

UNIVERSITY OF PORT HARCOURT

***MICROORGANISMS AND QUEST FOR
FOOD PRESERVATION AND MICROBIAL
FOOD SAFETY: PROSPECTS AND
CHALLENGES***

An Inaugural Lecture

By

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My academic achievements may not have been possible without the sponsorship of the following: The then Bendel State Government; The Federal Government of Nigeria, The International Foundation for Science (Sweden); The Netherlands Organisation for Scientific Research, The Hague. I am therefore very grateful for the financial support and exposure through these sources.

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1.0 PREAMBLE

Vice-Chancellor Sir, I am highly humbled, privileged and honoured to be given the opportunity to deliver the 114th lecture in the Inaugural Lecture Series of this Unique University.

Although some earlier inaugural lecturers have spoken on why some inaugural lectures are delivered later than expected, suffice it to say, that the desire and the interest to deliver one's inaugural lecture are not in doubt but the delay is often compelled by circumstances. For instance, I was to deliver my inaugural lecture in 2007 but for my invitation to serve (appointment) as Deputy Vice-Chancellor (Academic). Again, in 2013, preparations for my inaugural lecture were at an advanced stage until delivery of inaugural lectures was put on hold by Senate. Therefore, I am particularly pleased that this lecture is taking place today.

Vice-Chancellor Sir, the significance of inaugural lectures is well-known globally and the choice of the topic is often difficult because of the several research interests of the lecturer having spanned so many years. However, reflecting on my early years of growing up in the village coupled with the scope of my research activities over the years facilitated my choice of today's topic.

At about the age of 9 years, I observed that once yam tubers were cut or damaged, they became discoloured and a few days later, slimes or wooly materials developed on the cut/damaged surface. Similarly, foods (especially mixed types) left at room temperature often developed off-odour and accompanied by gas bubbles. These food products were considered spoilt and dangerous. As a child, I was warned not to eat such foods because they were unsafe.

Furthermore, as a child, I was cautioned not to eat any raw (unfermented) cassava product/tuber. These warnings became the *golden rules* with respect to food safety. As usual (in those days), these observations and warnings or commands were without explanations but as expected, I obeyed them fully without hesitation. However, these commands/warnings generated much curiosity

within me yet, there were no ready answers. As a result of this lingering curiosity over time, I began to show much interest in Biology (today, termed, Life Sciences) and more specifically known today as Microbiology.

Microbiology as a discipline is highly diverse and has several sub-disciplines. Irrespective of the sub-discipline of Microbiology, the study of microorganisms and their activities in the given ecosystem in question is paramount. Expectedly, the sub-disciplines interface/overlap. Microorganisms are both beneficial and harmful (sometimes referred to as “friends” and “foes”). Their activities can be harnessed for the services of humanity but they are also major *competitors* for food because they need the nutrients (just as we do) that are contained in foods thereby, causing substantial food spoilage and losses. Therefore, as competitors, the quest for strategies to control or eliminate their destructive effects in foods has always been of concern and research interest to man.

Vice-Chancellor Sir, to prevent or control food spoilage and enhance shelf-life of foods as well as improve on microbial food safety to ultimately achieve food security is a global concern. These are major areas of focus in the sub-discipline of Food Microbiology which is my scientific domain.

Vice-Chancellor Sir, distinguished ladies and gentlemen, today’s inaugural lecture is therefore to share our research experiences with you having collaborated with many colleagues and students both here and abroad for about thirty years.

2.0 INTRODUCTION

Vice-Chancellor Sir, globally, we are becoming increasingly aware that microorganisms are the basis of the biosphere (i.e. zone of living things/life on Earth). They are the ancestors of all living things and the support system for all other forms of life. However, certain microorganisms pose a threat to human health as well as to the health of plants and animals. Thus, microorganisms being the foundation of the biosphere and major determinants of human

health, they therefore play primary and fundamental roles in life on Earth. Consequently, the study of microorganisms is critical to the study of all living things and Microbiology is essential for the study and understanding of all life on this planet (AAM, 2004).

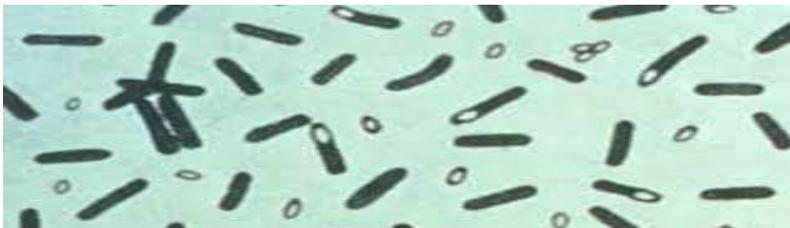
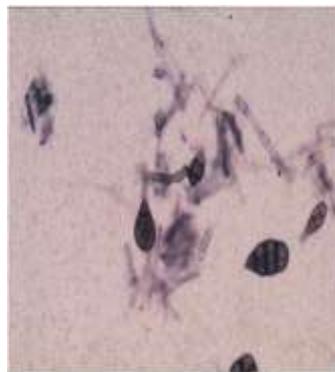
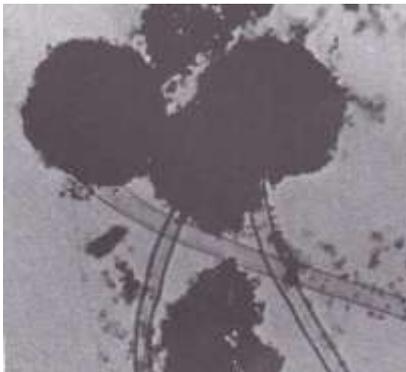
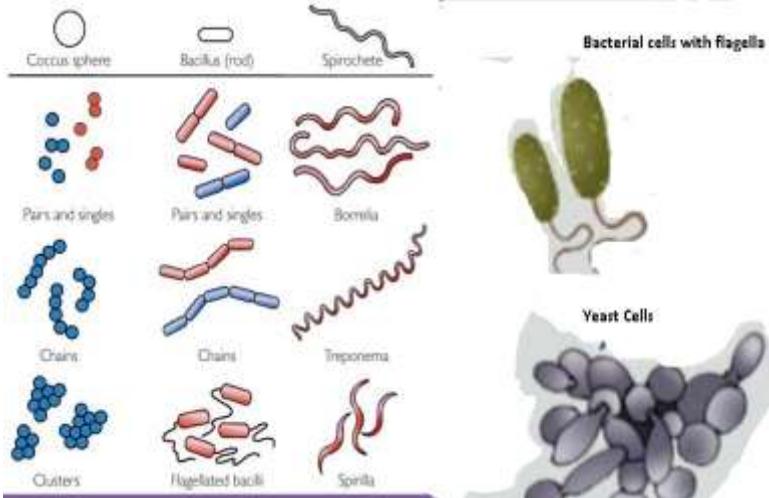
Vice-Chancellor Sir, based on the diversity and background of this distinguished audience, it becomes appropriate to define/explain some of the concepts and *keywords* in this lecture for better understanding.

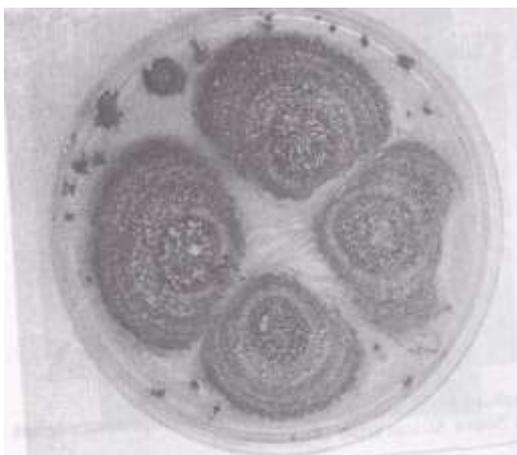
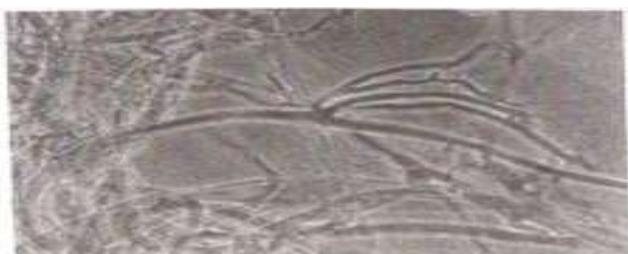
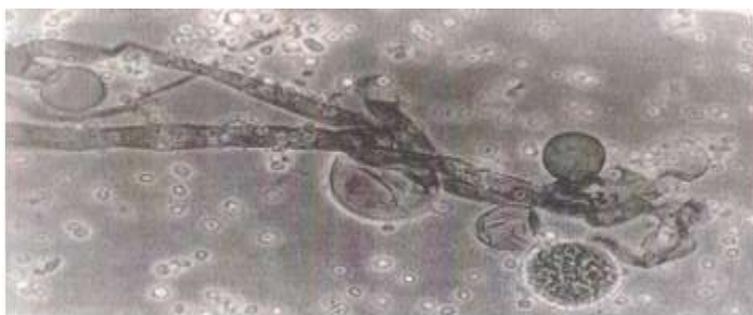
2.1 Definitions/Explanations

2.1.1 Microbiology is often defined as the study of microscopic organisms and agents (e.g. viruses) too small to be seen by the naked eye.

2.1.2 Microorganisms are living minute (ranging in size from $0.2 \times 0.5\mu\text{m}$ to $0.3 \times 2.0 \mu\text{m}$) organisms which can only be seen with a microscope and they include bacteria, moulds, yeasts, protozoa, algae and viruses (classified as microorganism due to their small size and not viability). However, bread moulds and some algae are studied by microbiologists (due to some of their characteristics) yet, they are visible to the naked eye. Interestingly, two bacterial genera (*Thiomargarita namibiensis*) and *Epulopiscium fishelsoni* were discovered recently (Prescott *et al.*, 2002). *Thiomargarita namibiensis* was discovered in 1999 off the coast of Namibia. It is about 3 million times the volume of a normal bacterial cell with width ranging between 0.1mm and 0.3mm but a few are approximately 1.0 mm in length. In contrast, *Epulopiscium fishelsoni* was discovered in 1985 inside the intestines of a brown surgeonfish and it ranges from a length of about 30 micrometer (μm) to 600 micrometer (μm) and a volume of greater than 2,000 fold (Prescott *et al.*, 2002). Figure 1 shows various types of microorganisms.

Some microorganisms and their shapes, sizes and arrangements.





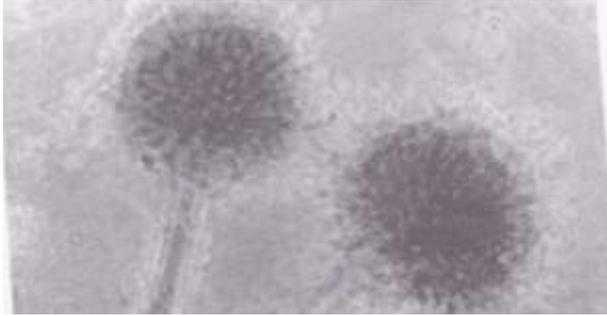
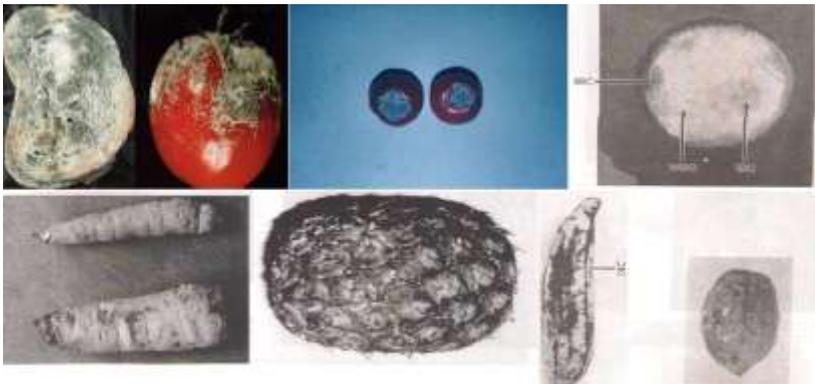


Figure 1- Different microbial types: bacteria, moulds and yeasts of various shapes, sizes and arrangements

Adapted from different sources by the author including: Efiuvwevwere, 1999; Efiuvwevwere, 2000;
www.microorganisms/shapes/morphology/images

2.1.3 Food spoilage is the alteration of the quality characteristics (such as appearance, taste, texture, odour) due to enzymatic and microbial attacks of food thereby making the food unacceptable. These changes are not always microbiological in origin but physical/chilling damage make the food become predisposed to microbial spoilage (Efiuvwevwere and Thorne, 1988; Jay, 1996; Snowdon, 1990). Several foods are spoilt by microorganisms (Figure 2).



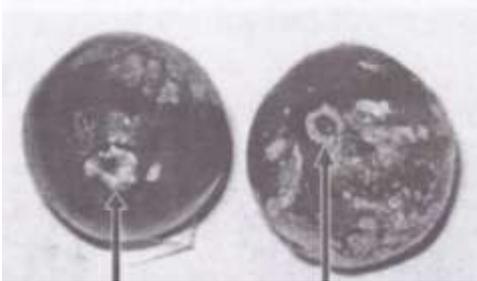


Figure 2. Microbial spoilage of different foods

Sources: Efiuvwevwere, 2000; www.microbialspoilage/images

2.1.4 Food preservation entails the manipulation of intrinsic/inherent factors of foods (such as pH, nutrient content, oxidation-reduction potential etc) and extrinsic parameters (e.g. relative humidity and temperature of storage) that affect microbial growth and microbial dynamics in food ecosystems.

Several methods are available for preservation of foods. These include use of preservatives, low or high temperatures, smoking etc (more discussion on these later in this lecture).

2.1.5 Food has several definitions but that by the Joint FAO/WHO Codex Alimentarius Commission (CAC) of the United Nations is most comprehensive and it defines food as “any substance, whether processed, semi-processed or raw, which is intended for human consumption and includes drink, chewing gum and any substance which has been used in the manufacture, preparation, processing or treatment of food but does not include cosmetics or tobacco or substances used only as drugs” (CAC, 1980).

2.1.6 Food safety is a major focus in *Food Microbiology* and it describes handling, preparation/ processing and storage of food in ways that prevent food-borne illnesses and food poisoning. This involves safety measures that emphasize “tracking” between

industry and the market and then between the market and the consumer (hence, there are cases of processed food recalls especially in developed countries). In developed countries, there are intricate and well-articulated standards for monitoring food safety but in many less developed countries (including Nigeria), these measures are at the formative/review stages. Questions concerning the relevance of microbiological standards in the country had earlier been raised (Ibe, 2008). However, it is gratifying to observe that a comprehensive review draft document on National Policy on Food Safety and its Implementation Strategy is now being considered under the leadership of the Federal Ministry of Health (FMOH) supported by some other Ministries, Parastatals and Agencies.

Globally, there is a growing concern that food often supports the survival and growth of pathogenic microorganisms thereby, exposing especially babies, children and the elderly to potential food hazards. Similarly, there are indications in the United Kingdom and elsewhere that the incidence of food poisoning is much higher than official statistics show due to under-reporting because it is common knowledge that many cases occur at home yet, are not reported since such victims do not necessarily consult their doctors when they suffer the symptoms of gastro-enteritis (inflammation of the gastrointestinal tract), abdominal pains, diarrhoea and vomiting (Pawsey, 2002). The situation is even worse in developing countries including ours where such statistics are virtually unavailable but food-borne illnesses of microbial origin are major causes of death in developing countries (WHO, 2002). In addition, the lack of proper medical consultation in this country often leads to self-medication and abuse which ultimately results in high rates of incidence of antimicrobial resistance in the country (Oladipo and Adejumobi, 2010).

It is noteworthy that toxins originating from bacteria or moulds (i.e. mycotoxins which are carcinogens) are not usually liable to heat conversion to non-toxic components because of their high temperature stability (usually above 150°C) (Samarajeewa *et al.* 1990). Therefore, the practice of trimming moulded/fungal-infected agricultural produce for consumption with the hope that microbial

hazards have been removed is very dangerous and consumers are therefore highly discouraged from such practices.

2.1.7 Commercial sterility is a term commonly used in the food (canning) industry meaning the condition achieved by the application of heat sufficient to render the processed food product free from viable (living) microorganisms (including those of known public health significance (such as *Clostridium botulinum*), capable of growing in the food under normal non-refrigerated temperatures at which the food is likely to be held during distribution and storage. These abusive storage conditions (including hoarding of foods in warehouses) are reminiscent of the era of “essential commodities” in the 1980s. The microbial hazards associated with both imported and locally canned tomato products showing defective appearance (yet, sold with impunity) as well as those with normal appearance have been reported and they are of serious health concerns due to the prevalence of *Clostridium botulinum* (that produces the most potent neurotoxin) and mycotoxigenic moulds (Efiuvwevwere and Atirike, 1998., Pawsey, 2002; Peck, 2005).

2.1.8 Shelf life is the length of time that a commodity may be stored without becoming unfit/ unwholesome for human use or consumption. It applies to foods/beverages, pharmaceutical products, chemicals and many other perishable items. The common labels on food with respect to shelf life are: *best before or sell by/use by* dates. However, shelf life is comparable with expiration date since both emphasise quality and safety. Therefore, food products should be removed from the shelf before the indicated dates. Unfortunately, this is not the practice in this country.

2.1.9 Food Security- According to FAO/WHO (2014), it is defined as a condition where all people, at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. It is complex and is built on three pillars:

- Food availability: sufficient quantities of food available on a consistent basis.
- Food access: having sufficient resources to obtain appropriate foods for nutritious diets.
- Food use: appropriate use based on knowledge of basic nutrition and care as well as adequate water and sanitation

2.1.10 Hazard Analysis and Critical Control Point (HACCP) is a systematic preventive approach (concept) to food safety from biological, chemical and physical hazards in food production processes to ensure the safety of food by reducing these hazards to acceptable safe level. Thus, this approach emphasises the prevention of hazards *at all the food production stages* of a food chain rather than the tradition of analyzing the finished food product (i.e. post-mortem approach). It therefore focuses on microbial/hazard control of foods from farm to table (**a total quality management approach**). HACCP was conceived in the 1960s when the US National Aeronautics and Space Administration (NASA) asked Pillsbury (a major food company in the US) to design and manufacture the first foods for space flights. Since then, HACCP has become adopted by the food industries and recognized internationally by government regulatory agencies and FAO/WHO (Efiuvwevwere, 2012; FAO, 2014).

2.1.11 Microbial food poisoning is a term that describes an illness caused by eating or drinking food contaminated with bacteria, viruses, parasites, toxins or chemicals. Two categories are known namely: **food infection and food intoxication**.

2.1.11.1 Food infection occurs where the microorganism itself grows inside the stomach (gastro-intestinal tract) of the victim to produce high microbial population to attain infective load/dose and is the source of the symptoms/illness including diarrhoea, stomach cramps, fever and chills. Due to this process of bacterial/viral multiplication/population increase, the symptoms take longer time (approximately hours to days) to appear (Jay 1996). The most common microorganisms responsible for food infection include

Salmonella spp., *Campylobacter jejuni*, *Listeria monocytogenes*, *Shigella* spp., *Vibrio parahaemolyticus* and Norwalk viruses.

The main treatment for severe bacterial food infection is the use of antibiotics. Unfortunately, the common practice in this country and many other countries is to prescribe multiple antibiotics or broad-spectrum antibiotics in place of narrow-spectrum drugs without the actual identification of the specific causative pathogen(s) (Prescott *et al.* 2002). This is often due to lack of appropriate diagnostic facilities and sometimes the expertise.

2.1.11.2 Food intoxication results from the consumption of a chemical or natural toxin (often an exotoxin i.e toxin produced by the organism and released to the immediate environment) and is consumed along with the food thereby causing the symptoms/illness. The symptoms appear sooner (within few hours of consumption of the contaminated food) because the toxins are already produced (pre-formed) in the food prior to consumption. This explains the variations in symptom/illness manifestation among people who must have consumed the same contaminated food. For example, a few may experience mild symptoms while others suffer more severe symptoms/illness depending on the amount of the toxin consumed along with the food. The most common pathogens responsible for food intoxications include *Staphylococcus aureus*, *Clostridium botulinum*, *Escherichia coli* O157:H7 and *Clostridium perfringens* (USFDA, 2014). Both food infection and food intoxication produce similar food poisoning-related symptoms and are not always easy to diagnose based on symptoms alone hence the causative microorganism(s) should be identified as quickly as possible using rapid diagnostic methods for adequate treatment. Regretably, most of the necessary facilities are not available in most parts of the country.

3.0 IMPORTANCE OF FOOD TO MAN

Whereas there are several views on essential human needs, the three basic needs are: (1) food (including water), (2) shelter and (3)

clothing. Food is therefore the most important to man and this is underscored by these various quotes and proverbs as follows:

- 3.1** The belly is a demon, it does not remember how well you treated it yesterday; it will cry out for more tomorrow (Russian writer and Noble prize winner in Literature-Alexander Solzhenitsyn (1918-2008).
- 3.2** One cannot think well, love well, sleep well, if one has not dined well (Virginia Woolf, British novelist (1882-1941).
- 3.3** When a man's stomach is full it makes no difference whether he is rich or poor (Euripides, a Greek tragic poet (BC 480-BC 406).
- 3.4** An army marches on its stomach (Emperor Napoleon Bonaparte (1769-1821).
- 3.5** The best way to reach a man's heart is through the belly (a common expression world-wide).
- 3.6** Food for all is a necessity. Food should not be a merchandise, to be bought and sold as jewels are bought and sold by those who have the money to buy. Food is a human necessity like water and air, it should be available (American Noble Prize Winning Author, Pearl S. Bulk (1892-1973).
- 3.7** If there is anything we are serious about, it is neither religion nor learning but food (a Renowned Chinese writer, Lin Yutang (1896-1976).
- 3.8** Words are sweet but they never take the place of food (Ibo/other Nigerians' proverb).
- 3.9** A hungry man is an angry man (Nigerian expression).

