nm (O--O), and peaks (P1, P2, P3), and fractions pooled for rechromatography on Sephadex G-200 (---).

causes an elevation of about 300ml flatus in man (equivalent to 5g raffinose intake), the level of raffinose and stachyose obtained after treatment with α-galactosidase will account for 52 and 69ml flatus elevation which represents 83 and 78% reduction respectively. The particle size of bean flours considerably influenced the effects of enzyme treatment (the finer the better) in terms of retaining accepted physico-chemical and functional properties. Since *A. niger* is not known to produce mycotoxins, its use for enzyme production seems to be safe, and opens up more versatile utilization of legumes in industrially scaled-up application (Somiari and Balogh, 1995).

Biotechnology in Nigeria

The 20th century was termed the industrial decade for Africa because of the implications of new technologies which became available and their implementations was expected to have considerable impact in up-grading traditional as well as recently transferred technologies. Biotechnologies in their earliest occurrences in West Africa – food fermentations – were analogues in some aspects to the early European practice in terms of its empirical character and scale, (Adeyemi and Balogh, 1985 and 1986). However, the recognition of the versatile nature of microorganisms, the mechanization of processes, scale-up, the

availability of trained man-power alongside with the fast developing science and technology advances in developed countries soon indicated the inevitable gap, which advanced rapidly in developing countries. In Nigeria, the period between 1950 – 1983 was very promising concerning the development of food industries (many established and efficiently producing), however due to restrictions arising from declining economy the elevation lasted only for a short period. Frantic efforts were made both by scientists and industrialists to cope with the multitude of problems surfacing from raw material, ingredients etc., shortage and processing problems related to it. By this time the bioprocess based on traditional biotechnologies were represented by innumerable progressive examples in other continents. The question arose as to the direction biotechnology should be oriented in developing countries in Africa. The Lagos Plan of Action was the first international effort (followed by several others) to specify and integrate biotechnology in Africa. It became clear in no time that the agriculture/food oriented biotechnologies, the microbial technologies (food and environment) and medical applications are likely to form the priorities. In Nigeria this tendency was basically accepted, but due to lack of clear policies, competition between administrative, industrial and research organizations and over eagerness to obtain fast solutions for

problems and become even commercially competitive for the time being seem to be a little over ambitious considering the time and proportions of industrial development in the country.

While one is optimistic, it needs to be realized that without serious investment concerning continuous services, stable infrastructural facilities, well coordinated research plans based on priorities. coordination between research interests and industrial needs and last but not the least, availability of not only qualified but well trained man-power is mandatory, (Mugabe, 1991; Sercovich, 1991; Oshinowo and Balogh, 1996).

Being a University lecturer, the latter one is of particular interest to me. The last three or so decades spent in teaching and researching with students – many who became learned colleagues of mine within and outside Nigeria – have shown that the classical systematic learning based on well designed University programme is absolutely necessary. Concerning biotechnology, despite the tremendous interest in the discipline, the foundation is poor, both in theory and particularly in practice. The basic courses like Genetics. Molecular Biology, Genetic Engineering, Microbial Technologies. etc., are scattered in different faculties and departments and often just barely passed b students as core courses and generall termed difficult, better-to-avoid courses as electives if one wants to

graduate without delay However, more often than not students strive to get projects, which in one way or the other contain some elements of biotech logy and begins to struggle to reorient to fill in the gaps arising from lack of solid background. It is therefore my wish to emphasize the need for a formal Department of Biotechnology with full programme for degree award and to strengthen the status of biotechnology courses in places where it is already existing. It is not enough to have **"biotechnology groups"**, we need to have inter-faculty and departmental programme, workshops within and outside the University to widen dissemination of knowledge in biotechnology (Balogh, 1995; Balogh, 1997; Balogh, 1998a, 1998b), with the same vigor as the fever of computerization is changing our l daily. The advances of biotechnology are already more than obvious in medicine and rapidly developing in other industries, including the food industries where the driving force is the increasing population and its demand to secure the daily needs or more in terms of food.

We, food scientists are applied scientists taking advantage of the limitless diversity offered by nature. It is therefore our task to exploit the versatility of nature by the judicious application of equally limitless biotechnologies through involvement in national/international policy making, research and teaching of

biotechnology to become firm residents of the future main discipline of science and technology in the 21st century all over the world.

Mr. Vice-chancellor, Sir, my Colleagues, my past and present Students, Ladies and Gentlemen, I respectfully offer this account of my research profile alongside with a **Subject Price for the Best Student in Biotechnology in the Department of Food Science and Technology.**

Thank you.

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PROFESSOR ESTHER BALOGH (1938 – 2000)

This inaugural lecture was supposed to have been delivered on Tuesday, 14th of November, 2000 by Professor E. Balogh. Unfortunately, she fell ill before then and did not recover until she passed on to the great beyond on Wednesday 13th of December, 2000.

Esther Balogh was born at Budapest in Hungary on 9th November 1938. In 1957, she was admitted to the famous Eotvos Lorrand University, Budapest where she graduated with a Master of Science degree in Biology/Chemistry in 1962. She obtained the Ph.D. degree in Biochemistry/Microbiology from the same University in 1967.

She arrived Nigeria in 1968 to accept a teaching position at the then University of Ife, now Obafemi Awolowo University (OAU), Ile-Ife. First, she served as a Research Biochemist in the Department of Animal Science. Then, she transferred to the newly created Department of Food Science and Technology in the Faculty of Technology in 1971 as a Lecturer I and one of the pioneer Lecturers of that Department. She was promoted to the rank of a Senior Lecturer in 1974, a Reader in 1990 and a Professor in 1992.

A dedicated teacher and a keen researcher, Esther Balogh will be remembered for a very long time for her dedication and meticulousness. Her remains were buried at the Obafemi Awolowo University Cemetery on Saturday 27th January, 2001. May her gentle soul find perfect rest with her creator.

We would like to thank the Acting Vice-Chancellor, Professor R.O.A. Makanjuola, for granting permission for the posthumous publication of this inaugural lecture.

Prof. G.A. Adegboyega
Dean, Faculty of Technology
Obafemi Awolowo University