3.10 Of the eight Millennium Development Goals (MDGs), the Number One is “Eradicate extreme poverty and hunger” (United Nations).

3.11 The disciples told Jesus that the multitude was hungry. He then fed them with five loaves and

two fish (Mark 6:35 and each gospel in the Bible has this parable).

Compiled by the author from several sources including:
www.brainyquote.com

www.en.m.wiktionary.org/wiki/the_way_to_man%27s_heart_is_thr_oug.....

www.goodreads.com/quotes/tag/food

Apparently, these quotes/proverbs from different parts of the world clearly reflect the importance man attaches to food hence the quest for food safety and food security will be a life-long undertaking/endeavour.

4.0 Brief Historical Perspectives on Microbiology and Food Microbiology

Microorganisms are ubiquitous (i.e. present everywhere on Earth, including humans, animals, plants and other living creatures as well as in different ecosystems- soil, water, atmosphere) and they can multiply everywhere except in the atmosphere (since it lacks nutrients). They were the first living cells to inhabit the Earth more than three billion years ago and since then, have played significant roles to man and other living systems (Beck, 2000; Ray and Bhunia, 2008). After Antonie van Leeuwenhoek’s series of microscopic observations and documentations on the “animalcules” (small worms) in the 17th century presented to The Royal Society of London, more improved microscopes became available due to the

outcome of the Industrial Revolution in the 19th century. As a result of his invention and development of the magnifying lenses/microscope, he became known as the “father of microbiology”. In spite of the interest generated by his observations, no one made a serious attempt either to repeat or extend his work/observations. However, during the 18th century as a result of the revival of a long-standing controversy about whether life could develop out of non-living material led to the significance of microorganisms in nature as well as in health and welfare of humans. This was centred around two schools of thought:

- a) That living things could originate from non-living things (matter); referred to as Abiogenesis (spontaneous generation) and that the goddess, *Gea* could create life from stones and
- b) That only pre-existing microbes could give rise to other microbes (referred to as Biogenesis).

These two concepts had very strong proponents and adversaries in this debate with forces of personality and individual WILL often obscuring /distorting the facts. However, Louis Pasteur (a French confectioner) and a proponent of biogenesis in 1861-1864 proved that bacteria were able to reproduce/regenerate and that life could not originate through spontaneous generation (Abiogenesis).

This led to more discoveries using improved microscopes which aided Ehrenberg (a German) by 1838 to introduce the term “bacteria”. In addition, Robert Koch in 1880 and 1890s isolated pure cultures of bacteria responsible for different diseases including anthrax (*Bacillus anthracis*), cholera (*Vibrio cholerae*), tuberculosis (*Mycobacterium tuberculosis*) to demonstrate clearly through his famous postulates (Koch’s postulates that a specific bacterium is the causative agent of specific disease. But John Tyndall, a 17th century European physicist discovered endospores (i.e. bacteria in dormant, inert, heat-resistant state). Thus, the quest for causative agents of more diseases, their structures and functions continued to be challenging and exciting. These became more evident with the invention of the electron microscope in the 1940s which led to the discovery of sub-microscopic entities called viruses

(Prescott *et al.* 2002). Today, viruses have become a household word due to the outbreak of Ebola Virus Disease (EVD).

All the developments described thus far occurred in Europe but as from early 20th century, Microbiology became established and flourished in the USA especially with regard to collaborative disciplines of Biochemistry, genetics (now genomics) and molecular microbiology. Some developments in Microbiology between 19th and 21st centuries included the following:

- | | |
|------|--|
| 1898 | Martinus Beijerinck discovered first known virus, tobacco mosaic virus (TMV). |
| 1905 | Fritz Schaudinn and Erich Hoffmann identified the causative organism of syphilis (<i>Treponema pallidum</i>) |
| 1908 | Paul Ehrlich developed a drug (Salvarsan) to treat syphilis |
| 1929 | Alexander Fleming discovered penicillin for treatment of bacterial infections, usually Gram-positive |
| 1976 | Peter Piot discovered Ebola virus (worm-like structure under electron microscope). |
| 2001 | Anthrax (<i>Bacillus anthracis</i> spore/powder) attack in the USA |
| 2003 | SARS (severe acute respiratory syndrome) epidemic caused by Coronavirus. |
| 2014 | Ebola Virus Disease/Outbreak in West Africa but spread later to other countries. |

Compiled by the author from several sources including:
www.wikipedia.org/ebola/tmv;
www.wikipedia.org/wiki/penicillin/Fritz_Schaudinn; Prescott *et al.* 2002

4.1 Early Developments in Food Microbiology

Evidently, the early man (Homo ancestors) who were hunters and gatherers of food were aware of food spoilage and food-borne diseases. Although, they had no perception of the causative agents, they used ice and fire to preserve foods and made them safe. At about 8000 BC, agricultural practices became adopted by the early civilisation and food supply became abundant during the growing seasons (this scenario is typical of ours). Preservation of foods became important for regular supply all year round and between 8000 and 1000 BC, many food preservation methods such as drying, baking, smoking, salting, low temperature storage (i.e. in ice), storage without air (i.e. in pits), fermentation, pickling were employed mainly to reduce food spoilage. Whereas it is doubtful that the society at that time recognised the implications of diseases transmitted through food, the scriptural injunctions stipulated by many religions suggest that the societies recognised the association of diseases with some foods. Such stipulations included not eating meat from a diseased animal or animal killed by a scavenger or not eating food that appeared unnatural or had been handled by an unclean person. These were emphasised to safeguard the health of citizens against unwholesome foods and food-borne diseases. Fermentation was used extensively by many societies (even till date) not only to preserve foods but also to achieve variety of desirable foods from milk, meat, fish, fruits and vegetables (Dirar, 1993., Ray and Bhunia, 2008).

The major developments of ideas on the possible roles of microorganisms in foods and their scientific proof were initiated by Louis Pasteur in the 1870s and followed by many other scientists before the end of the 19th century. Some of the major developments in food microbiology in the 19th century are shown in Table 1.

These paved the way for the establishment of early Food Microbiology in the 20th century.

Table 1. Summary of some of the major developments in food microbiology in the 19th century

Food Fermentation

Date	Development
1837	Theodor Schwann named the organism involved in sugar fermentation as <i>Saccharomyces</i> (sugar fungus).
1860	Louis Pasteur showed that fermentation of lactic acid and alcohol from sugar was the result of growth of specific bacteria and yeasts respectively.
1883	Emil Christian Hansen used pure culture of yeasts to ferment/produce beer.

Food Preservation

1804	Francois Nicolas Appert developed food preservation using sealed glass bottles by heating in boiling water (canning). He won the prize of 12,000 francs given by the French government (Emperor Napoleon Bonaparte) for the invention.
1819	Peter Durand developed food preservation using steel cans.
1860	Louis Pasteur demonstrated that heat destroyed undesirable microorganisms in wine and beer.
1870	Louis Pasteur invented heating of wine at 62.7°C for 30 minutes to destroy bacteria that cause souring but this method was later modified to kill many vegetative pathogens and spoilage bacteria and was named after him as “pasteurisation”. As a result of the importance of his work, Pasteur is known as the “founder of food microbiology”. He also demonstrated that air did not have to be heated to

remain sterile using his famous Swan-neck flask (Figure 3) that finally disproved the theory of spontaneous generation.



Figure 3. Pasteur's Swan Neck Flask

Food borne diseases

- 1857 Milk was incriminated as a vehicle of typhoid fever by W. Taylor of Penrith, England.
- 1894 J. Denys associated pyrogenic (fever-related) *Staphylococcus* with death of a person who ate meat prepared from a diseased cow.
- 1895 Marie von Ermengem isolated *Bacillus botulinus* (*Clostridium botulinum*) from contaminated meat and proved that it caused botulism (acute paralytic disease caused by botulinum toxin).

1971 First USA food-borne outbreak of *Vibrio parahaemolyticus* gastroenteritis occurred in Maryland.

Surprisingly, in the United States, many food industries hesitated to adopt industry-wide microbiological standards until they were economically threatened by the publicity which surrounded outbreaks of food-borne diseases. As a result of several notorious outbreaks of botulism (very deadly paralytic toxin in the early 1920s), the US canning industry decided to adopt a very conservative heat treatment, known as the 12-D process that reduces the probability of survival of the most heat resistant *Clostridium botulinum* spores to one in a billion (usually expressed as 10^{12}). This practice continues till date and since 1925, the food canning industry has produced more than a trillion containers with only 5-6 known incidents of botulism (Ray and Bhunia, 2008).

At about the same time, the dairy industry was urged to implement microbiological control over milk because of numerous notorious outbreaks of milk-borne typhoid fever, tuberculosis, diphtheria and brucellosis. As a result of these, public health authorities established requirements that addressed animal health, sanitation, pasteurisation and refrigeration coupled with reinforced bacterial standards. Based on these measures, pasteurised milk became one of the safest foods by the mid-1900s.

The history of food microbiology will be incomplete without mention of “Typhoid Mary”. Mary was an asymptomatic typhoid carrier (i.e. did not show any symptoms of typhoid) who worked as a cook for several families in about 1910 in New York. For over ten years, 7 outbreaks of typhoid were directly traced to her and it was estimated that she may have been responsible for 51 cases of typhoid fever. New York authorities arrested her and sought to have her gall bladder (organ for colonisation and reservoir for *Salmonella typhi*) removed but eventually released her when she agreed never to work as a cook again. But when another outbreak was traced to her a few

years later, she was arrested as a threat to public safety and detained until her death in 1938.

4.2 Advances in Food Microbiology and Current Status

During the early 20th century, studies continued to emphasise the association of foods with microorganisms especially pathogenic bacteria in foods. Additionally, specific methods were developed to prevent microbial growth and enhance destruction of pathogenic and spoilage microorganisms in foods. There was also interest in isolation and characterisation of beneficial bacteria (especially probiotics i.e. microorganisms very useful for life, more details on this later) associated with food fermentation particularly dairy product fermentation. However, after the 1950s, Food Microbiology entered a new era focusing on diverse types of foods as well as microbial interactions, microbial physiology, development of strains and food biotechnology which have helped to open new frontiers in food microbiology (Beck, 2000; Hartman, 2001).

Before the 1970s, food microbiology was regarded as an applied science mainly involved in the microbiological quality control of food. However, since then, the technology used in food production, processing, distribution (including international trade) and retailing as well as food consumption patterns (with urbanisation) have changed drastically. Thus, these changes have introduced new problems that can no longer be solved by merely using applied knowledge. Consequently, the modern day food microbiologist needs considerable knowledge of both basic (including microbial ecology, genomics and physiology) and applied science to understand and effectively solve the microbiological problems associated with foods. Acquisition of such expertise/training is to help develop novel methods for rapid and effective detection of spoilage and pathogenic microorganisms (as evidenced by the Ebola virus outbreak). In addition, development of desirable microbial strains to produce fermented foods of better quality with emphasis on shelf-life extension and improved safety is of current concern.

Obviously, the human civilisation began with production and preservation of food but efforts were made later to understand the principles of food spoilage, microbial interactions and dynamics. Current investigations and research interests are therefore directed towards better understanding of microorganisms and development of molecular methods (nanotechnology involving application of very small particles i.e. nanoparticles) that can serve as nanosensors for rapid detection of pathogenic and spoilage microorganisms in foods to help predict their potential shelf-life and safety (Martirosyan and Schneider, 2014; Sozer and Kokini, 2008). In addition to the application of nanotechnology in food microbiology, a novel area of focus termed *Predictive Microbiology* involves the use of mathematical models to predict growth of pathogenic and spoilage microorganisms in foods based on generated data (data bank) by studying microbial growth at different pH, water activity, temperature and effects of different preservative concentrations in laboratory media (now including food ecosystems). Although, very promising results have been achieved, there are some limitations which are being addressed. www.fsai.ie/food_business/topics_of_interest/predictive_micro.html

5.0 Microbiology and its sub-disciplines

Microbiology as a discipline is vast, intricate, challenging and exciting. As a result, it is divided into two broad categories namely; 1) General/Pure Microbiology (for the purpose of classification/organism-related studies) and 2) Applied Microbiology.

5.1 General/Pure Microbiology includes:

5.1.1 Bacteriology is the study of bacteria and their characteristics for classifications and identification.

5.1.2 Mycology is the study of fungi (moulds and yeasts), their genetic and biochemical properties well as their uses.

5.1.3 Phycology is the study of algae (seaweeds, blue-green algae or cyanobacteria).

5.1.4 **Parasitology** is the study of parasites, their hosts and the relationship between them.

5.1.5 **Virology** is the study of viruses and viral diseases.

5.2 **Applied Microbiology** includes:

5.2.1 **Medical Microbiology** is the study of pathogenic i.e. disease causing microorganisms and their roles in human illnesses.

5.2.2 **Pharmaceutical Microbiology** is the study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, other pharmaceutical products as well as those involved in the contamination and spoilage of pharmaceuticals.

5.2.3 **Food Microbiology** is the study of microorganisms and their involvement in food spoilage, food preservation and food-borne diseases as well as their use for production of foods to enhance quality through fermentation such as yogurt, beer and other products.

5.2.4 **Environmental Microbiology** is the study of microorganisms in different environmental ecosystems and their activities including microbial resource management/bioremediation, microbial ecology.

5.2.5 **Industrial Microbiology** the study and exploitation of microorganisms for use in industrial processes such as industrial fermentation involving production of organic acids, antibiotics, alcohols etc and waste water treatment.

Apparently, there is considerable interface (overlap) between the sub-disciplines of Microbiology and with other disciplines. For example, Food Microbiology interfaces closely with Environmental Microbiology (since all foods originate from different environments), Industrial Microbiology and Medical Microbiology. Due to its obvious overlap with many other disciplines (i.e. being inter-disciplinary/multi-disciplinary in nature), Food Microbiology

is globally domiciled in several different departments. These departments include: Biological Sciences, Microbiology, Epidemiology and Public Health, Food Science/Technology/Biotechnology, Bioscience and Biotechnology.

6.0 Sources of Microorganisms, Their Growth and Involvement in Food Fermentation

The sources of microorganisms in foods are numerous and they include, soil, water, air. However, their ability to survive and grow in food is dependent on several factors including the intrinsic and extrinsic parameters (more details on these in the course of this lecture).

Although numerous types of microorganisms are found in foods, bacteria constitutes the major important groups. This is due to their diverse characteristics including their rapid growth rate, ability to utilize different nutrients, ability to grow under different temperatures, pH, water activity as well as their survival under adverse/unfavourable conditions.

6.1 Bacterial growth

Bacteria reproduce or multiply in favourable environments through a process called *binary fission* (i.e. one cell divides into two, two into four and so forth). The time that a single cell takes to divide into two is called *generation time or doubling time*.

Generally, bacteria have the shortest generation time followed by yeasts and moulds under optimum conditions. The generation time of microbial population can be calculated mathematically from the differences in population during a given time period using logarithms (base 10 i.e. \log_{10}) and the formula:

$$G = \frac{0.3t}{\log_{10} Z - \log_{10} X}$$

where G is the generation time (often in minutes); 0.3 is a constant (i.e. value of $\log_{10} 2$ indicating doubling time), t is the duration of study (min), $\log_{10} x$ is the initial population and $\log_{10} z$ is the final population per milliliter or colony forming units (CFUs) per milliliter (Prescott *et al* 2002).

For instance, if a given bacterial species grows under a given condition starting with initial population of 100,000 (10^5) cfus and increases to 100,000,000 (10^8) cfus/ml in 90 minutes, its generation time will be:

$$G = \frac{0.30 \times 90}{8-5} = 9 \text{ minutes}$$

The bacterial growth curve (Figure 4a) shows the typical four phases as follows: (1) lag phase (2) exponential/logarithmic phase (3) stationary phase and (4) the death phase. The growth behaviour of the organism at the different phases varies with the type of organism. However, at the lag phase, assimilation of nutrients and increase in size occur but no change in population while in the exponential phase, the cell number/population increases slowly at first and then very rapidly (following a first-order reaction kinetics) and often used to determine the generation. In contrast, the stationary phase shows the death of few cells with a few cells multiplying due to nutrient depletion and accumulation of metabolic waste products with the net-effect of a stable population. Finally, the growth curve now enters the last phase in which the rate of cell death is higher than the rate of cell multiplication. It is noteworthy that some microorganisms will remain viable for a long time and could be sources of food pathogenicity and food spoilage. Figure 4b shows the inverse relationship between the bacterial growth activities and the food mass depletion.

6.2 Microorganisms and their involvement in food fermentation

Among the most important activities of microorganisms in foods is the fermentation process (Dirar, 1993., Efiuvwevwere and Akoma, 1995., Ray and Bhunia 2008., Njoku and Okemadu, 1989). Food fermentation involves a process in which raw materials are converted to fermented foods by the growth and metabolic activities

(usually) of the desirable microorganisms. The raw materials include milk, meat, fish, vegetables, fruits and others.

Fermentation is one of the oldest methods used to preserve food and converts its quality to desirable attributes. Globally, over 3,500 types of fermented foods are available and many ethnic types are produced in several localities and regions (Dirar, 1993., Efiuvwevwere and Ezeama, 1996; Ray and Bhunia, 2008).

The basic principles of fermentation developed by the ancient civilizations are still in use today especially those involving natural/spontaneous fermentation processes. These methods involve using either the desirable microbial population naturally present in the raw materials or some products containing the desirable microorganism(s) from a previous fermentation (termed back-slopping) are added to the raw materials. This is the most commonly used method of fermentation in developing world due to lack of culture collection centres and relevant infrastructure. Food products such as iru, fufu, ugba, kunun-zaki etc are produced using this method (Efiuvwevwere and Akoma, 1995; Njoku *et al.* 1991; Oyewole and Isah, 2012).

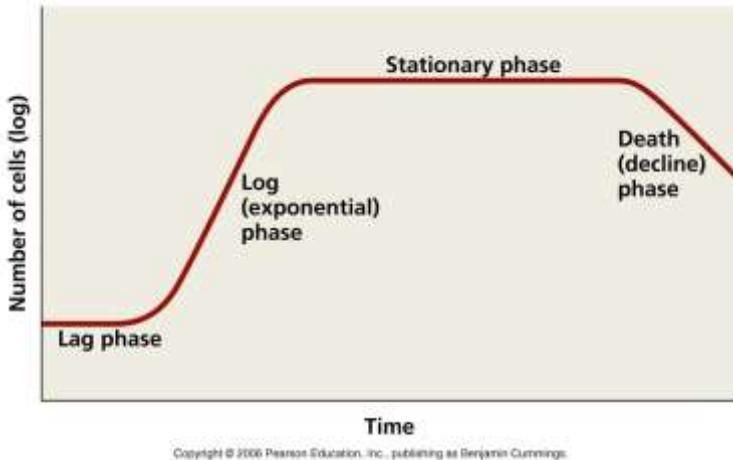


Figure 4a. Bacterial growth curve

BACTERIAL GROWTH

Food to mass ratio decreases →

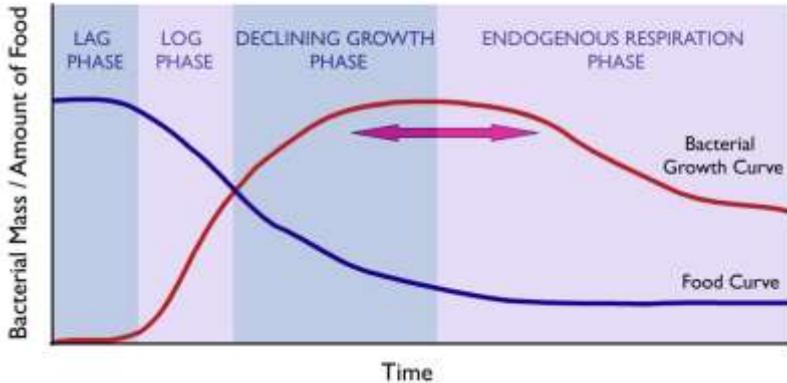


Figure 4b. Two curves showing the bacterial growth curve and food mass curve (inverse relationship)

Another type of food fermentation involves using purified, identified cultures that are maintained in the laboratory (referred to as starter cultures) for future use. This is typical of current biotechnologically fermented foods including beer, wine, bread, yogurt etc and they are products of consistent desirable quality and safety. Such processes make the food product/microenvironment of the food unfavourable to other microorganisms including pathogenic and spoilage types thereby, extending the shelf-life and the safety of the food. Table 2 shows a list of some microorganisms used in food and beverage production.

Table 2: Some food products of fermentation, their raw materials and fermenting microorganisms

Raw material	Microorganisms involved	Product
Milk	<i>Streptococcus</i> spp	Yogurt
Milk	<i>Lactobacillus</i> spp	Cheese
	Lactic starters, <i>Lactobacillus</i> + others	
Sorghum/millet	<i>Lactobacillus</i> , <i>Streptococcus</i> , <i>Saccharomyces</i>	Kunun-zaki
Cassava	<i>Lactobacillus</i> spp <i>Leuconostoc</i> spp <i>Streptococcus</i> spp, <i>Geotrichum candidum</i>	Garri
Cassava	Lactic acid bacteria, <i>Corynebacterium</i> spp <i>Geotrichum candidum</i> , <i>Candida tropicalis</i>	Fufu
Rice	Lactic acid bacteria, <i>Streptococcus</i> spp	Rice-masa
African oil bean seed	<i>Corynebacterium</i> spp, <i>Bacillus</i> spp	Ugba
African locust bean	<i>Bacillus</i> spp	Iru

Compiled by the author from several sources: Efiuvwevwere and Ezeama, 1992; Efiuvwevwere and Akoma, 1995; Njoku *et al.* 1991; Odunfa and Oyewole, 1986; Okorie and Olasupo, 2013; Oyewole and Isah, 2012; Wikipedia. en.m.org/wiki/food-microbiology

6.3 Starter Cultures as Probiotics

Starter cultures are derived mainly from the group of bacteria known as lactic acid bacteria (LAB). They have the ability to metabolize carbohydrate and produce large amounts of lactic acids. They include the genera: *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and others (Efiuvwevwere and Akoma 1995; Jay, 1996; Ray and Bhunia, 2008).

Many of these microorganisms are used to produce yogurt, cheese, sausages, pickles as controlled fermentation. However, under natural/spontaneous fermentation processes, many of them are

involved as mixed microflora hence the products are characterised by inconsistent quality and questionable safety.

Starter cultures in addition to be of considerable importance in food production, play major roles in conferring a wide range of health benefits including immune system modulation, increased resistance to malignancy and infectious illness (Soccol *et al.* 2010).

Since their discovery, LAB have been of much research interest in various applications as starter cultures in food/fermentations, pharmaceuticals, probiotics (i.e for life) and as biological control agents (biopreservation). LAB are “generally recognized as safe” (GRAS, just like some food chemical preservatives) to the consumer hence such products (e.g. yogurt) are consumed containing high populations of live starters/probiotics. *Probiotics* was initially used as synonym of the word “antibiotic” and used therapeutically (Vasiljevic and Shah, 2008). Similarly, FAO/WHO (2012) working group on the evaluation of probiotics in foods indicated that they are live microorganisms that when administered in adequate amounts confer health benefits on the host (Sanders, 2008).

Louis Pasteur convinced the scientific world that all fermentative processes were caused by microorganisms and that specific types of fermentations (such as alcoholic, lactic or butyric) were due to the specific types of microorganisms.

At about the same time, the dairy industry was urged to implement microbiological control over milk because of numerous notorious outbreaks of milk-borne typhoid fever.

Nevertheless, as we eliminate microorganisms from food we create an environment free of competition which may allow opportunities for other microorganisms to grow and cause disease. As a result, there is considerable interest in identifying safe bacteria (e.g probiotics: lactic acid bacteria) which when deliberately added to

food (e.g. yogurt) inhibit the growth of pathogens but would not rapidly spoil the product themselves (USDA FSIS, 2010).

7.0 Microbial food spoilage

In general, the internal tissues of healthy animals (meat) and plants (fruits and vegetables) are essentially sterile. However, microorganisms enter foods from both natural and external sources to which they are exposed from the time of production/capture/harvest until the time of consumption. Natural sources for foods of animal origin include skin, hoofs, hair, feathers, gastro-intestinal tract, urinogenital tract, milk duct (teat canal). In contrast, natural sources for foods of plant origin include the surfaces of fruits, vegetables, grains and pores of tubers and onions. In addition, foods can be contaminated with different types of microorganisms from the external sources including air, soil, water, feeds, humans (food handlers), processing equipment, packages and others.

7.1 Microbial growth and necessary factors

The phenomenon of microbial food spoilage occurs due to either microbial growth in a given food or release of microbial enzymes (both extracellular and intracellular) in the food micro-environment. Generally, microbial food spoilage involves several events that usually take place in sequence (Efiuvwevwere and Oyebanji 1999; Efiuvwevwere, 2000; Jay 1996; Ray and Bhunia, 2008; Snowdon, 1990). Following the contamination of the food from any of these sources (air, soil, water etc), the food inherent parameters (pH, oxidation-reduction potential, nutrients, antimicrobial substances) as well as the storage conditions must favour the growth of the contaminant and finally, the food must be stored for sufficient length of time to enable the microorganism(s) attain the high population needed to induce undesirable quality changes in the food (Efiuvwevwere and Izakpa, 2000; Efiuvwevwere and Amadi 1992 Jay 1996).

Multiplication of microorganism(s) in foods is a very important factor in food spoilage hence bacteria are the major spoilage agents

because of their short generation time compared with fungi and other microorganisms (Table 3).

Table 3. Generation times for some selected microorganisms

<u>Microorganism</u>	<u>Temperature (°C)</u>	<u>Generation Time (min)</u>
<u>Bacteria</u>		
<i>Bacillus subtilis</i>	40	26
<i>Escherichia coli</i>	40	21
<i>Staphylococcus aureus</i>	37	28
<i>Pseudomonas aeruginosa</i>	37	35
<i>Clostridium botulinum</i>	37	35
<i>Mycobacterium tuberculosis</i>	37	720
<i>Treponema pallidum</i>	37	1,980
<u>Algae</u>		
<i>Chlorella pyrenoidosa</i>	25	465
<i>Euglena gracilis</i>	25	654
<i>Ceratium tripos</i>	20	4,968
<u>Protozoa</u>		
<i>Paramecium caudatum</i>	26	624
<i>Acanthamoeba castellani</i>	30	720
<i>Giardia lamblia</i>	37	1,080

Fungi

<i>Saccharomyces cerevisiae</i>	30	120
<i>Monilinia fructicola</i>	25	1,800

Source: Prescott *et al.* (2002)

7.2 Microbial types and population

Several types of bacteria, yeasts and moulds are normally found in raw and processed foods and they cause the spoilage due to their ability to multiply in the foods (but viruses do not multiply in foods being non-living systems). Bacteria cause most food spoilage most rapidly due to their short generation times compared with fungi (Table 3). In order for microbial spoilage of food to occur, the microorganism(s) must multiply and attain certain population often referred to as the “spoilage detection level” (Ray and Bhunia, 2008). Generally, the spoilage detection level can range from 1,000,000 ($\log_{10} 6$) cells/g/ml or cm^2 to 100,000,000 ($\log_{10} 8$) cells/g/ml or cm^2 (Figure 4c) (Efiuvwevwere and Amadi, 1992; Efiuvwevwere and Ajiboye 1996; Jay, 1996; Ray and Bhunia, 2008). Evidently, a food product that has higher initial microbial populations and contains microorganisms having shorter generation times will spoil more rapidly than a food product with a low initial microbial load containing microorganisms having longer generation times (Ray and Bhunia, 2008).

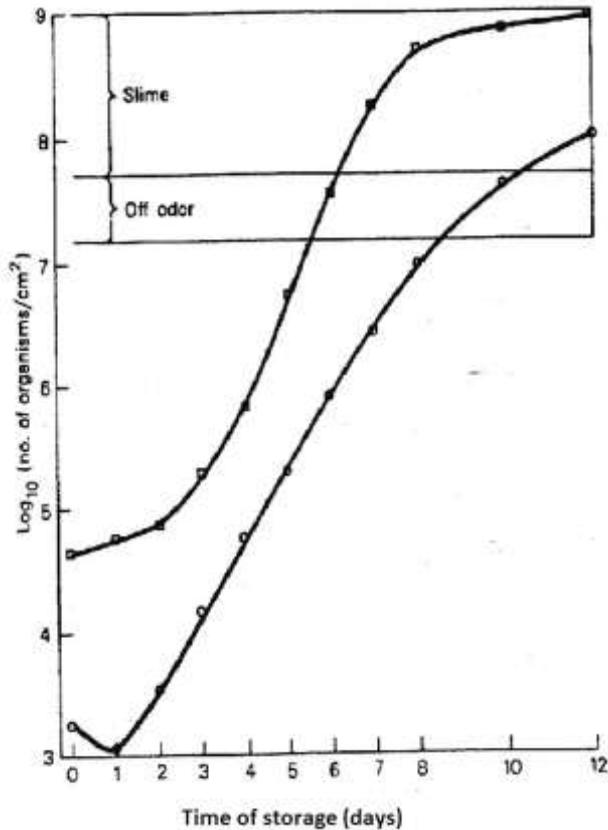


Figure 4c. Detection of food spoilage at certain microbial populations (\square = chicken; \circ = beef)

Source: Jay, 1996

In general, the initial microbiological profile of food is diverse consisting of several bacterial and fungal types. However, when the same food is spoilt, it is found to contain predominantly one or two types which may not have been present initially in high numbers in the unspoiled/fresh product. Thus, the different species (microbial types) initially present and capable of growing in a particular food;

only those that have the shortest generation times under the storage conditions attain the spoilage numbers rapidly and cause spoilage. For example, when meat containing initial mixed microbial flora of mainly *Acinetobacter*, *Moraxella*, *Brochothrix thermoosphaeta*, *Staphylococcus*, lactic acid bacteria, *Enterobacteriaceae* but few *Pseudomonas* was stored aerobically at 2⁰C, the *Pseudomonas* became the predominant microorganism due to its shortest generation time and the favourable optimum storage condition at 2⁰C (Jay, 1996). Other studies have shown microbial successions in different foods (Efiuvwevwere and Akoma, 1995; Efiuvwevwere and Chinyere, 2001; Efiuvwevwere and Eka, 1991; Ray and Bhunia, 2008).

7.3 PARAMETERS OF FOODS THAT AFFECT THEIR SPOILAGE

Two categories of parameters critical to microbial food spoilage are: (1) intrinsic and (2) extrinsic parameters (Jay, 1996). The intrinsic (inherent) parameters serve as defence mechanisms against the invasion and proliferation of microorganisms in foods. Therefore, the discussion of microbial spoilage of foods will be incomplete without emphasis on these parameters.

7.3.1 intrinsic parameters

These inherent parameters are found in animal and plant tissues and they help to prevent or retard the microbial spoilage of foods that are derived from them. The parameters are as follows:

- pH
- Moisture content
- Oxidation- reduction potential (Eh)
- Nutrient content
- Antimicrobial constituents/substances
- Biological barriers/structures

These parameters are discussed briefly as follows:

7.3.1.1 pH

In general, most bacteria grow best at about pH 6.6 – 7.5 while yeasts and moulds grow at below pH 5.0. It is therefore important to know the minimum pH values for the growth of some food-borne bacteria and fungi (Table 4).

Table 4: Minimum pH values for the growth of some food-borne bacteria and fungi.

Microorganism	pH
<i>Aeromonas hydrophila</i>	6.0 – 6.5
<i>Bacillus cereus</i>	6.0 – 7.0
<i>Clostridium botulinum</i>	5.0 – 6.0
<i>Clostridium perfringens</i>	6.0 – 6.5
<i>Escherichia coli</i> 0157:H7	5.0 – 6.0
<i>Lactobacillus plantarum</i>	4.5 – 5.5
<i>Leuconostoc cremoris</i>	5.0 – 5.5
<i>Listeria monocytogenes</i>	5.5 – 6.0
<i>Pseudomonas aeruginosa</i>	6.0 – 6.5
<i>Propionibacterium</i>	5.5 – 6.5
<i>Salmonella</i> species	5.5 – 7.0
<i>Shigella flexneri</i>	6.0 – 6.5
<i>Staphylococcus aureus</i>	5.5 – 6.0
<i>Vibrio parahaemolyticus</i>	6.0 – 7.0
Moulds	
<i>Alternaria citri</i>	4.0 – 5.0
<i>Alternaria tenuis</i>	4.0 – 6.0
<i>Aspergillus flavus</i>	4.5 – 5.5
<i>Colletotrichum falcatum</i>	4.5 – 5.5
<i>Fusarium moniliforme</i>	4.5 – 5.0
<i>Gloeosporium papaya</i>	5.0 – 6.5

<i>Gloeosporium citricolum</i>	5.0 – 6.3
<i>Penicillium crustosum</i>	5.0 – 5.5

Yeasts

<i>Hansenula</i>	4.0 – 4.8
<i>Pichia</i>	5.0 – 5.5
<i>Saccharomyces cerevisiae</i>	4.0 – 4.5

Compiled by the author from several sources including: Banwart, 2004. Jay, 1996; Madan,1998.

Table 5. pH values of some selected foods

Food	pH range	Food	pH range
Beef	5.3 – 6.2	Oysters	5.8 – 6.5
Onions	5.0 – 5.8	Bananas	4.5 – 5.0
Catfish	6.5 – 7.0	Tomatoes	3.7 – 4.8
Shrimps	6.8 – 8.0	Pineapple	3.2 – 4.1
Milk	6.3 – 6.8	Oranges	2.8 – 4.0
Egg white	7.5 – 9.5	Lemons	2.2 – 2.4
Bread	5.0 – 6.0	Limes	1.8 – 2.0
Carrots	4.7 – 6.2		

Sources: Banwart, 2004; Efiuvwevwere and Ajiboye, 1996; Efiuvwevwere and Akoma, 1997b

Whereas several factors influence the growth of microorganisms and their ability to cause food spoilage, an obvious relationship/correlation exists between the type of species, the initial population and the type of food (Table 5). For example, while bacteria are mainly responsible for spoilage of pH neutral foods (meat, fresh milk, poultry), yeasts and moulds are the main spoilage

agents of acidic products such as fruits, alcoholic products/soft drinks as well as high sugar products (Efiuvwevwere and Akoma, 1997b, Jay, 1996; Ray and Bhunia, 2008).

The food composition influences the microorganisms and at the same time, the microorganisms influence the food ecosystem. For instance, the initial reaction of most microorganisms in food is acidic because they breakdown carbohydrates to produce organic acids. This alteration of pH by production of acids is often used in the food fermentation industries. However, growth of moulds in acidic foods such as tomatoes/tomato products results in pH increase leading to proliferation of food borne pathogens and potential health hazards (Efiuvwevwere and Eka, 1991; Mundt and Norman, 1982). Thus, lactic acid bacteria (acid producers) tend to lower the pH by production of lactic acid while proteolytic microorganisms such as *Pseudomonas* spp tend to increase the pH by production of ammonia and other proteolytic compounds.

Microbial spoilage of foods is therefore mainly influenced by the acidic nature of the food and the type of microorganisms present. For example, from Table 5, fruits, fruit juices, beer and wine are spoilt mainly by lactic acid bacteria (acidophiles), moulds and yeasts while most of the other foods will be spoiled by bacteria (neutrophiles). It is important to note that microorganisms have special mechanisms to adapt to their environment (including food ecosystems) for survival (Griffiths, 2005).

2. Moisture content/ Water activity

Microorganisms can remain viable in a dried condition but cannot multiply in absence of water or in pure water (i.e no nutrients/solutes). The water in food is both bound and free. Bound water is held by physical forces to macromolecules and is not available to microorganisms for metabolic activity.

Water Activity

It is now accepted that the water/ moisture requirements of microorganisms should be described in terms of the water activity

(a_w) in the environment. This parameter is defined as the ratio of the vapour pressure (V.P) of food subtracted solution to the vapour pressure of pure water at the same temperature i.e $a_w = p/p_o$ where p is the vapour pressure of the solution and p_o is the vapour pressure of the solvent normally water. The values of water activity range from 0-1.0.

The VP of a liquid depends on the rate of escape of water to the air is measured by the equilibrium relative humidity (ERH). Consequently, VP and ERH are related. For example, when pure water is altered by the addition of a solute (e.g sodium chloride), the concentration of water is decreased and the rate of escape from the surface is reduced.

Thus, the water activity of solution is defined in terms of VP and ERH by the formula:

$$a_w = p/p_o = ERH/100$$

This formula has been used to determine the water activity of several substances including foods (Nunes *et al.* 1985).

Microorganisms have minimum, optimum and maximum a_w for growth. Since the a_w of pure water is 1.00 and microorganisms cannot grow in pure solvent, the maximum or upper limit for microbial growth is an a_w less than 1.00 (approximately 0.99). In general, bacteria require a higher a_w (0.92-0.99) than yeasts and yeasts require a higher a_w (0.80-0.90) than moulds (0.70-0.82) (Table 6).

However, there are other interacting factors such as the type of solute and the microorganisms involved as well as the type of food.

Table 6. The a_w of some selected foods

FOOD	a_w
Fresh fruit and vegetables	0.97 – 0.99
Fresh poultry	0.98 – 0.99
Fresh meats	0.97 – 0.99
Nuts	0.66 -0.84
Cereals/grains	0.12 – 0.25
Rice	0.80 – 0.87
Flour	0.70 – 0.88
Honey	0.54 – 0.75
Noodle	0.50 – 0.55
Biscuits	0.30 – 0.40

Source: Banwart, 2004;

It is apparent from Table 6 that shelf life stability/storage-life is highly dependent on the a_w values.

3) **Oxidation-reduction potential (Eh)** is a measure of the tendency of a reversible system/reaction to give or receive electrons. The usual method of measuring OR/Eh is with a platinum redox electrode attached to a pH/mV (millivolt) meter.

The importance of redox potential in foods is that it creates two major Eh requirements for microorganisms where some microorganisms require positive (i.e. oxidized: =+50mV) Eh for growth while others require negative (i.e. reduced: = -40mV) condition. The aerobic microorganisms such as fungi and some bacteria (e.g. *Bacillus* spp) require positive Eh while anaerobic (absence of free oxygen-loving microorganisms (e.g. *Clostridium* spp) require negative Eh values (typically – 200mV and below).

However, some microorganisms (e.g. *Lactobacillus* sp and *Campylobacter* spp) prefer slightly reduced Eh conditions and these are referred to as microaerophiles. In addition, others grow under either aerobic or anaerobic conditions and they are termed facultative anaerobes.

Changes in Eh of food occur as the microorganisms grow in such foods. In general, aerobic microorganisms (especially fungi) cause the spoilage of fruits due to the relatively high Eh and low pH (as earlier indicated).

However, the microbial spoilage of meat due to Eh is highly dependent on the type of meat. For example, uncut/solid meat samples (carcasses) have reduced Eh due to less total surface area as compared with comminuted (ground) meat. Thus, microaerophilic and anaerobic microorganisms (that prefer negative Eh value) will grow and spoil the former as compared with the latter that have positive Eh values which support aerobes (fungi and some bacteria). Similarly, if these products are packaged, the Eh/oxygen content will change and consequently, the microbial profiles and the spoilage mechanism become altered (Efiuvwevwere and Uwanogho 1990; Efiuvwevwere and Nwachukwu, 1998; Efiuvwevwere and Eka 1992; Jay, 1996).

4. Nutrient content

Microorganisms as earlier indicated need adequate nutrients for their growth and proliferation. Thus, the nutrient content comprising water, source of energy, source of nitrogen, vitamins and other growth factors and minerals is essential. However, there are variations in microbial requirements for these components. For example, few microorganisms are able to utilise complex carbohydrates such as starches and cellulose as sources of energy by first degrading these compounds to simple sugars. Generally, simple components such as amino acids will be utilised by almost all organisms before any attack is made on the more complex compounds including high molecular weight proteins and polysaccharides. In addition, some microorganisms may require B

vitamins in low quantities yet they cannot synthesise them. Interestingly, Gram-negative bacteria and moulds are able to synthesise most or all of their vitamin requirements. Thus, these two groups of microorganisms are capable of spoilage of fruits as result of their lower B vitamin contents, low pH as well as positive Eh all of which contribute to their spoilage by these microorganisms.

5) Antimicrobial constituents/substances

Several antimicrobial substances are naturally present in some food and these provide some degree of stability against their spoilage. Examples of such substances and their antimicrobial activities are shown in Table 7.

Table 7. Antimicrobial substances naturally present in some foods

Antimicrobial substances(s)	Food	Organisms inhibited
*Lactoperoxidase thiocyanate/ hydrogen peroxide system	Bovine/cow fresh milk	<i>Pseudomonas</i> spp <i>Listeria monocytogenes</i>
Lysozyme (muramidase)	Eggs, oysters, clams	Gram-negative and Gram-positive bacteria
Isohumulone	Hops	Gram positive bacteria
Hydroxycinnamates, <i>P</i> - coumaric acid	Fruits, vegetables, grapes	<i>Saccharomyces</i> <i>cerevisiae</i> <i>Bacillus</i> <i>cereus</i> , <i>Staphylococcus</i> <i>aureus</i>
Caffeic, ferulic and chlorogenic acids	Fruits, vegetables, tea etc	<i>Fusarium</i> spp., <i>Aspergillus</i> spp and some bacteria

*Can be used to preserve raw milk in countries where adequate refrigeration is uncommon.

Compiled by the author from several sources including: Banwart, 2004; Jay, 1996; Ray and Bhunia, 2008.

6. Biological Barriers/Structures

These natural protective structures against the entry of microorganism and spoilage of foods include the testa of seeds, peels of crops, e.g cassava, paw-paw, shell of eggs, skin of animals and fishes. Evidently, once these barriers are damaged, invasion by microorganisms becomes accelerated and spoilage is much faster (Efiuvwevwere and Hobson, 1989., Efiuvwevwere and Nwachukwu 1998; Snowdon, 1990). Overall, these six intrinsic parameters play major roles in determining the extent of microbial growth/retardation and spoilage of foods. However, other factors such as temperature, gaseous composition of the environment contribute to these effects in terms of interactive growth behaviour (Jones, 1990; Ray and Bhunia, 2008) .

7.3.2 Extrinsic parameters

These are the parameters involving the strage environment that affect the food itself and the microorganism. They are as follows:

- 1). Temperature of storage
- 2). Presence and concentration of gases in the microenvironment
- 3). Relative humidity of the microenvironment

These parameters are critical for storage- life and control of microbial growth thereby influencing the safety of the food. They are discussed briefly as follows:

7.3.2.1 Temperature of storage

Temperature is one of the most important environmental factors that influences the growth of microorganisms. Generally, microorganisms are classified into three categories based on their temperature of growth (Banwart, 2004).

- a) Psychrophiles /psychrotrophs (those that prefer 5°C refrigeration temperature and those that grow at between 5°C and 15°C respectively).
- b) Mesophiles have 25°C to 40°C as their optimum growth temperature range (most dangerous for pathogenic growth).
- c) Thermophiles prefer 45°C to 65°C

Examples of microorganisms in the three categories are shown in Table 8.

Table 8. Some examples of microorganisms in the three categories of their temperature of growth

Psychrophiles	Psychrotrophs	Mesophiles	Thermophiles
<i>Flavobacterium</i> , <i>Moraxella</i> , <i>Pseudomonas</i> , <i>Shewanella</i> , <i>Vibrio</i> , <i>Bacillus</i> , <i>Brochothrix</i> , <i>Clostridium</i> , <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Listeria</i> , <i>Micrococcus</i> and others	<i>Pseudomonas</i> , <i>Serratia</i> , <i>Psychrobacter</i> , <i>Bacillus</i> , <i>Carnobacterium</i> <i>Listeria</i> and others	<i>Staphylococcus</i> , <i>Salmonella</i> , <i>Clostridium</i> , <i>Shigella</i> , <i>Bacillus</i> and others.	<i>Bacillus</i> <i>stearothermophilus</i> , <i>Clostridium</i> <i>botulinum</i> , <i>Clostridium</i> <i>sporogene</i> , <i>Bacillus</i> <i>coagulans</i> and others.

Sources: Banwart, 2004; Jay, 1996.

Of these three categories, the psychrophiles/psychrotrophs are more responsible for spoilage of foods (milk, meat poultry, etc) under refrigeration temperature. However, while mesophiles cause food spoilage, the greater concern is with respect to their ability to cause food borne illnesses. On the other hand, the generation time is shorter for thermophiles than for either psychrophiles or mesophiles when respectively grown at their optimum temperature (Banwart, 2004). Due to the limited availability of “cold chain food system” in the developing countries, the mesophiles and thermophiles (and not the psychrophiles and psychrotrophs) are more important as food spoilage organisms in the developing countries (Banwart, 2004; Efiuvwevwe and Chinyere, 2001).

7.3.2.2. Presence and concentration of gases in the microenvironment

Microorganisms vary significantly in their gaseous concentration requirement. For example, the type of gas in the microenvironment of the food will determine the types of microorganisms that become

dominant. While presence of free oxygen in the atmosphere favours the growth of aerobic microorganisms especially fungi, lack of oxygen (e.g carbon dioxide increase) supports facultative anaerobes and they become dominant (Jay, 1996). The main purpose for gaseous composition manipulation in food microenvironments is to achieve significant commercial benefits. For instance, the use of modified atmosphere storage (MAS) i.e increase in CO₂ and reduction in oxygen content is widely employed in international food trade (Gorris and Heppelenbos, 1992; Sugar, 2001) especially for shipment of apples, pears and grapes (particularly to many developing countries including ours). The quest to exploit this concept of oxygen manipulation to enhance the shelf-life of fruits has been of research interest to us and others (Efiuwewwere and Uwawagho, 1990; Efiuwewwere and Oyelade, 1991; Jay, 1996; Jones, 1990).

However it has been shown that increase in CO₂ could also result in anaerobic condition that will encourage the growth of food-borne pathogens such as *Clostridium botulinum* (Jones, 1990).

7.3.2.3 Relative humidity of the microenvironment

The importance of relative humidity in relation to water activity is the induced spoilage of foods.

However, it is important to know that when foods having low a_w values (such as flour) are placed in microenvironment of high relative humidity, the food will pick up (absorb) moisture until equilibrium is established and spoilage especially on the surface will occur. In contrast, foods containing high a_w value (e.g.meat) will lose moisture when stored in an environment of low RH thereby, leading to spoilage by moulds, yeasts and some bacteria. Therefore, adequate consideration must be given to the packaging and relative humidity of storage of foods (Efiuwewwere and Oyebanji, 1998 ; Jay, 1996).

8.0 Food Preservation Methods and Strategies

The rationale for food preservation is primarily two-fold: (i) to prevent or minimize the growth of pathogenic and spoilage microorganisms and (ii) to preserve the quality attributes of the food. All foods following harvest, slaughter or manufacture lose quality at some rate which is dependent on the initial microbial profile, food type and composition; formulation (for manufactured/processed foods), storage conditions and so forth. However, quality loss may be accelerated or minimized at any of the stages and the total preservation strategy is therefore often multi-component since one factor/approach is often inadequate (Efiuvwevwere and Amadi, 1992; Efiuvwevwere and Ajiboye, 1996; Efiuvwevwere and Isaiah, 1998; Gould, 1989; Jay, 1996). Several food preservation methods exist and they include the following:

Drying, smoking, use of low/high temperatures, food preservatives, fermentation, radiation, modified/ controlled atmosphere (MA/CA). These are discussed briefly as follows:

8.1 Drying

This involves the lowering of the moisture content of food so that microorganisms and their enzymes become inactive. Foods are dried to different levels of moisture and water activity using several drying systems.

Some of the foods are referred to as low moisture (LM) (i.e. contain not more than 25% moisture and intermediate moisture (IM) foods that contain between 15% and 50% moisture.

In general, bacteria are the most inhibited by drying since they require higher moisture content than both yeasts and moulds. Thus, dried foods such as maize, garri, wheat flour, cassava flour etc are often spoilt by these groups of microorganisms e.g *Rhizopus stolonifer*, *Alternaria citri*, *Aspergillus glaucus*, *Zygosaccharomyces rouxii* which are usually termed xerophiles i.e. dry-loving microorganisms. The microbial hazards associated with these foods are due to the growth of these moulds which produce

mycotoxins (carcinogenic substances) as earlier indicated in this lecture.

Generally, in order to maintain the storage stability of dried foods, the “alarm water” content should not be exceeded to prevent mould growth, hence adequate packaging after drying is very important. The “alarm water” content of some foods is presented in Table 9.

Table 9. “Alarm water” content of some foods

Foods	% “Alarm water” content
Rice	13-15
Wheat flour	13-15
Powdered milk	15
Dried vegetables	14-20
Dried fruits	18-25

Source: Jay, 1996

8.2 Smoking

In addition to its preservative effect, is the distinctive and desirable flavours imparted in certain foods such as meat, fish, shellfish, poultry etc. The preservative effect is mainly due to the combination of drying and the deposition of the chemicals resulting from the heat (thermal) decomposition of wood. However, the chemical composition of wood smoke depends upon factors such as the type of wood (e.g. “Ingala”, hard wood is preferred because it impacts desirable flavours), the temperature and duration of smoking. In contrast, soft wood is known to impact unpleasant flavours in the food hence choice of wood for food smoking is important. Among the several chemicals identified to be associated with smoked foods (smoke condensate) include acids, alcohols, aldehydes, ketones, phenols, waxes, resin, tars, benzopyrene and others) (Fretheim, 1976; White *et al.*, 1971) which are carcinogenic and populations in which smoked foods are consumed in large quantities have relatively high incidence of carcinoma of the gastro- intestinal tract (Banwart, 2004). Similarly, the microbial hazards associated with smoked foods have been highlighted. For example, commercially smoked

fish was found that about 0.9% to 2.0% contained *Clostridium botulinum* type E spores even though the fish had been subjected to smoking temperature of 82°C for 30 minutes (Banwart, 2004). In addition, Efiuvwevwere and Ajiboye (1996) showed that combination of smoking and preservative treatment was more effective in prevention of food pathogens and spoilage microorganisms than smoking alone.

8.3 Use of Low temperatures

Since foods are usually not sterile, the use of low temperatures is to retard their growth but if not properly stored (i.e. abused), the microorganisms will grow and cause spoilage or health hazards. It is important to note that even at low temperatures ($\leq 10^{\circ}\text{C}$), psychrophiles (low temperature-loving microorganisms) and psychrotrophs (low-temperature tolerating microorganisms) will grow but more slowly as the temperature is reduced. Importantly, as the refrigerator temperature is reduced from 10°C, fewer types of microorganisms can grow (due to bacteriostatic effect) and cause spoilage hence food will spoil about four times as fast at 10°C and twice as fast at 5°C as at 0°C. Also, with every 10°C rise in temperature, the catalytic rate of an enzyme doubles and also reduced to half (50%) by decreasing the temperature by 10°C. The implications of these temperature changes in food safety and spoilage are enormous where there is erratic power supply which is a serious challenge in the country. Similarly, it has been shown that microorganisms will remain viable even at freezing temperatures (hence it is not a sterilisation process) but grow at a much slower rate to cause food spoilage (Banwart, 2004; Efiuvwevwere and Oruwari, 2014; Ray and Bhunia, 2008). However, some foods especially tropical and sub-tropical crops are sensitive to certain low temperatures thereby, resulting in chilling injury which predisposes them to microbial invasion and growth leading to substantial economic losses (Efiuvwevwere and Thorne, 1988; Snowdon, 1990).

8.4 Use of High Temperatures

The two commonly used methods involving high temperatures are pasteurization (named after Louis Pasteur, the French Chemist and Microbiologist) and sterilization/canning (named after another French, Appert, termed Appertisation).

Pasteurization is often used to preserve liquid foods especially milk and alcoholic beverages (beer and wine) by subjecting them to temperatures below 100°C while use of above 100°C is termed sterilization. Generally, pasteurization is at about 63°C for 30 minutes or at about 75°C for 15 to 30 seconds to kill vegetative spoilage or specific types of microorganisms (e.g. *Mycobacterium tuberculosis*) without adversely affecting the quality of the food. On the other hand, sterilization/thermal processing is aimed at destroying pathogenic microorganisms and spore-formers such as *Clostridium botulinum* but complete sterilization is not achievable (hence the practice of “commercial sterility” earlier indicated).

8.5 Use of food preservatives/additives

These substances (additives) that are used as food preservatives must be approved by regulatory agencies (e.g. the Joint FAO/WHO Codex Alimentarius Commission of the United Nations, US Food and Drug Administration, NAFDAC and others). They are often referred to as “Generally Regarded As Safe” (GRAS). They are added to food to serve as essential aids in food processing, making food more attractive (e.g. bacon), enhancing the keeping quality and shelf-life, reducing microbial growth and minimizing food safety risks (public health hazards etc). The necessity for use of preservatives in foods is better captured by this quote “**Food, one assumes, provides nourishment: but Americans eat it fully aware that small amounts of poison (preservatives) have been added to improve its appearance and delay its putrefaction (spoilage)**” (Cage, 1990 American avant-garde composer, 1912-1992). (www.quotesdaddy.com/author/John+cage).

Thus, the choice between consumption of approved preservatives versus consumption of harmful/toxic microorganisms is becoming

clearer in the light of more recent scientific findings. However, by and large, most consumers prefer consuming food containing small amounts of approved and effective preservatives to consumption of neurotoxic/paralytic substances produced especially by *Clostridium botulinum*. This was the main reason for introduction of sodium nitrite in the production of bacon and some other meat products (although its use has been questioned recently due to its carcinogenic potential).

In Table 10 is shown some examples of food preservatives, their concentrations and applications.

Table 10. Some examples of food preservatives, their concentrations and usage

Preservatives	Usage level	Microorganism (s)	Affected Foods
Sorbic acid/sorbates	0.2%	Moulds	cake, syrups Salad dress cheese
Benzoic acid/benzoates	0.1%	Yeasts & moulds	Soft drink tomato Ketup, margarine Salad dressing
Sulphur dioxide/sulfites	200-300ppm	Insect, microorganisms	Molasses, fruits Wine products
Ethylene/propylene Oxide	700ppm	Yeasts, moulds	Fumigants spices, nuts
Sodium nitrite	120ppm	<i>Clostridium</i>	meat curing
<i>Nisin</i> (probiotic)	1%	Lactic acid bacterial <i>Clostridium</i> spp	certain pasteurized cheese

Source: Jay, 1996.

8.6 Food Fermentation

Globally, numerous food products are produced through fermentation (bioconversion) processes. These are aimed at achieving better preserved products (having extended shelf-life) with special desired aroma and flavour characteristics impacted by the microorganisms. Some of the products include garri, fufu, yogurt, beer, cheese, ugba etc. In general, these products have longer shelf-life than the raw materials from which they are produced. In addition, the fermentation process reduces the toxicity of some of these products (Efiuvwevwere and Orelesi, 2014; Lennox and Efiuvwevwere, 2014; Oyewole and Isah, 2012).

Many microorganisms are employed in the fermentation of different foods. However, the lactic acid bacteria are the most commonly involved in the fermentation processes (Efiuvwevwere and Akoma, 1995; Jay, 1996; Ray and Bhunia, 2008).

The shelf-life of these products was enhanced through additional preservation strategies such as pasteurisation (Efiuvwevwere and Akoma, 1997a) or addition of salt (Ademola *et al* 2013) or dehydration (Okorie and Olasupo, 2013).

8.7 Food Radiation

Whereas the use of radiation for preservation of food has been recognized for a long time, its application has been slow due to some challenges especially concerns/public perception about the safety of such irradiated foods (Jay, 1996). There are several types of radiations but the radiation of primary interest in food preservation is the electromagnetic. The electromagnetic spectrum is further divided into: microwaves, ultraviolet rays, x-rays and gamma rays which are the radiation of primary interest in food preservation. These radiations destroy microorganisms without raising temperatures appreciably hence the process is often termed “cold sterilization”. In general, gram-negative bacteria are more sensitive to irradiation than gram-positive bacteria. Also, spore formers are more resistant than non-spore-formers. However, many factors such as age and type of microorganisms, population, composition of the food contribute to

the radiation resistance or sensitivity of the microorganisms in the foods. For better appreciation of the application of radiation for food preservation, these terms have been adopted (Goresline *et al.* 1964) and they are used as follows:

1. **Radappertization:** equivalent to radiation sterilization or “commercial sterility” as in the food canning industry (30-40 kGy)
2. **Radication:** equivalent to pasteurization e.g. milk (2.5 – 10 kGy)
3. **Radurisation:** also considered as equivalent of pasteurization by different radiation dose used for different foods such as fresh meats, poultry, fruits, vegetables and cereal grains (0.75-2.5 kGy).

These different dosage levels are to effectively destroy microorganisms without adversely changing the quality of the food (Jay, 1996).

Globally, the current legal status of the application of irradiation for food preservation indicates that several countries have approved the use of irradiation for food preservation (WHO, 1999) but complaints about safety concerns are not uncommon.

8.8 Modified Atmosphere and Controlled Atmosphere Storage

The use of modified and controlled atmosphere storage for food preservation and shelf-life extension has national and global economic importance. This preservation strategy usually involves manipulation of the gaseous composition of the food environment. For example, increasing the carbon dioxide level at the expense of oxygen enhances the storage life of agricultural produce (e.g. apples, pear etc) because the growth of fungi (aerobes) which are the major spoilage microorganisms is highly inhibited. Many other foods have been shown to benefit from this application/ method of food preservation (Figures 5a and 5b) (Efiuvwevwere and Oyelade, 1991; Efiuvwevwere and Nwachukwu 1998; Sugar, 2001)



Figure 5a Benefits of modified/controlled atmosphere storage in pear (cultivar, Bartlett)



Figure 5b. The benefits of modified/ controlled atmosphere storage are shown in pear (cultivar, Bosc).

Source: Sugar, 2001

9.0 Microbiological Criteria and Standards For Foods

Several microbiological criteria/standards exist in different parts of the world (Table 11). However, many of these criteria/standards are often reviewed in the light of changes occasioned by production processes and scientific findings due to improved scientific infrastructure/facilities and expertise.

Among the various food products of particular safety concern at national and international levels are “the ready-to-eat-foods” which are very popular in the developing world. These are foods intended by the producer/manufacturer for direct human consumption

without the need for further cooking or other effective processing to eliminate or reduce to an acceptable level the specific microorganisms/microbial groups of concern (CFS, 2014).

Table 11. Microbiological criteria/standards for some ‘ready-to-eat foods’

Food Category	Examples	Microbiological Criteria (Log₁₀ cfu/g/ml)		
		Satisfactory	Border line	Unsatisfactory
Ambient/room temperature stable canned/bottled foods	Corned beef, canned fish/sardine, pasteurised products (juices/milk)	<10	N/A	Presence of spore-formers
Foods cooked immediately prior to sale/consumption	Take away foods: Burgers, pizza, fried rice, noodles etc	<10 ³	10 ³ -<10 ⁵	≥10 ⁵
Bakery and confectionery products without dairy cream/powdered items	Cakes without dairy cream, milk powder, reconstituted powdered foods (i.e. ready to eat/drink milk after reconstitution or warming)	<10 ⁴	10 ⁴ -<10 ⁶	≥10 ⁶
Cooked foods chilled but with some handling prior to sale/consumption	Meats, sandwiches without salad, smoked fish, shell fish, periwinkles, oysters, non pre-packaged cold beverages etc	<10 ⁵	10 ⁵ -10 ⁷	≥10 ⁷

Table 11 Cont'd

Foods mixed with dressings, dips, pastes	Coleslaw, salads etc	$<10^6$	10^6-10^7	$\geq 10^7$
Extended shelf life food products requiring refrigeration	Modified atmosphere packaged (MAP) or vacuum packed products e.g. meat, fish, fruits and vegetables	$<10^6$	10^6-10^8	$\geq 10^8$
Dried foods	Fruits, nuts, spices, dried fish etc	NA	NA	NA
Fermented, cured and dried meats, other fermented products	Cheese, butter, yogurt, sausage, sauerkraut (from cucumber)	NA	NA	NA

NA denotes ‘not applicable’ thus, the criteria are not adequate for the assessment e.g. fermented products such as fresh yogurt normally has 10^7 cfu/g.

Sources: Compiled by the author from several sources including: Efiuvwevwere and Isaiah, 1998; CFS, 2014; Codex, 2013; HPA, 2009.

Several food-borne pathogens are associated with occurrence of food-borne diseases but they have different infective doses/levels (Table 12).

Table 12. Some food borne pathogens, their infective dose and other characteristics

Food borne pathogen	Infective dose (cfu/g or ml)	Incubation period	Associated foods
<i>Campylobacter jejuni</i>	Generally 10^4 but as few as 500 cfus have led to disease manifestation	2-5 days	Raw and under cooked poultry
<i>Bacillus cereus</i>	Greater than 10^6 but pathogenicity/disease arises from pre-formed toxin	Usually 1-6 hours	Meat, stews/sauces improperly refrigerated cooked/fried rice
<i>Clostridium perfringens</i>	Greater than 10^6 or greater than 10^6 spores of food; toxin production in the GIT associated with sporulation	Ranges from 6-24 hours but usually 10-12 hours	Meat, poultry and sauces/gravies
<i>Escherichia coli</i> : 0157	As low as 10 organisms	Ranges from 2-10 days usually 3-4 days	Raw or undercooked ground meat products, fruits and vegetables
<i>Listeria monocytogenes</i>	Less than 10^3 especially in susceptible individuals	Range 3-70 days but 3weeks on the average	Ready-to-eat foods under refrigeration e.g. salads, cheese

Table 12 cont'd

<i>Salmonella</i> spp	Typhoid fever: less than 10^3 cfu	Range from 7-21days	Food or water contaminated with faeces and urines of an infected persons; oysters, fruits and vegetables, unpasteurized milk.
<i>Staphylococcus aureus</i>	Less than 1 μ g pre-formed heat stable toxin; greater than 10^5 cfu/g to produce this toxin level	Range from 30 minutes to 8hours; usually 2-4 hours	Any food contaminated by food handlers with skin infection or nasal carriers, e.g. sandwiches, cakes pastries etc
<i>Vibrio parahemolyticus</i>	Approximately 10^8 cfu/g	Usually 12-24hours	Seafoods, salted foods
<i>Shigella</i> spp	As few as 10cfu/g	Usually 1-3 days but can be up to 7days	Contaminated raw foods e.g. salads and sandwiches

Adapted by the author from different sources:

1. The US FDA Bad Bug Book, Food-borne Pathogenic Microorganisms and Natural Toxins Handbook, 2nd edition. (Accessed 4 March, 2014).
2. Diagnosis and Management of Food-borne Illnesses- A Primer for Physicians and Other Health Care Professionals: Food borne Illnesses Table: Bacterial agents. AMA/CDC/FDA/USA Department of Agriculture, Feb. 2004.
3. Ray and Bhunia, 2008.

Table 13 shows top five pathogens associated with foodborne illness.

Table 13. Top five pathogens contributing to domestically acquired foodborne illnesses

* Pathogens	Estimated number of illness	%
<i>Norovirus</i>	5,461,731	58
<i>Salmonella</i>	1,027,561	11
<i>Clostridium perfringens</i>	965,958	10
<i>Campylobacter spp</i>	845,024	9
<i>Staphylococcus aureus</i>	3,641,148	39
Unknown microorganisms		9
Total		100

Note: * This distribution varies from time to time.

Compiled by the author from several sources:

www.salmonella/index.html

www.foodborneburden/clostridium-perfringens

www.nczved/divisions/fnd/diseases/staphylococcal

10.0 NATIONAL AND GLOBAL PERSPECTIVES ON FOOD SAFETY

The importance of food microbiology in national and global food standardization is effected through the working of the Codex Alimentarius Commission (CAC) of the joint FAO/WHO Food standards programme of the United Nations. CAC was established in 1962 by FAO and WHO and held its first session in 1963 in Rome.

The main goals of the commission are to protect the health of consumers and ensure fair practices in the international food trade {WHO/FAO, 2008}. CAC is recognized by the World Trade Organization as an international reference point for the resolution of disputes concerning food safety and consumer protection especially in the twenty- first century (FAO/WHO, 2008).

Nigeria is a signatory to this commission by the virtue of her membership of the United Nations. The country is usually represented by delegates from the National Agency for Food and Drug Administration and Control (NAFDAC) and the Standard Organization of Nigeria (SON).

Harmonisation of respective national food standards by the CAC is one of her major responsibilities. However, foodborne diseases are widespread and growing public health problems with the recognition that food is a major vehicle of food borne illness both in developed and developing countries (WHO, 2005). In industrialised countries, the percentage of people suffering from foodborne diseases each year has been reported to be about 30% (WHO, 2005). Several guidelines (Articles) have been adapted to help minimize or prevent incidences of food borne illness on global scale (CAC, 2010). However, for this lecture, I will focus on Article 3 and its principles as follows:

3.1 International trade in food should be conducted on the principle that consumers are entitled to safe, sound and wholesome food and protection from unfair trade practices.

3.2 No food (including re-exported food) should be in international trade which:

- a) has in or upon it any hazard in an amount which renders it poisonous, harmful or otherwise injurious to health, taking into account the application of risk analysis principles; or
- b) consists in whole or in part of any filthy, putrid, rotten, decomposed or other substance or foreign matter which renders it unfit for human consumption; or
- c) is adulterated; or
- d) is labeled or presented in a manner that it is false, misleading or deceptive; or
- e) is prepared, processed, packaged, stored, transported or marketed under unsanitary condition; or
- f) has an expiration date, where applicable which does not leave sufficient time for distribution in the importing country.

Source: www.codexalimentarius.net/injuit/download/i/cxp_020....

As a result of these Articles, countries are at liberty to detain foods at destination points for several reasons (Kenny, 2000). For example, USFDA effected detentions of foods for different reasons as shown in Table 14.

Table 14. Detentions of foods for a number of reasons

Reason for detention	Number of detentions Jan-June 1997]
Food additives	339
Pesticide residues	364
Heavy metals	249
Mould	313
Microbiological contamination	585
Decomposition	412
Filth	1,688
Low-acid canned food	647
Labelling	524
Others	147
Total number of reasons (contraventions cited)	5,268
Number of consignments	4,795

Source: www.fda.gov/ora/ids/ora/ids/ora_ids_access.html

The international food trade is on the increase as countries rely on the harmonization of food standards by CAC and other agencies such as WTO. However, the United States is not sitting on its oars (laurels) especially since the incidence of food borne diseases is 1 in 6 Americans suffers from food borne illness annually resulting in 128,000 hospitalizations and 3,000 deaths per annum (CDC, 2011). This is marked improvement because the earlier statistics was 76 million cases of foodborne diseases resulting in 325,000 hospitalisations and 5,000 deaths per annum (WHO, 2005) and this could be attributed to the creation by President Barak Obama in 2009 of Food Safety Working Group (FSWG) as a central coordinating mechanism for the United States Federal Government Food Safety activities headed by the Departmentt of Health and Human Services with other agencies such as USFDA, Food Safety

and Inspection Service (FSIS), Centres for Disease Control (CDC) and Prevention, EPA etc.

10.1 NEW FOOD SAFETY MEASURES AND GLOBALIZATION

The issue of food safety and food security is always of concern to the food regulatory agencies world-wide. It is therefore not surprising that the USFDA recently introduced a new Act through Congress termed “Food Safety Modernization Act (FSMA). This was signed into law by President Obama in 2011 and is the most sweeping reform of United States Food Safety laws in more than 70 years by shifting the focus from responding to contamination of food supply to preventing it (USFDA, 2014) . In addition, USFDA has created a new office known as “Coordinated Outbreak Response and Evaluation (CORE)” Network within FDA to ensure rapid and effective emergency response and more systematic follow up investigations in collaboration with CDC and other agencies.

In this regard, new impetus by the USFDA with respect to international food trade and globalization has been put in place.

10.2 FOOD STANDARDS/SAFETY: INTERNATIONAL FOOD TRADE AND GLOBALIZATION

The issue of food standards, food safety and globalization is now a major concern to the global community (WHO, 2014). Whereas the US encourages free international food trade, the regulatory agencies ensure that the standards are not compromised. As a result of the FSMA, USFDA will now focus on import safety tool which states that “Food importers will be responsible for providing documented assurances to USFDA that the food they import has been produced under the same prevention-oriented standards as domestic food”. This is to prevent occurrence of food borne illnesses and ensure food safety. Furthermore, US Congress has directed USFDA to establish new prevention oriented standards for the food industry that imported food meets the ***SAME MODERN STANDARDS*** as domestically produced food. Whereas globalization has its own merits and demerits, most developing countries appear to be disadvantaged since international trade (including trade in foods)

and cross-border investment flows are the main elements for this integration (globalisation). However, some major challenges are apparent for developing countries as indicated in USFDA requirements based on the Food Safety Modernisation Act for food imports. This is particularly obvious when quality assurance system in the food sector (i.e involving food producers and manufacturers) are not in place. Thus, much needs to be done so that all countries can take full advantage of the new opportunities (liberalization) for international food trade in order to achieve the comparative advantages in each country. .

11.0 TOP SEVEN MEASURES/STEPS TO PREVENT FOOD-BORNE ILLNESS

It is important to note that one cannot tell from the way food looks (i.e. from the visual appearance), smells or tastes if it is safe or not but here are top seven measures to prevent food-borne illness following production, preparation or processing.

11.1 Improper cooling or holding

Cooling food too slowly is a major cause of food borne illness. Potentially hazardous foods such as meat, seafood, poultry and dairy products must be rapidly cooled from 60°C to 21°C within two hours and from 21°C to 5°C within four hours (please, remember the generation times of microorganisms earlier discussed).

- Store food to be cooled in shallow pans/containers not deeper than 3-4 inches.
- Stir the food (if liquid) often while cooling.
- Refrigerate hot foods uncover in shallow containers immediately (use a freezer to quicken the process).
- Do not place tight covers on container of food during cooling.
- Allow air circulation in the refrigerator.
- Do not cool food to room temperature longer than 30 minutes.

11.2 Contaminated raw foods or ingredients

- Many raw foods such as meat, fish, poultry, periwinkles, snails, milk are usually contaminated with bacteria or viruses. These

microorganisms can be spread during processing and preparation and can easily survive in the food due to inadequate heating.

- Cook foods to the proper temperature (internal tissue about $\geq 72^{\circ}\text{C}$)
- Wash all raw fruits and vegetables thoroughly (studies have shown drastic microbial reduction due to washing).
- Keep all cold foods properly refrigerated (safety implications of erratic power supply are obvious).
- Avoid cross-contamination by using a separate cutting board/utensil for raw and cooked products unless they are sanitized between use. Use a different cutting board for fruits, vegetables and bread as for meats.

11.3 Infected person handling foods

Persons with poor handling habits and poor personal hygiene are the greatest contributors to food borne illness outbreaks (please, remember “Typhoid Mary” as earlier discussed).

- Do not handle food if you have colds, flu, diarrhoea or hepatitis.
- Do not handle food if you have infected cuts, burns or lesions on the hands or lower arm.
- Wash hands effectively before handling foods.
- Wash hands after eating, smoking, blowing nose.
- Picking nose (reservoir for *Staphylococcus aureus*)
- Do not wipe hands or utensils/cutlery on apron or cloth towels
- Do not touch ready-to-eat foods with bare hands (use disposable gloves / others).
- Use hand sanitizers after washing hands (especially with the EVD outbreak).

11.4 Inadequate cooking or heating of food

- All potentially hazardous foods (e.g. meat, poultry, seafood must be cooked/heated to a safe internal temperature (avoid red/rare meat very common abroad) before consumption.
- Cook ground beef and pork to 68°C for at least 60 seconds; this may be adequate where proper sanitary/hygienic practices for handling animals are regulated/enforced.

- Do not rely on the colour of the food but use a food thermometer to check the temperature.

11.5 Inadequate reheating

Reheating leftovers and refrigerated foods to improper/inadequate temperature is also a major cause of food borne illness. Often, leftovers and refrigerated foods are just “warmed up” rather than heated thoroughly.

Therefore, always reheat such foods RAPIDLY to 74°C before serving or hot holding but if it is liquid, bring it to boil.

11.6 Obtaining food from unsafe sources

In all food establishments, all food received must be from approved and inspected sources. Food processed at private homes may not be offered for sale to the public (please, note carefully because this is a common practice in the country and a major source of food poisoning).

11.7 Time lapse between food preparation and consumption

- As earlier discussed, given sufficient time, bacteria in food can grow depending on the type of food, the temperature of storage, its moisture content, its acidity and other factors. Therefore, such foods must be properly cooked, cooled to proper temperatures and stored at 5°C or below.
- Do not forget to reheat all leftover foods to 74°C rapidly.

Adapted by the author from several sources: Efiuvwevwere and Amadi, 1992; Efiuvwevwere and Akoma, 1997b; Jay, 1996; Ray and Bhunia, 2008; www.houstontx.gov/health/food/FOODBORNEILLNESS.html;

12.0 NEW FOOD SAFETY GLOBAL CONCERNS

Recently, the issues of food safety have become serious national and international concerns especially involving:

(1) Genetically modified foods (GMFs) and (2) Emerging food-borne pathogens.

12.1 GENETICALLY MODIFIED FOODS

Briefly, genetically modified foods (GMFs) are foods derived from animals and plants in which certain genes for particular desired characteristics are added to the organism's deoxyribonucleic acid (DNA), the hereditary material. Consequently, the animal or plant grows and develops to express the proteins of the inserted genes thereby, leading to changes in the organism's molecular structure, biochemistry, physiology, anatomy and shape with the net-effect of creating a new living organism/entity very different from the original organism (Osuji, 2012; Pattron, 2006).

However, the safety of GMFs is being questioned by scientists, researchers, medical doctors/health professionals and is a major challenge facing the food industry in the 21st century worldwide (Pattron, 2006) because it is believed that these foods have potential of posing serious public health risks, especially for the young, elderly, pregnant women and immune-compromised (HIV/AIDS) persons (Pattron, 2006). While it is argued that the products of this new genetic engineering result in cheap, nutritious foods and solve the problems of hunger and food security, there is little reputable scientific evidence to clearly show that GMFs are safe for human consumption and health (The Pew Institute of Food and Biotechnology, 2005; Pattron, 2006). It has been reported that consumption of GMFs results in allergies, increase in antibiotic bacterial resistance, diarrhoea, hormone imbalances, increase in susceptibility to colds and infections (Pattron, 2006). Unfortunately, the current food standards and regulations in many countries do not emphasise the demerits and the safety implications of genetically modified foods. For example, to worsen the serious concerns of public health food safety, the United States Food and Drug Administration (USFDA) does not require mandatory labelling of GMFs (Pattron, 2006). In addition, USFDA only requests that firms/companies conduct their own tests on new GMFs but makes no review of such tests unless voluntarily requested by the company (The Pew Institute on Food and Biotechnology, 2005). The food safety implications are therefore worrisome since consumers cannot distinguish GMFs from non-GMFs without appropriate labels. It is

now known that GMFs are exported from many developed countries to developing countries without the knowledge of such countries or consumers (Patrons, 2006). This has compounded the food safety concern globally.

Interestingly, Nigeria signed and ratified an internationally binding biosafety protocol known as Cartagena Protocol on Biosafety in 2000 and 2002 which came into force on 11th September, 2003 addresses the safe transfer, handling and use of living modified organisms (LMOs) that may have adverse effects on conservation and sustainable utilisation of biodiversity, taking into account risk to human health (Babatunde, 2014). Unfortunately, many years (about 11) after signing the protocol, the debate as to passing the National Biosafety law is now before the National Assembly (Babatunde, 2014). Whereas globally, safety considerations are emphasised, the focus by the farmers (and perhaps, the law-makers) is the financial benefits of “moving from subsistence farming to commercial farming” (Babatunde, 2014).

12.2 EMERGING FOOD BORNE PATHOGENS

The epidemiology of food borne diseases is changing rapidly due to the emergence of “new” food borne pathogens. Emerging food borne pathogens are divided into three categories namely: 1) microorganisms that are totally new (e.g. human immunodeficiency virus, HIV), 2) microorganisms that were previously known but only recently identified as pathogens (e.g. *Helicobacter pylori*, causative/associated agent of peptic ulcer and gastritis and 3) microorganisms that are old/have long been known but have undergone changes [(e.g. antibiotic resistant microorganisms such as Methicillin-Resistant *Staphylococcus aureus*, MRSA)]. It was speculated some years ago that diarrhoeal diseases due to contaminated food and water as causes of death will decline worldwide. However, there is no evidence to support such downward trend particularly due to production of food increasingly by the developing countries for a global market (Newell *et al.* 2011). The issue of food safety is complex and multi-factorial involving food security, climate change, sustenance of food safety standards

and constantly monitoring such standards. Unfortunately, the behaviour of food pathogens relevant to food safety are not static (but dynamic) hence their new resurgence and the phenomenon of emerging pathogens.

Food is an excellent vehicle through which many pathogens can reach an appropriate colonisation site in a host. Interestingly, as food production practices change, well-known food-borne pathogens (e.g *Salmonella* spp..) also adapt and evolve to exploit novel opportunities such as minimally processed fruits and vegetables. Thus, previously unknown food-borne pathogens particularly *Campylobacter jejuni*, *Listeria monocytogenes* are constantly emerging as food borne pathogens (Griffiths, 2005; Newel *et al* 2011). It is therefore necessary to understand the multiple interactions that exist between these food pathogens and their food environments (Efiuvwevwere and Kets, 2000; Ezeama Efiuvwevwere, 2006, ICMSF, 1998) during transmission along the food chain (from farm to table) in order to develop effective preventive and control strategies for enhanced/improved food safety on national and global scale.

13.0 OUR MODEST CONTRIBUTIONS TO SCIENTIFIC KNOWLEDGE

It is evident from the lecture thus far that several mechanisms and phenomena are involved in the sub-discipline of food microbiology. However, the primary focus is to enhance food security in recognition of the geometric population growth especially in the developing world. Thus, our research activities over the years have focused on the **quest** to achieve adequate and safe food supply bearing in mind global considerations with emphasis on compliance with international standards.

Vice-Chancellor Sir, distinguished ladies and gentlemen, may I at this point, therefore present our (i.e. my associates and I) modest contributions to knowledge as follows:

13.1 Microbiological Assessment of Foods (Fresh and Processed) for Microbial Quality and Safety

13.1.1 Microbial Quality of Fresh Produce

Many fruits and vegetables are now consumed raw or following minimal processing. We therefore investigated the microbial quality of freshly harvested and market retailed cucumber. Fourteen bacterial flora were found in market- retailed cucumber as compared with only four from freshly harvested samples. Many food borne pathogens were observed in the market-retailed produce while only one was isolated from the freshly harvested cucumber (Lennox and Efiuvwevwere, 2012). Ten moulds were isolated from the market-retailed samples as compared with four from the freshly harvested crops.

Similarly, much higher microbial load was found in the market-retailed as compared with the freshly harvested cucumber. Therefore, much higher food safety risks are associated with the market-retailed cucumber and should be washed thoroughly before consumption.

13.1.2 Imported and Local Brands of Canned Tomato Paste: Microbial Quality and Safety

Occurrence of defective/swollen canned foods is wide-spread in the country and more worrisome is the sale of such products (i.e. non-removal from the shelf) to consumers with impunity. In our quest to address these safety concerns, we embarked on the investigation of the microbiological and pH (safety indicators) of defective and normal brands of imported and locally produced tomato paste (Figure 6).

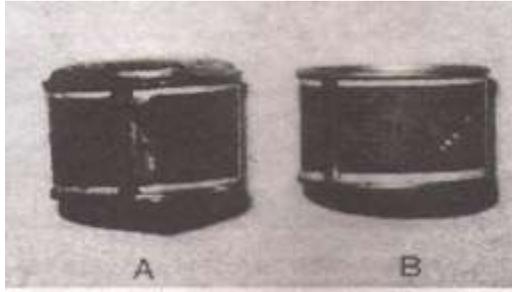


Figure 6. Defective (A) and normal (B) appearance of canned tomato paste

Significantly ($P \leq 0.05$) higher microbial populations and more microbial diversity were observed in the defective product but the lowest population was found in the local brand. Anaerobic populations were higher than the aerobic populations in both normal and defective cans with four dominant bacterial genera (*Bacillus*, *Clostridium*, *Lactobacillus* and *Leuconostoc*) occurring in the samples but the spore-formers dominated (Efiuvwevwere and Atirike, 1998).

Percentage occurrence of *Clostridium thermosaccharolyticum* and *Aspergillus fumigatus* were much higher in defective samples while the *Lactobacillus* spp were dominant in normal cans coupled with *Fusarium* spp and others. The pH values of the defective samples were much higher than those of the normal samples and they were above the critical safe level of 4.6. Overall, the imported brands showed more undesirable microbial quality and pH values, making them more potentially hazardous (being more associated with food poisoning). It is therefore evident that imported tomato paste is not microbiologically safer than locally produced. However, the conditions to which they were exposed/subjected prior to distribution/retailing in the country are important variables which may have affected their quality.

13.1.3 Bottled Soft drinks

Complaints by consumers concerning spoilage and questionable safety of manufactured soft drinks including the most popular brands are common. We therefore carried out research work on these two brands (based on “tracking” after production) held under two marketing conditions (ambient *ca* 28⁰C and open air *ca* 34⁰C) to investigate microbial and chemical changes during the two storage conditions. Our findings showed the occurrence of several types of microorganisms in the two brands. Much higher incidence (30%) of *Staphylococcus* spp (food-borne pathogen) occurred in brand B compared with 10% in brand A. The two brands were dominated by the bacterial group, *Bacillus* spp (food-borne pathogen and spore-former) and two moulds: *Aspergillus* spp (mycotoxigenic) and *Cladosporium* spp. (Efiuwewwere and Chinyere, 2001).

Exposure of the samples to the two conditions resulted in two-fold increase in *Lactobacillus* spp (acid- loving bacteria) in brand B on day 14 of storage. More types of microorganisms and higher populations were found in the brands after 14 days of storage especially in samples held in open air (Efiuwewwere and Chinyere, 2001). Among the other changes observed in both brands after 14 days included increase in pH and colour alteration as well as sedimentation due to *Saccharomyces*. These changes must have been induced by utilization of the preservatives (a de-acidification phenomenon) and photooxidation processes. Conclusively, consumption of these products is therefore not advised after 14 days of exposure to these two marketing conditions (but worse with 34⁰C exposure) investigated in this work. This study further confirms the concept of “commercial sterility” and the potential hazards associated with processed (canned/bottled) foods.

13.2 Use of Various Preservation Strategies

13.2.1 African breadfruit (*Treculia africana*) preserved with sodium chloride

This is very popular in the South Eastern part of the country and many of its products (ready to eat) are consumed without much

preparation, several yeasts and moulds (including these mycotoxins producing mycote) were isolated from these product and even those treated with high concentration (10 or 15%) of sodium chloride. Lower concentrations of 0 or 5% sodium chloride had no appreciable effect on the microbial quality but 10% or 15% drastically reduced the microbial hazards and safety risks (Nwaiwu and Efiuwewwere, 1995).

13.2.2 Use of Low temperature storage of tomato fruits

Temperature is considered the most important parameter that affects all living organisms positively or negatively. We have used *temperature* in a number of research work to clearly demonstrate its importance in food preservation. For instance, we established the critical temperature/time relationship (5°C/9days or 7°C/12 days) at which the phenomenon of chilling injury/low temperature breakdown of tomato fruits occurred (Figure 7) and results in tremendous economic loss (Efiuwewwere and Thorne, 1988). Also, we demonstrated the lower organic acid (citric, malic, oxalic etc) content of the



Figure 7. Tomato fruits showing pitted tissues

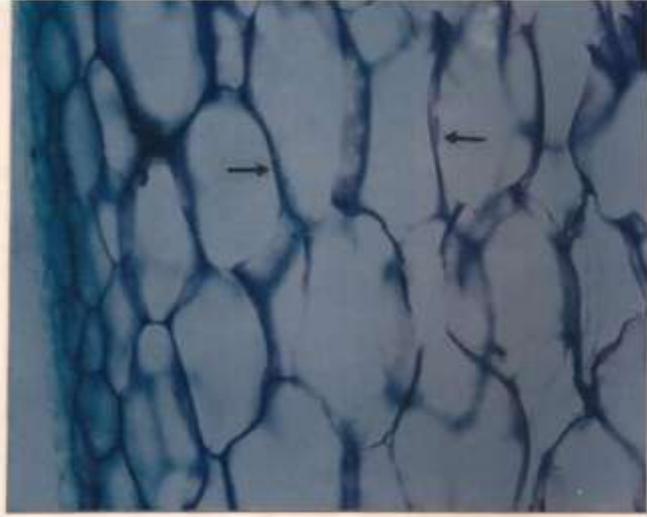


Figure 8a. Unpitted tomato tissues



Figures 8b. Photomicrograph of pitted tomato tissues



Figure 9a. Investigating/analysing the unpitted and the pitted extracted tissues for organic acids using High Performance Liquid Chromatography (HPLC)

chilled/pitted versus unpitted tissues of tomato fruits (Figures 8a and 8b) (using state-of-the-art equipment, High Performance Liquid Chromatography) (Thorne and Efiuvwevwe,1988) (Figures 9a and 9b). In addition, the physical distortions of the chill- damaged tissues were elucidated using photomicroscopy. All these changes facilitated the invasion and infiltration of the fruits by several microorganisms particularly mould (Figures 10a-10d).

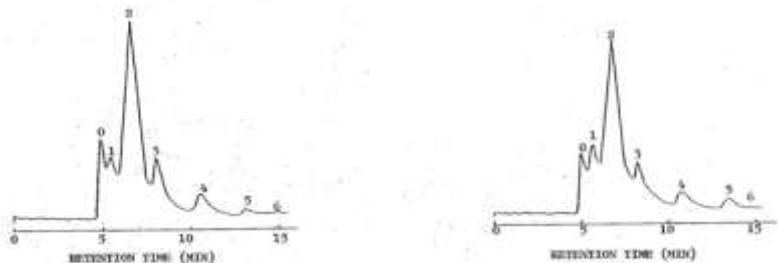


Figure 9b. HPLC chromatograms showing peaks of organic acids of unpitted and pitted tissues of tomato fruits.



Figure 10a. Photomicrograph of *Penicillium* spp



10b. Photomicrograph of *Aiternaria* spp

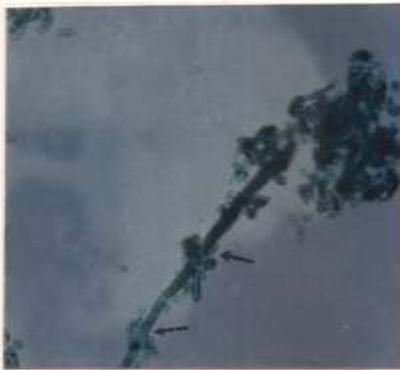


Figure 10c. Photomicrograph of *Aureobasidium* spp



Figure 10d. Photomicrograph of *Stemphylium* spp



Figure 11a. Mould infiltrated tomato tissue
(initial invasion/ cell death)

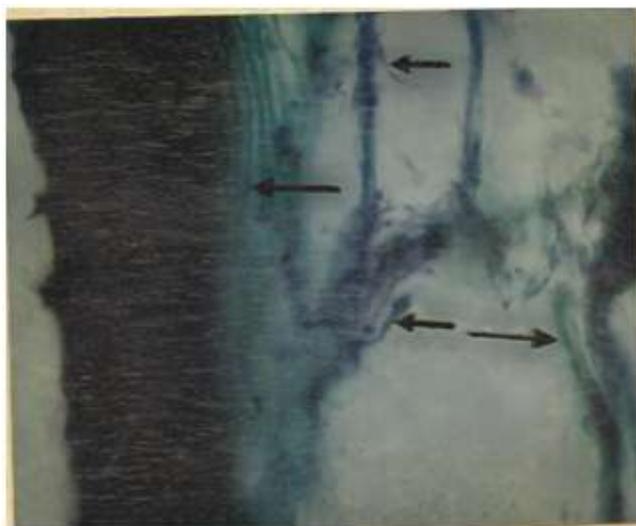


Figure 11b Extensive mould infiltration



Figure 11c Pronounced extensive damage /infiltration with several hyphae

The invasion by the moulds of the chill-damaged tissues clearly shows the susceptibility of such tissues (Figures 11a-11c).

These findings especially the correlation between the chemical and physical parameters as well as the microbial invasion are novel and several scientists and entrepreneurs were highly interested in the work (especially since tomato fruit is a commodity of global acceptance).

13.2.3 Varying storage temperature of tomato fruits and quality changes

Whereas relatively low temperature is desirable for storage of tomatoes/ other produce, regular power supply is a challenge in Nigeria and some other developing countries. This work was therefore undertaken to evaluate the effects of storing tomato fruits at 27°C continuously or at varying temperatures of 35°C, 18°C and 27°C for 3, 2, and 4 days respectively before inoculation with mould (*Alternaria solani*). We found decreased resistance in fruits stored at

varying temperature and significantly ($P \leq 0.05$) larger lesions occurred (Figure 12). This demonstrates the adverse effects of irregular (erratic) temperature of storage of fruits (Efiuvwevwere and Hobson, 1989).



Figure 12. Varying (irregular) storage temperature effect on tomato fruit susceptibility to mould infection

13.2.4 Preservation of Seafoods

Seafoods are special delicacies in the international market hence we focused on their preservation to ensure long distance distribution while maintaining their quality attributes for potential international trade.

13.2.4.1 “Ngolo” (*Thais califera*)

“Ngolo” *Thais califera*, an economically important seafood particularly in the Niger Delta region has potential for international trade. Yet, there is little or no scientific information on its very limited shelf-life, chemical and microbiological quality characteristics. In our quest to provide some answers regarding its storage-life and safety we then embarked on its preservation using

different temperatures ($29\pm 2^{\circ}\text{C}$; $4\pm 2^{\circ}\text{C}$ and $-15\pm 2^{\circ}\text{C}$) commonly employed in Nigeria and other developing countries.

The changes in the microbial populations clearly showed the benefits of low temperature usage. Whereas samples stored at ambient temperature attained the maximum of $\log_{10} 8.72\text{cfu/g}$ on day 3, those stored at $4\pm 2^{\circ}\text{C}$ showed initial decrease indicating cell death due to adaption to low temperature but eventually reached the peak of $\log_{10} 7.48\text{cfu/g}$ on the 12th week. In contrast, samples stored at $-15\pm 2^{\circ}\text{C}$ had $\log_{10} 2.12\text{cfu/g}$ on the 12th week of storage, suggesting their enhanced storage-life and safety (Efiuvwevwere and Oruwari, 2014) (Figure 13).

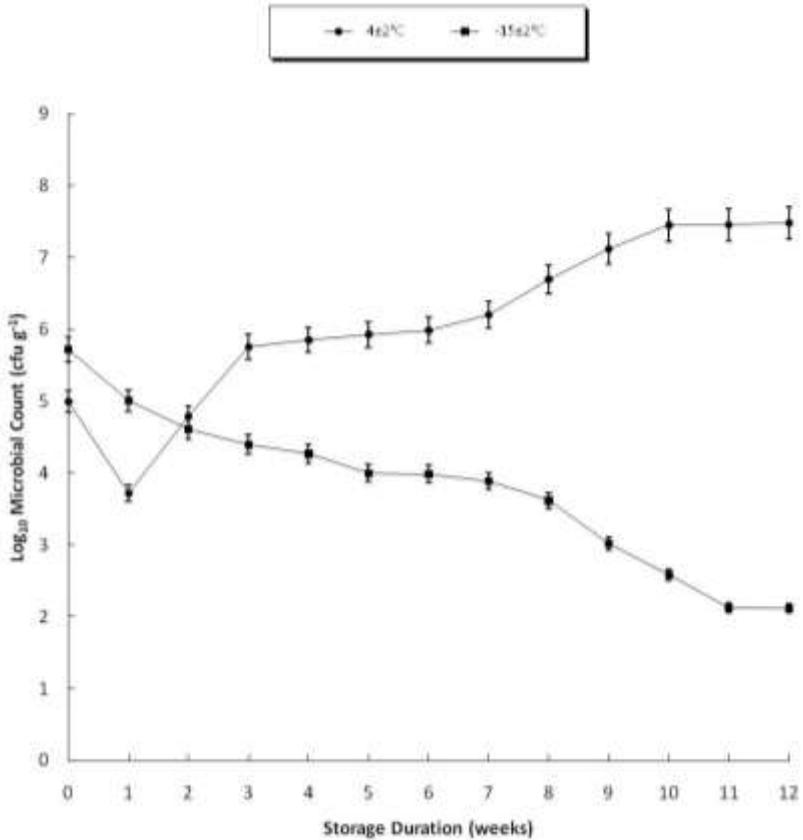


Figure 13. Changes in microbial population of “Ngolo” during storage at 4±2°C and -15±2°C.

Based on the three quality parameters (chemical, microbiological and organoleptic), samples stored at -15±2°C remained acceptable for 12 weeks while those stored at 4±2°C and 29±2°C were rejected by the 4th week and 1st day (data not shown) respectively.

Table 15: Microorganisms Isolated from “Ngolo” Samples During Storage at Different Temperatures

MOCRO ORGANISMS	29±2⁰C	4±2⁰C	-15±2⁰C
<i>Bacillus</i> spp	+	+	+
<i>Pseudomonas</i> spp	+	+	+
<i>Flavobacterium</i> spp	+	+	-
<i>Micrococcus</i> spp	+	+	+
<i>Staphylococcus</i> spp	+	+	-
<i>Vibrio</i> spp	+	-	-
<i>Aeromonas</i> spp	-	+	-
<i>Escherichia coli</i>	+	-	-

+ = Isolated
 - = Not Isolated

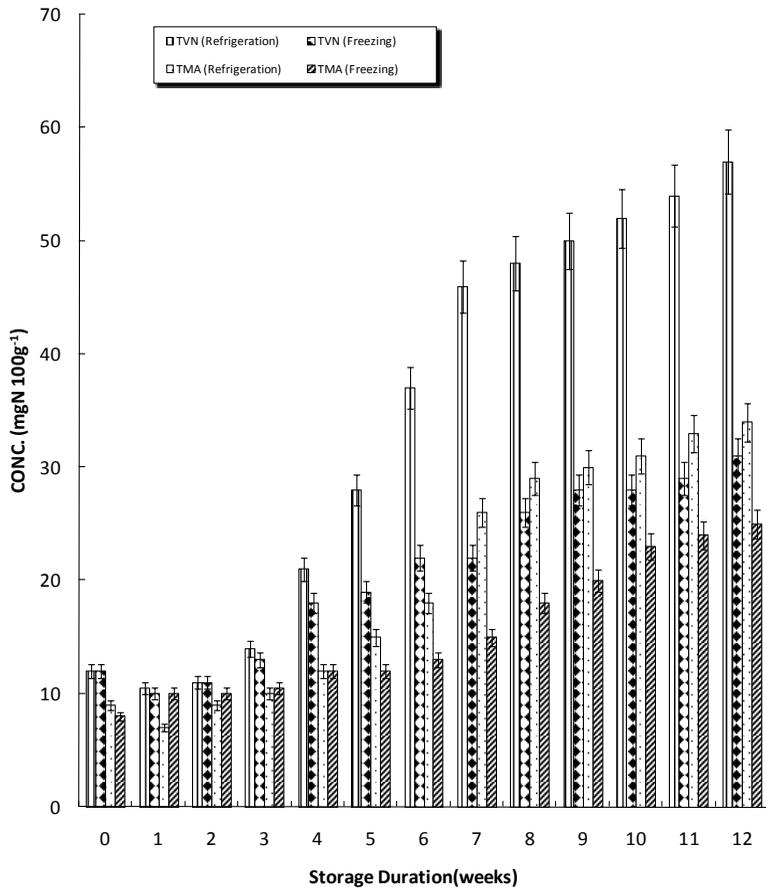


Figure 14. Changes in chemical indices of “Ngolo” during storage at $4 \pm 2^{\circ}\text{C}$ and $-15 \pm 2^{\circ}\text{C}$

Table 16: Changes in Sensory Quality of “Ngolo” Samples During Storage at $4\pm 2^{\circ}\text{C}$ and $-15\pm 2^{\circ}\text{C}$

TIME (Weeks)	TEMP/ORGANOLEPTIC INDICES							
	$(4\pm 2^{\circ}\text{C})$				$(-15\pm 2^{\circ}\text{C})$			
	Appearance	Taste	Flavour	Texture	Appearance	Taste	Flavour	Texture
0	7.3±0.12	7.8±0.07	6.9±0.04	7.2±0.05	7.3±0.03	7.6±0.03	6.9±0.09	7.2±0.04
3	6.7±0.09	5.8±0.12	5.9±0.06	6.1±0.07	7.3±0.01	7.2±0.03	6.8±0.04	6.9±0.03
6	4.3±0.04	3.9±0.04	3.6±0.08	3.6±0.09	7.1±0.04	7.0±0.06	6.4±0.06	6.7±0.03
9	3.7±0.06	3.3±0.06	3.4±0.05	3.2±0.03	6.9±0.03	6.7±0.06	6.1±0.05	6.4±0.02
12	3.0±0.08	2.9±0.09	2.4±0.05	3.1±0.08	6.7±0.09	6.5±0.05	5.8±0.07	6.1±0.05

Each value represents the mean of four determinations

Each value represents the mean \pm standard error

Thus, samples stored at $4\pm 2^{\circ}\text{C}$ can be distributed within a short distance while those stored at $-15\pm 2^{\circ}\text{C}$ can be transported for weeks without deterioration in their quality.

13.2.5 Preservation of oyster using combination of low temperature and preservative

The search for shelf-life extension of oysters (a well-known shellfish for local and international trade) prompted our interest to study the

effects of its storage at low temperature of 5°C in combination with 0.2% potassium sorbate (KS) (an approved preservative). Initial microbial composition of Gram-positive to Gram-negative ratio of 51: 49 occurred in freshly shucked samples. But changed to a reverse ratio of 22:78 (i.e. Gram-positive to Gram-negative and 80:20 (i.e. Gram-positive to Gram-negative) in 0.2% KS treated samples stored at 5°C and 29°C respectively with *Pseudomonas* spp dominating (65%) at 5°C while *Bacillus* spp (40%) and *Micrococcus* spp. dominated at 29°C. Significant ($P \leq 0.05$) correlations were observed between total microbial population and faecal coliforms (sanitary indicators) in samples stored at 5°C but no significant relationship was found in samples stored at 29°C. Thus, the use of correlation studies/analyses with respect to groups of microorganisms to predict the presence or absence or group of microbial quality indicators is complex and related to other variables such as temperature of storage and preservative treatment of the samples (Edberg and Smith, 1989; Efiuvwevwere and Izakpa, 2000). The combined use of low temperature with the preservative enhanced the shelf-life by 4 days as compared with rejection of samples after 36 hours of storage at 29°C. This clearly demonstrates the commercial benefits of combination treatments using low temperature with preservative on oysters (Efiuvwevwere and Izakpa, 2000).

14.2.5.1 Further preservation of oysters to enhance shelf-life

Enhancement of the shelf-life of oysters is critical for national and international seafood trade. The **quest** for such enhancement led us to investigate subjecting freshly harvested and shucked oysters to several preservative treatments: sodium benzoate (NaB), sodium chloride (NaCl), potassium aluminum sulphate (PAS) and green lime juice filtrate (LJF) before storage at room temperature. Control samples (i.e not treated with preservatives) showed nine bacterial genera but only five were observed in PAS-treated samples (Efiuvwevwere and Amadi, 2014). The bacterial populations of all samples increased with storage time with control showing \log_{10} 8.30 cfu/g by day 2 compared with significantly lower population of \log_{10} 4.50 cfu/g found in PAS-preserved samples. Also, PAS- and

NaCl-preserved samples showed significantly lowest pH. Of all the treatments, the PAS-preserved samples showed the best bacteriological and sensory qualities as well as most extended shelf-life of 3 days which was followed by lime juice preserved samples. These findings are therefore remarkable contributions since PAS (Alum) has not been previously investigated for this purpose. This is novel because it also reduced the trimethylamine (TMA) content (a seafood spoilage indicator) which coincided with the low bacterial population. Thus, PAS (Alum) is highly recommended for shelf-life extension of oysters to improve its commercial potential but the concentration of usage should be monitored (Figure 15) (Efiuvwevwere and Amadi, 2015).



Processed (untreated/control) oysters on day 0



i. Sample A (untreated) Control day 3
Oysters: opaque, soggy and off- odour



ii. Sample B (KS treated) day 3
Oysters: pale, soggy and off-odour



iii. Sample D (Lime JF) treated day 3
Oysters: pale, soft and off-odour



iv. Sample F (alum treated) day 3
Oysters: slightly pale and wholesome

Figure 15. The PAS- preserved oyster samples showed extended shelf life

13.2.6 Combination of pasteurisation with preservatives to extend shelf-life of beverages

13.2.6.1 Kunun- zaki

Production of Kunun-zaki (a popular fermented beverage) is widespread among small scale entrepreneurs in the country. However, its shelf-life is usually about 24 hours and therefore a major limitation with respect to its safety and commercial potential. We therefore carried out an investigation to extend the shelf-life using combination of pasteurisation and preservatives. Through our research work we found that pasteurization in combination with preservative (sodium benzoate), resulted in a sharp reduction in microbial population and enhanced the shelf-life by 4 days (Efiuvwevwere and Akoma, 1997a). This improved the quality and commercialization potential of kunun-zaki compared with samples subjected to pasteurization alone which had shelf-life of only two days while those not pasteurized had remarkably high microbial population of about \log_{10} 6.64 cfu/ml which is above the recommended microbial load for beverages and liquid foods and became unacceptable within 24 hours. Many “brewers” of kunun-zaki have benefitted from these findings.

13.2.6.2 Orange Juice

Similarly, orange juice extracted from variously packaged oranges using polyethylene bags or paper cartons showed differential microbiological qualities after pasteurization and addition of preservative (potassium sorbate, PS). Our findings showed that the orange juice pasteurized and treated with preservative had significantly low (≤ 30 cfu/ml) lactic acid bacteria (LAB) but they increased dramatically to \log_{10} 8.60 cfu/ml in unpasteurized samples after 15 days of ambient temperature storage and showed haziness/spoilage. (Efiuvwevwere and Oyelade, 1991). Through this work, we showed that the previous history of a raw food material (i.e. in this case, packaging) plays a critical role in the quality of the final product but pasteurization in combination with PS was most effective and is recommended for use in fruit juice preservation (Efiuwevwere and Oyelade, 1991).

13.2.6.3 Tomato juice: metabiosis and food safety

Use of high temperature treatment destroys many microorganisms in foods while some others survive since they are thermo-resistant. In addition, post-process contamination in the food industry is a major concern. In order to demonstrate the safety risks associated with processed tomato juice, the effects of pasteurisation, acidification and post-process contamination (i.e. deliberate inoculation as a “challenge test”) using *Alternaria* spp (a common contaminant of tomatoes) before storage of the samples at room temperature (29°C) for investigation. Through our work (Efiuwewere and Eka, 1991), we demonstrated that the phenomenon known as *metabiosis* in which moulds utilise acidic components in acid food products and raise the pH beyond the critical 4.6 level resulting in a more favourable condition for growth of microorganisms to hazardous levels (Figure 17a). In addition, the acidified inoculated sample showed a “separation” due to the mould growth (Figure 17b). Consequently, the product was made extremely unsafe for consumption (Efiuwewere and Eka, 1991). Our findings have generated considerable interest and awareness especially in the food industry and underscore the need to adhere to Good Manufacturing Practices/HACCP approach to avoid post-process contamination in spite of acidification.

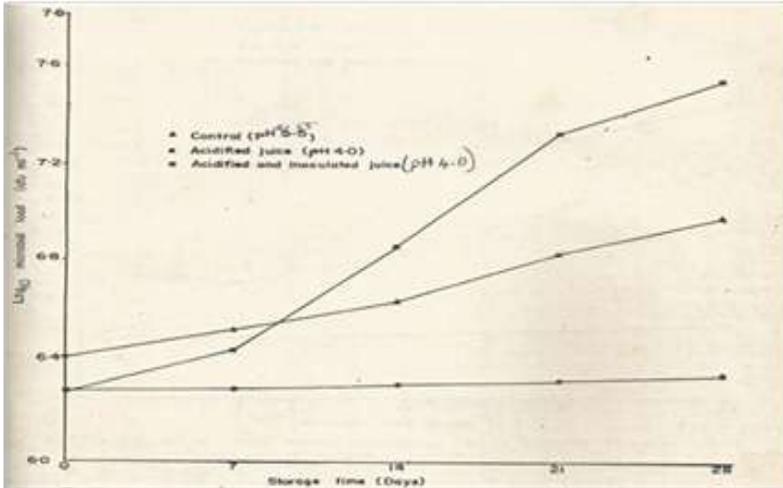


Figure 17a. Changes in microbial populations of tomato juice as affected by acidification and inoculation with mould (*Alternaria* spp)

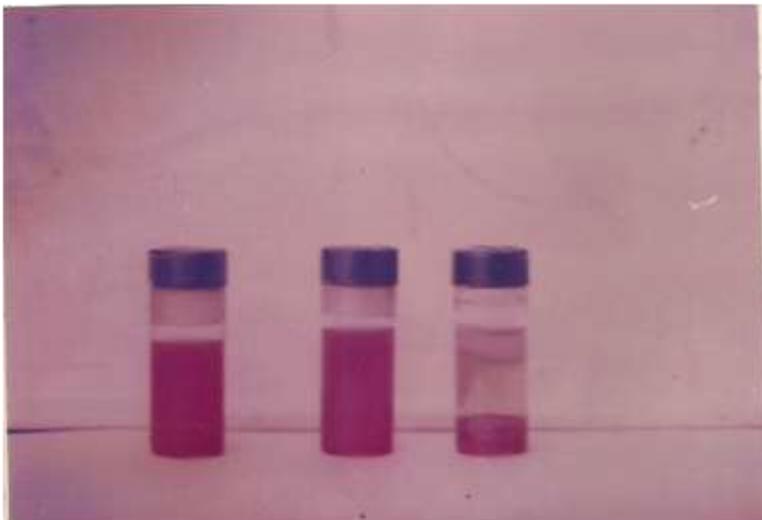
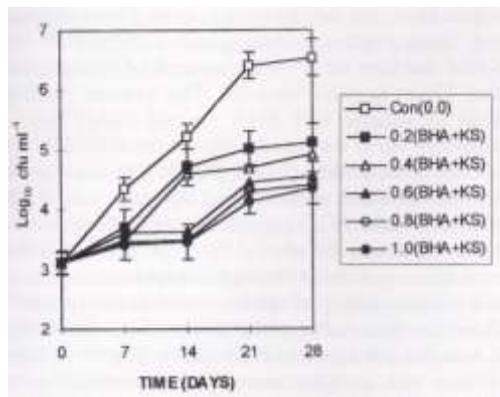


Figure 17b. Bottled tomato juice after 28days of storage (L to R) control; Acidified (pH 4.0); Acidified (pH 4.0)/inoculated

13.2.7 Use of Sterilization in combination with preservatives and inoculation (challenge tests)

13.2.7.1 Palm oil

Palm oil is the second (next to soybean oil) most important edible oil in global trade yet, its deteriorative changes have received very limited attention. Our quest was to evaluate the effects of antioxidant in combination with a known broad-spectrum antimicrobial preservative (potassium sorbate, PS). We therefore evaluated the effects of the antioxidant, butylated hydroxyanisole (BHA) alone or in combination with the preservative (PS) following mixed-inoculation with two microorganisms (*Aspergillus flavus* and *Bacillus spp.*, mould and bacteria) of major concern in palm oil as “challenge test”. We found that bi-phasic minima-extended-lag-phases occurred in the samples treated with BHA + KS as compared with the remarkable high microbial load and mycelial weight in samples treated with BHA alone or sterilized only (Figure 16) (Efiuvwevwere and Efi, 1999). Our findings underscored the importance of synergism (i.e. BHA and KS effects) resulting in improved microbial quality and other attributes (chemical and sensory). These findings are of considerable benefits to palm oil processors and exporters as well as the regulatory agencies.



Figures 18. The microbial populations of palm oil as affected by combination treatments and inoculation

13.2.7.2 Post- Process Contamination of Sterilised Soymilk

Soymilk is one of the world's most popular soybean products. However, it is exposed to a wide range of microorganisms. Production of soymilk by many local food processors is thriving yet, there is little or no scientific information on the growth behaviour of microorganisms that contaminate this product. Since microorganisms co-exist in nature (including food systems), this study therefore investigated the induced changes in sterilized soymilk deliberately inoculated/contaminated with single culture of *Aspergillus flavus* (AF) or Enteropathogenic *Escherichia coli* (EEC) or *Staphylococcus aureus* (SA) or mixed cultures of AF + EEC or AF+ SA or SA + EEC or AF + EEC + SA and uninoculated control. We found little or no change in the microbial population within the first 18hr but significant increase occurred after 36hours particularly in soymilk samples inoculated with mixed cultures containing *Aspergillus flavus* (Efiuvwevwere and Nwanebu, 1998). This observation demonstrated the concept of metabiosis since the pH was elevated and food-borne pathogens (*Staphylococcus aureus* and Enteropathogenic *E. coli*) proliferated to high populations of log₁₀ 8-12 cfu/ml except the controls and those inoculated with *Staphylococcus aureus* alone or *Staphylococcus aureus* + EEC making them hazardous for consumption. However, all the samples became unacceptable after 48 hours except the controls (uninoculated) and those inoculated with *Staphylococcus aureus* alone.

These findings highlight the danger of using only one quality criterion because the samples inoculated with *Staphylococcus aureus* alone were accepted using sensory/organoleptic scores after 48 hours but were unacceptable microbiologically. This study has demonstrated the importance of microbiological control using GMPs and HACCP concept to prevent post-process contamination in milk processing operations.

13.2.7.3 Post-Process Contamination of Sterilised Fresh Water Snail

Fresh water snails are popular seafoods in some localities in the Niger Delta region. However, they are often contaminated with

pathogens and their shelf-life is about 24 hours. We therefore embarked on measures to enhance the shelf-life and safety of this delicacy. Comparison between the impact of readily available local “preservative” (lime juice, LJ) versus a well known broad-spectrum preservative (potassium sorbate) on the growth behaviour of food-borne pathogens (*Salmonella enteritidis* and *Listeria monocytogenes*) inoculated into fresh water snail (*Pila ovata*) meat after sterilisation was investigated. Samples treated with LJ and inoculated with *Salmonella enteritidis* and stored under refrigeration showed decrease in the pathogen population while increase occurred in ambient stored samples. Increase of about 2.5 log cfu/g was found in samples inoculated with the mixed cultures of two organisms under ambient storage but no changes in the population under refrigeration. Treatment with LJ had significant reduction in *Salmonella* population under refrigeration storage but treatment with KS showed negligible inhibitory impact. Conversely, the population of LM increased to a hazardous level of log₁₀ 8.12 cfu/g irrespective of KS treatment and refrigeration storage. These growth dynamics displayed by the pathogens indicated potential safety risks to consumers of freshwater snail when contaminated with these food-borne pathogens and not properly processed before consumption (Ezeama and Efiuvwevwere, 2006). Use of lime juice in combination with low temperature was more effective in control of these pathogens in fresh water snail and is therefore recommended for its preservation.

13.2.8 Preservation of Fish Using Combination Treatments

13.2.8.1 Combination of Preservative with Smoking on Microbial Profiles of Fish Tissue Types

Several complaints received from smoked fish retailers indicated that smoked fish spoilage is not uniform. In addition, the quest to establish if there is microbial variation with the tissue types especially with recognition to the African tradition which dictates that the head of the fish be reserved for the head of the family became a research interest. We therefore studied the effects of smoking and potassium sorbate treatment on the microbiological and

physico-chemical quality of the flesh, the gill or head of mullet (*Mugil cephalus*), a common affordable fish . We found that the microbial load was significantly higher in the head than in either flesh or gills of the fresh fish (Efiuvwevwere and Iweanoge, 1991). In contrast, the highest microbial population was observed in the flesh of the potassium sorbate-treated and in untreated smoked fish but the gills had the lowest population.

The pH initially increased but decreased sharply after final smoking (being a two- stage smoking process; initial and final). Moisture content decreased drastically with smoking but the gills of potassium sorbate treated or untreated had the highest moisture content and this explains why onset of spoilage originated from the gills as from day 6 of ambient storage (Efiuvwevwere and Iweanoge, 1991). Whereas the tradition of reserving the head of the fish with reverence for the head of the family may be commendable, microbiologically, the head is the most hazardous. Overall, these findings are highly beneficial to the smoked fish cottage industry and the regulatory agencies.

13.2.8.2 Smoking of fish with combination of preservatives

Extension of the shelf-life of perishables (especially seafoods) is a major research interest globally. Smoking is a common preservation method but its benefits are inadequate and usually inconsistent. Based on complaints from smoked fish processors/retailers, it became necessary to investigate the effects of smoking alone or in combination with use of different concentrations of preservatives (sodium berizoate or potassium sorbate) on the microbial quality, physico-chemical parameters and shelf-stability of catfish (*Clarias gariepinus*), a common and popular fish.

We found that the Gram-negative bacteria were eliminated by the smoking resulting in the dominance of the smoked samples by Gram-positive bacteria (*Bacillus*, *Staphylococcus* and *Streptococcus*) and spoilage moulds (*Aspergillus flavus*, *Penicillium verrucosum* and *Achlya* spp.). The moulds became evident (Figure 18a). However, packaging the smoked fish in polyethylene bags (Figure

18b) prevented mould spoilage for some days. Smoking alone reduced the microbial populations but combination of smoking with 0.4% w/v potassium sorbate was most effective in reducing the microbial loads, moisture content and enhanced the shelf-life by 8 days at ambient temperature storage (Efiuvwevwere and Ajiboye, 1996).

However, in general, the effects were transient since the microbial populations increased to and above populations that occurred prior to smoking which are higher than the recommended limits of $\log_{10} 7$ cfu/g (ICMSF, 1986).



Figure 18a Smoked fish spoilage by moulds



Figure 18b. Absence of mould spoilage in polyethylene packaged smoked fish

The public health implication of this work is that smoked fish are consumed in the country with little or no further cooking/processing yet, these are high-risk category of foods (ICMSF, 1986). Whereas the benefits of smoking and addition of preservatives have been shown, the effectiveness is dependent upon the type of preservatives, the concentration and other variables such as duration of smoking.

13.2.8.3 COMBINATION OF THREE PRESERVATION STRATEGIES

In order to further enhance the shelf-stability of sea-foods, the **quest** for additional strategies was embarked upon and this involved hygienic handling in combination with preservative (potassium sorbate, KS and smoking (a multi-component approach). This work investigated the effects of de-contamination (i.e. use of sodium hypochlorite to decontaminate containers for handling the fish samples), dipping of the samples (Croaker, *Micropogonia furnieri*) in potassium sorbate for 30 or 60 seconds in 3% w/v prior to smoking. Our findings showed more bacteria (12) and moulds (5) occurring in unhygienically handled control samples without preservatives but 6 bacteria and moulds (3) types occurred in hygienically handled samples. Gram-positive bacterial flora consisting of *Staphylococcus*, *Bacillus*, *Clostridium*, *Lactobacillus*

and *Streptococcus* dominated the smoked and preservative- treated samples. However, moulds (*Aspergillus niger*, *Penicillium* spp and *Rhizopus stolonifer*) constituted the major spoilage microorganisms. Significant reduction in microbial load occurred in all samples after smoking but much more reduction in samples held in decontaminated containers occurred and coliform bacteria (sanitary indicators which are more heat-sensitive) were undetected on the 4th day in samples handled hygienically and dipped in KS for 60 seconds (Efiuvwevwere and Isaiah, 1998). The combination of hygienic handling with extended dipping time (60 seconds) and smoking resulted in maximum positive impact on the microbial quality and shelf-life extension of 8 days. Again, this showed the synergistic effects of combination treatments [preservative (KS), extended treatment time (60 seconds) and smoking]. However, the increase in microbial population with storage time is of public health concern and confirms the need to reheat such high-risk products before consumption and at the same being aware of the associated mycotoxigenic moulds) (Efiuvwevwere and Ajiboye, 1996; Efiuvwevwere and Isaiah, 1998).

13.2.8.4 Combination treatments (“Hurdle technology”)

The **quest** for combination treatment (“hurdle technology” to inactivate microbial growth in food ecosystems is a global research interest. We therefore investigated the growth responses of a starter culture (very useful in the food fermentation industry) and a food-borne pathogen, *Listeria monocytogenes* subjected to various pH conditions (acid and alkaline): 4.5, 5.5, 7.0 and 8.0; temperature of 28°C, 38°C or 37°C or combination of hydrostatic pressure/temperature/time of 25 bars/25 °C/63h; 50 bars/30 °C/63h; 150 bars/20 °C/15h or 150bars/30 °C/63h following these exposures. Whereas pH 4.5 medium completely inhibited the growth of both organisms, maximum growth response was exhibited by *L. lactis* and *L. monocytogenes* in pH 7.0 medium. However, the growth of and *L. monocytogenes* was much lower than that of *L. lactis* at all the pH value (media). Following initial lag (adaptive) period of 2.3h, marked growth was displayed by *Ll* and *Lm* at 30 °C and 37 °C respectively. But growth of *Ll* was particularly inhibited at 37°C.

Combination of low hydrostatic pressure of 25bars/ 25 °C/63h showed no microbial inactivation resulting in \log_{10} p.11 efu/ml but samples subjected to hydrostatic pressure of 50 or 150bars combined with 30°C and 63h exposure resulted in total microbial inhibition which was similar to sterilization of food (Efiuvwevwere and Kets, 2000).

13.2.8.9 FERMENTATION PROCESSES OF DIFFERENT FOODS: MICROBIAL DYNAMICS AND FOOD SAFETY

13.2.8.9.1 Fermented beverage (Kunun - zaki)

Kunun-zaki was not given serious research attention until about thirty years ago in spite of its popularity especially in the Northern part of the country. Most research work was on its chemical composition and nutritional quality. Our curiosity and quest concerning the quality changes associated with kunun-zaki prompted our research into the dynamics of microbial succession during its fermentation. We found that a wide range of microorganisms occurred after the steeping (soaking of the millet) for 24hours but the moulds became eliminated and the ratio of 1:1 (Gram- negative bacteria to Gram –positive bacteria) emerged. By the end of fermentation (8 hours), all the Gram-negative bacteria were eliminated and lactic acid bacteria predominated but *Saccharomyces cerevisiae* became evident due to increased acidity (Efiuvwevwere and Akoma, 1995). A positive correlation ($r=0.81$) was found between the acidity and the lactobacilli population. This work reflects the microbial ecology of food ecosystems particularly in fermentation processes. The significantly high lactic acid bacteria population (\log_{10} 6.98 cfu/ml) and the high acidity may have provided some assurance of microbiological safety of the product. These findings have become very useful to “brewers” of kunun-zaki with respect to selection of raw materials and hygienic measures during the fermentation process. HACCP approach is highly recommended for improved quality and safety.

13.2. 8.9.2 A Nigerian fermented rice product (rice ‘masa’)

Several fermented food products are available in the country and elsewhere but the quest for ‘new’ fermented products is of major research interest especially rice and its products. Therefore, we embarked on the investigation of the influence of fermentation time, use of indigenous tenderizer (“kanwa”) and potassium sorbate on the microbial profile, chemical attributes and shelf-life of rice ‘masa’, (comparable product available in China, India, Sudan and Indonesia). Our findings showed that various types of microorganisms (bacteria and fungi) were present at the initial stages of rice slurry fermentation but beyond 8 hour fermentation time, only *Lactobacillus* spp, *Saccharomyces* spp and *Rhizopus* spp were present. The sour-sweet spongy characteristic of the product was attributed to the dominance by LAB and the yeast (*Saccharomyces* spp) (Efiuvwevwere and Ezeama, 1996). After fermentation and baking process, the product became dominated and spoilt by bacterial spores former (*Bacillus* spp), Lactic acid bacteria and *Saccharomyces* spp with storage time. Use of “kanwa” improved aroma but was not beneficial to visual appearance and spoilage was accelerated due to induced higher pH but addition of potassium sorbate resulted in reduced microbial load and prolonged shelf- life of the product by 3 days. In addition to the improvement on the shelf life of the product, inclusion of this product in the world data base of fermented products is of considerable significance.

13.2.8.9.3 Fufu Production: Traditional Versus Modern Method Of Fermentation And Safety Implications

As a result of the relative popularity of fufu in the country, many fufu producers are eager to make quick financial gains through the introduction of a new faster fermentation method. We therefore carried out comparative investigation on the quality attributes of the product (fufu) produced using the respective traditional and modern method. We found higher microbial loads (\log_{10} 10.04cfu/g) compared with \log_{10} 8.38 cfu/g in cassava mash from “modern” and traditional method respectively but lactic acid bacteria counts increased until day 4 of fermentation using both methods while sanitary indicators (coliforms) became non-detectable as from day 3

of fermentation in both methods (Efiuvwerwere and Orelesi, 2014). However, fufu produced using traditional method had better organoleptic (taste, aroma except texture) attributes, lower hydrogen cyanide (HCN) (poisonous substance) content as well as lower total viable (microbial) counts (Table 13 and Figure 19).

Table 17: Comparison of Changes in Physiochemical Parameters of Cassava Mash During Fermentation of Cassava for Fufu Production Using Traditional and “Modern” Methods (Control)

Fermentation Time (hours)	Traditional method				Modern method			
	pH		Moisture Content (%)	Titratable Acidity. (% lactic acid)	pH		Moisture Content (%)	Titratable Acidity. (% lactic acid)
	Cassava Mash	Stepping water			Cassava mash	Stepping water		
0	6.14 ±0.01		54.50±1.50	0.0565 ±2.34 x 10 ³	6.12±0.01		53.50±4.50	0.0560 ±3.14 x 10 ³
24	5.87 ±0.01	4.19±0.01	65.50±1.25	0.0642 ±4.68 x 10 ³	5.82±0.01	4.16±0.01	64.00±2.75	0.0656 ± 3.60
48	4.00±0.00	3.79±0.01	57.25±1.52	0.0758 ±2.55 x 10 ³	3.98±0.01	3.76±0.01	56.50±1.75	0.0708 ±0.00
72	3.78±0.01	3.69±0.01	62.20±1.00	0.1260 ±3.60 x 10 ³	3.55±0.00	ND	59.50±1.25	0.2818 ±3.6 x 10 ³
96	3.60±0.01	3.51±0.01	66.50±0.00	0.1522 ±3.60 x 10 ³	3.43±0.01	ND	67.30±0.75	0.2946 ±2.55 x 10 ³
120	3.52±0.01		56.50±0.00	0.0920 ±3.60 x 10 ³	3.40±0.01	ND	51.50±1.50	0.148 ±3.6 x 10 ³

Mean ± Standard deviation; ND = Not determined

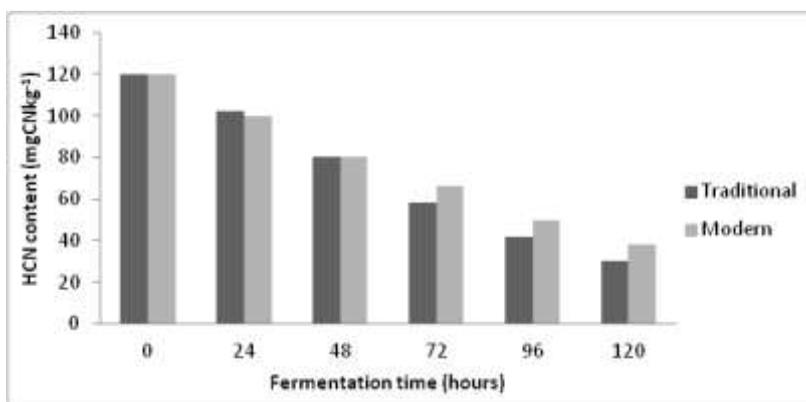


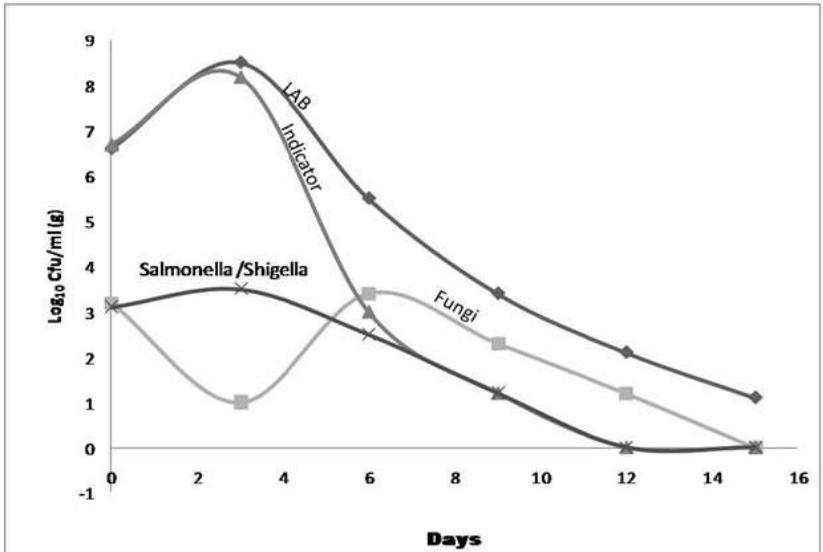
Figure 19. Changes in hydrogen cyanide content during fermentation of cassava for fufu production using traditional and “modern” methods

This work has therefore shown that microbiologically and chemically (HCN), fufu produced using the traditional method is relatively safer. Therefore, the financial benefits associated with the non-continuous fermentation regime (2 days before grinding and 2 days after) of “modern” fermentation method is not worth the health benefits derivable from the traditional method. Thus, the traditional method should be the method of choice until there is further improvement on the fermentation process based on the modern method.

13.2.8.9.4 Fermentation of cucumber (pickles)

Fermentation of food is aimed at obtaining desirable products with unique/special quality attributes. However, in the developing countries, spontaneous/ natural fermentation processes are used due to unavailability of culture collection centres and other facilities. The quest for novel fermented food products and improvement on existing products led us to these investigations.

Cucumber (*Cucumis sativus*) is widely consumed but highly perishable. The need to have cucumber converted to a different product of special quality and improved shelf-life led to this work. We investigated the microbial dynamics of cucumber (*Cucumis sativus*) during fermentation using 10% (w/v) sodium chloride solution. We found that initially both desirable and undesirable bacteria (including sanitary indicators and *Salmonella-Shigella*) were present in high populations. However, lactic acid bacteria became more dominant and the pathogenic microorganisms as well as other contaminants became eliminated with sharp decrease in pH as from day 6 of fermentation while the fungi and *Salmonella – Shigella* spp were totally inhibited as from 12 day of the fermentation process (Lennox and Efiuvwevwere, 2013). This work showed the interactions and competitive nature of the initial diverse microbial flora during the fermentation process and the subsequent dominance by LAB resulting in enhanced shelf-life and safety of the product (pickles) (Figure 20) .



Figures 20. Microbial dynamics during fermentation of cucumber

Vice-Chancellor Sir, I wish to state that these microbial dynamics/successions in foods may be likened to a sport arena for contestants but in this context, “food as an arena for display of microbial supremacy where the most endowed conquer the others and become the dominant microorganism, the “superstar” and confers its characteristics on the food”.

13.2.8.9.5 Fermentation of garden egg (*Solanum melongena*)

Traditionally, cucumber has always been the raw material for pickling but recently other fruits and vegetables have been evaluated as pickles such as pears, beets, peaches (Lee and Kang, 2004) to create more variety of fermented foods. We therefore investigated the fermentation of garden egg samples obtained from two sources (direct from the farm and market-retailed) and subjected respectively to 7% (w/v) sodium chloride for 15 days. Market-retailed samples had more types of microorganisms (*E. aerogenes*,

Salmonella spp, *E.coli*, *Staphylococcus aureus*, *Lactobacillus* spp, *Pseudomonas* spp, *Shigella* spp., *Bacillus* spp and *Leuconostoc* spp.) than the freshly harvested samples which had only four types (*Bacillus* spp, *Leuconostoc* spp, *Pediococcus* spp and *Lactococcus* spp.). Irrespective of the source of the samples, the microbial succession pattern was similar showing marked decrease in the population of the sanitary indicators and pathogenic microorganisms with significant increase in the lactic acid bacteria load accompanied by lowering of the pH as the fermentation progressed especially as from the 12th day (Lennox and Efiuvwevwere, 2014). This work demonstrated that the fermentation process has made the product relatively safe (due to the high population of LAB and low pH) for consumption and is therefore a good biopreservation method (Lennox and Efiuvwevwere, 2014).

13.3 Novel method for preservation of starter culture (*Lactococcus lactis*)

Freeze-drying (lyophilisation) is the standard method for preservation of starter cultures (microorganisms used in food/dairy industries) which are kept in the culture collection centres in different parts of the world. Unfortunately, we do not have these important facilities for microbiological work virtually throughout the country. We therefore deemed it most appropriate to investigate the preservation of this commercially important starter culture (*Lactococcus lactis*) using a novel and inexpensive method and thereafter evaluated its viability and fermentative ability (Efiuvwevwere *et al.*, 1999). The microorganism was grown, harvested and subjected to vacuum desiccation/drying) using different protective agents (sucrose, trehalose, betaine or mannitol) at different a_w levels (0.12, 0.33, 0.43, 0.76, 0.97 or 1.00). Mannitol (sugar alcohol) was found to be most effective in preservation of the starter culture and its recovery. The fermentative ability (including acid production) was comparable to that of the control (i.e. freshly harvested and not preserved) except that the lag phase was slightly extended by 4hours (Efiuvwevwere *et al.* 1999). However, no growth or acidification of the starter culture or medium was observed for 12 hours for rehydrated cells dried in the absence of

mannitol (Fig. 21a and 21b). This work attracted much interest by the fermentation industries (particularly the dairy industry) and the patenting office but the interest waned due to irregularity in follow up and also, the fact that standard methods for starter culture preservation are readily available in developed countries (including The Netherlands).



A

B

Figure 21a. *Lactococcus lactis* (starter culture) preserved with (A) or without (B) mannitol

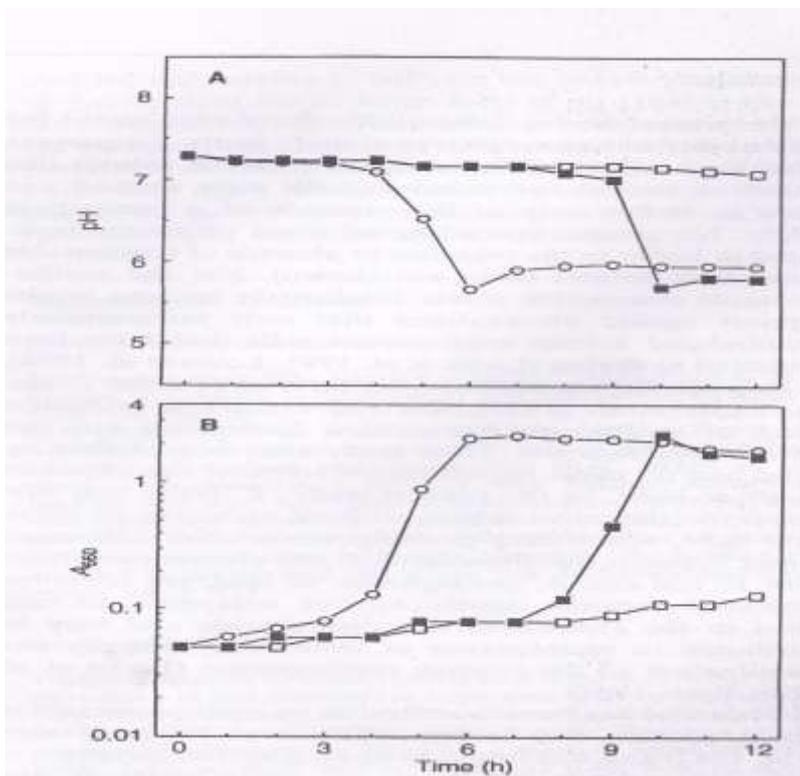


Figure 21b. Recovery and fermentative ability of *Lactococcus lactis* (starter culture) following preservation using mannitol

13.4 NON-FERMENTED FOOD PRODUCTS AND THEIR MICROBIAL SAFETY

13.4.1 Kwoka

Current trends globally show that new food products are being produced and marketed even without approval by the regulatory agencies. Therefore, the need to investigate first, a non-fermented maize product with a long history of production and popularity, known by various names in the south of the country as ‘Kwoka’ and ‘Ekusu’ in Delta and Edo States respectively; ‘Ngwugu-Ikpa’ or ‘Epiti’ in Rivers State, ‘Asuruasu-Oka’ and ‘Egbo’ in Anambra and Ogun States respectively. Our results showed that the

slurry (i.e mixed ground maize with condiments before steaming) and 30minutes steamed ‘Kwoka’ had more diverse bacteria and moulds than “Kwoka” steamed for 60minutes (with or without preservative, potassium sorbate). Among the microorganisms isolated from the raw materials and “Kwoka” were *Bacillus*, *Staphylococcus*, *Lactobacillus*, *Enterobacter*, *Klebsiella*, *Aspergillus* and *Fusarium*. However, heat sensitive bacteria (coliforms) and fungi were not detected on day 0 in KS- treated and 60minutes steamed “Kwoka”. Significant reduction in microbial populations to safe levels occurred following steaming (Efiuvwevwere and Amadi, 1992). However, sharp increase to hazardous levels was observed within 1day of ambient temperature storage. Control samples (i.e.. without KS treatment) became unacceptable after 1day but the samples produced using KS treatment and 60 min- steaming had extended shelf-life of 2 days (Figure 22).

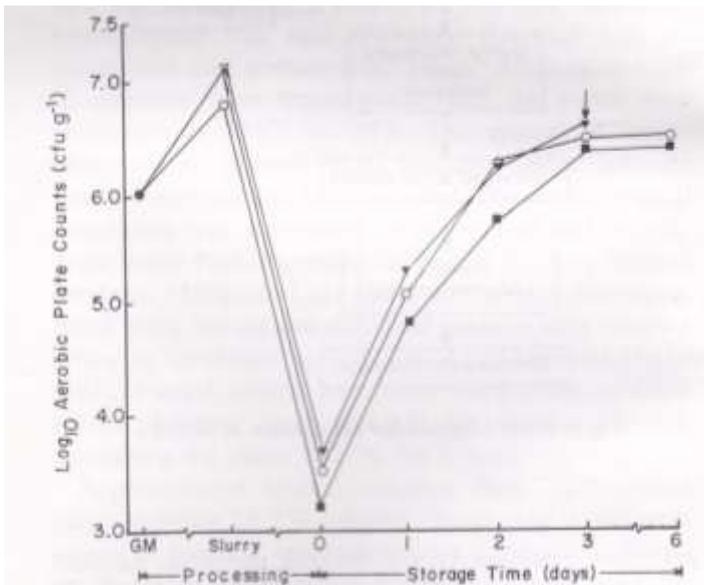


Figure 22. Dramatic increase in microbial load after day 1 of ambient storage of “Kwoka” (∇ = 30min; o = 60min; ■ = KS + 60min steaming)

13.4.2 Non- fermented maize product supplemented with soybean (“Soy-Kwoka”) and the microbial safety

Soybeans are a major source of nutrients hence they are used for supplementation of m maize products which are deficient. However, there is no information on the microbiology of such supplementation (20 or 30%). In addition. Because of increased and emigration, there is increased international concern for microbiological data and safety of traditional/ ethnic foods (Bryan 1988). Soybean-supplemented product had greater microbial diversity and higher population than the unsupplemented control. Whereas a sharp increase occurred in the microbial populations of all samples maximum load was observed in 20% soybean supplemented “Kowa” at the end of storage (Efiuwewwere and Akoma, 1997b). Apparently, packaging in tracature leaves the shelf-life and potential safety did not exceed 24hours after production. Therefore, control measures to minimize health risks include consumption of the product soon after production and avoidance of left-over since heat-stable microbial toxins are likely to occur in the product.

13.4.3 Soybean supplemented maize (“Soy-Kwoka”) was subjected to acidification and antioxidants (BHA/BHT) A drastic reduction of approximately 4-log microbial population was attributed to the acidification (pH 3.5) using lime juice and the use of BHA/BHT. The storage stability and microbial safety potential of the acidified and antioxidant-preserved products were approved by 3days (Efiuwewwere and Akoma, 2000).

13.4.4 Reconditioned maize moisture content and mycotoxin production

Maize is an important cereal world-wide but unfortunately it is also a suitable substrate for mould growth, development and production of mycotoxins (carcinogens). Of these metabolites, Aflatoxins B₁, B₂, G₁, and G₂ are the most potent and B₁ occurs in the highest concentration and is the most toxic (Lacey, 1990), we investigated the effects of re-moistened maize to different moisture contents (m.c) (13,15,17,20,25,30 or 35%) and stored with the natural microflora or sterilized before artificial inoculation with either single

or mixed moulds. Fungal population of naturally contaminated maize of 13% m.c decreased significantly with storage but 17 and 20% m.c. maize increased and the latter attained the peak of \log_{10} 7cfu/g. Production of hazardous levels (>20ppb) occurred only in samples of $\geq 20\%$ m.c. Also, these were the only samples that showed significant positive relationship/correlation ($r=0.92$) between m.c and fungal load. Aflatoxin B₁ content of 25% m.c. maize increased with increase in inoculum concentration of *Aspergillus flavus*. Interestingly, mixed mould inoculation of maize samples resulted in reduction in Aflatoxin concentration with co-cultures of *Aspergillus flavus* and *Penicillium purpurogenum* showing the lowest production while sample inoculated with *Aspergillus flavus* alone (control) exhibited the maximum production. Initiation time (onset) to moulding was most rapid in $\geq 20\%$ m.c. maize irrespective of inoculum type but *Aspergillus flavus* was the most invasive in singly inoculated samples (Oyebanji and Efiuvwevwere, 1999) (Figure 23). However, variations in their competitiveness were dependent upon the m.c of the maize with *Fusarium moniliforme* being most competitive in the 35% m.c. maize. This work showed the critical importance of moisture content in spoilage of maize and production of mycotoxins to hazardous levels.

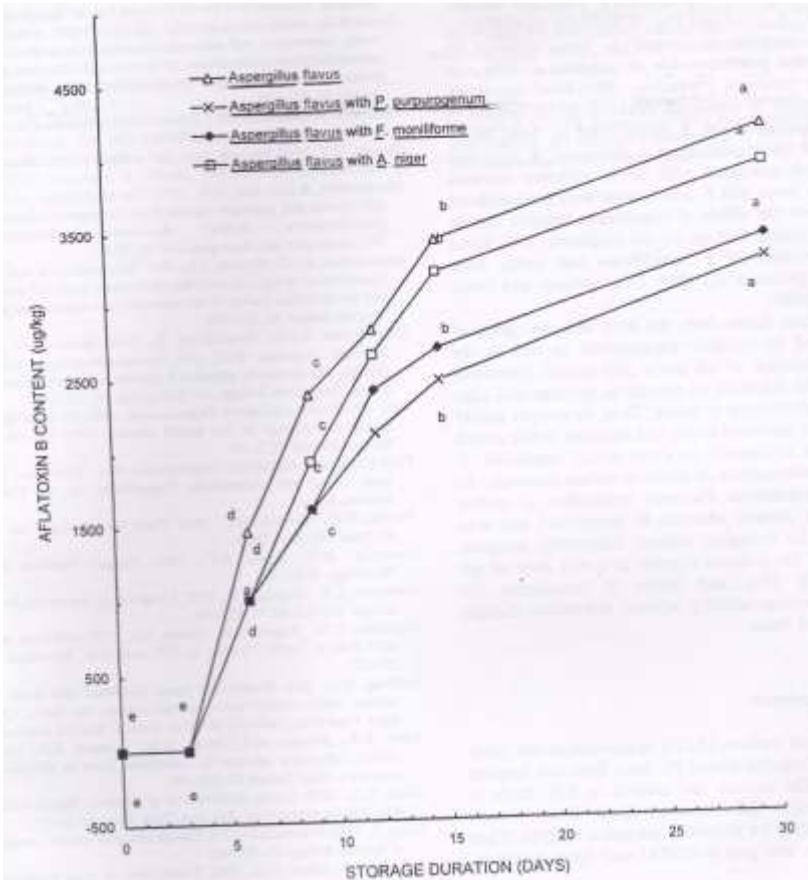


Figure 23. Production of Aflatoxin B₁ (mycotoxin) in 25% moisture content Maize singly or co-inoculated

13.5 MODIFIED ATMOSPHERE STORAGE AND SHELF-LIFE EXTENSION

The application of MAP in international food trade is wide-spread (Gorris and Heppelenbos, 1992). However, the quality of such produce is not always regulated using the same international food standards such that some countries take advantage of the others (Figure 24a and 24b)



Figure 24a.



Figure 24b.

Photographs of cartons used for Modified Atmosphere Packaging of apples shipped to Nigeria

(Note: Inscription on the packaging ‘For Africa Only’)

13.5.1 Pre-packaging Chemical Treatments of Tomato Fruits Before Packaging Using Polyethylene Bags

Alteration of the gaseous composition of food microenvironment has beneficial effects on the shelf-life of the food. Several packaging materials have different gas permeability properties which invariably contribute to the microbial behavior and shelf-life. In addition to use of different polyethylene bags (HDPE or LDPE) tomato fruits were pre-treated with 70% ethanol or 0.2% benomyl and packaged using the polyethylene bags or raffia palm basket. LDPE most effectively maintained the fruit quality compared with basket packaged and control fruits. Benomyl pre-treatment resulted in lowest microbial load and lower spoilage incidence than in ethanol treated fruits (Efiuvwevwere and Uwanogho, 1990).

13.5.2 Unconventional Packaging of Tomato Fruits Using Chemical Treatment, Packaging with Sawdust in Polyethyethylene Bags and Storage Under Two Temperatures

The quest to further reduce the incidence of tomato fruit spoilage, led us to investigate an unconventional method of packaging the Benomyl or Dithane- treated fruit in either HDPE or LDPE with sterile sawdust before storage at 10⁰C or 29⁰C. Dithane M45 treated fruits packaged in HDPE showed the lowest incidence of deterioration. Fruits inoculated with *Fusarium* spp and *Aspergillus* spp before packaging induced the most severe lesions at 29⁰C but fruits packaged in polythene bags with sterile sawdust showed the smallest lesions (Efiuvwevwere and Eka, 1992). This multi-component preservation strategy is often referred to as ‘hurdle technology’ since the microorganism(s) has to “scale” or ‘jump’ several barriers/hurdles which results in pronounced inhibitory effects thereby, enhancing the storage-life or shelf-life of the produce. This unconventional preservative approach using sawdust prevented fruit-fruit contact contamination and reduced incidence of lesion formation (Figure 25). Many colleagues requested reprints of this work and is a good example of “multi-target” food preservation strategy.



Figure 25. Different packaging of tomato fruits in LDPE (1), HDPE (2), LDPE/S (3), HDPE/S (4) and control (unpacked)

13.5.3 Combination of chemical fungicide and MAP for control of yam tuber rot

Yam tubers are usually damaged during harvest or transportation/distribution which often results in substantial economic losses. We therefore investigated the effectiveness of fungicides and modified atmosphere packaging following single culture or pair- culture inoculation of yam tuber samples at two wound depths of 10mm and 15mm. Lesions/rots produced at 10mm depth were significantly larger than those induced at 15mm depth. Pair-inoculation of *Aspergillus* and *Rhizopus* induced the most extensive rots and minimum rot size occurred when *Fusarium* was co-inoculated with either *Botryodiplodia* or *Penicillium*. Dithane M-45 (fungicide) significantly reduced the rots while sodium benzoate (antimicrobial preservative) was least effective. Use of polyethylene bag of 18 μ m thickness significantly maintained the quality of the samples (Efiuvwevwere and Nwanchukwu, 1998). Inoculation of the samples with *Aspergillus* spp resulted in a significant decrease in carbohydrate and moisture content but enhanced crude protein content indicating utilisation of the carbohydrate by the organism. Application of combined preservation strategies (“hurdle technology”) was most beneficial and several scientists, producers of crops and food-chain distributors showed considerable interest in this work.

13.5.4 Application of MAP to extend the shelf-life of local plantain cultivars and the hybrid fruits

Use of MAP for shelf-life extension of temperate produce has been very successful. However, very limited scientific information is available on its application on tropical produce. We therefore investigated use of transparent polyethylene films on the quality of local plantain cultivars and the hybrid fruits. The results showed delayed biodeterioration, ripening and weight loss, thereby enhancing the shelf-life of both the local cultivar and the hybrid without use of chemical preservatives. However, pathogenicity tests (i.e. microbial invasion) showed that the local cultivars were selectively more susceptible to different moulds (*Botryodiplodia theobromae* and *Fusarium moniliforme*) while the hybrid fruits were

more susceptible to a different mould (*Colletotrichum musae*), a more aggressive biodeteriorative agent (Nwaiwu *et al.* 2003).

Interestingly, higher fungal counts were obtained from the hybrid and this was attributed to the availability of more nutrients in this improved fruits. This work in addition to the others highlights the benefits of MAP in plantain and its possible adaptability to other produce. These benefits are well demonstrated in Figure 26.



Figure 26. Effects of Modified Atmosphere Packaging on Plantain

13.6 FOOD MICROBIOLOGY AND RELATED IMPACTS OF ECOLOGICAL PARAMETERS

13.6.1 Fresh Water Snails

Seafoods are usually exposed to several ecosystems which contribute to their contamination and questionable safety (Ezeama and Efiuvwevwere, 2004; Izuchukwu and Efiuvwevwere, 2007; Odu *et al.* 2010).

Fresh water snail (*Pila ovata*) is a cheap source of animal protein but as a filter-feeder, it accumulates large amounts of microorganisms and other pollutants. Therefore, the aquatic environment in which they are found plays a major role in their microbial quality and safety. In the **quest** to know the microbial status of this seafood under different conditions, we investigated the impacts of subjecting

them to different microcosms simulating storage conditions namely; body of stagnant water (BSW), body of water changed intermittently (BCWI), hibernating condition (HC i.e. buried in the sand) and depuration process (DP i.e. self-purification as water runs continuously through the samples).

Our findings showed that exposure of the samples to BSW and BWCI were not beneficial but HC resulted in initial (i.e. within the first 3months) microbial decrease with increase thereafter. A more diverse bacterial flora was observed at the initial stage of hibernating condition but anaerobic spore-formers dominated at the end of the HC. However, significant microbial decrease was found within 2days of DP but thereafter remained practically unchanged (Efiuvvwere and Ezeama, 2004). These findings have underscored the benefits of depuration process compared with the others in reduction of the potential risks and safety implications but the exposure time is critical. It has been demonstrated that these shellfish are highly contaminated and should be adequately processed /cooked before consumption to avoid food-related diseases.

13.6.2 Prawns (*Macrobrachium vollebovenii*)

These are special seafoods because they are highly valued in the international food trade. Yet, little or no scientific information is available on the microbial profiles especially in relation to the habitats from which they are harvested.

We therefore investigated the impacts of the physico-chemical parameters of three ecosystems (Port Harcourt Marine Creek, Ogbakiri, and Rumuapareli) in Rivers State in relation to the microbiological characteristics and safety of prawns (Figure 27). Our findings demonstrated that the highest total microbial population occurred in PHMC while the lowest was found in Ogbakiri (Figure 28). Similar observations were made on sanitary indicators (faecal coliforms) (Izuchukwu and Efiuvwevwere, 2007). But *Pseudomonas* spp. populations were comparable in the three habitats. Evaluation of the two portions (Cephalothorax i.e. head and tail) showed the

dominance by *Staphylococcus aureus* and other species of *Staphylococcus* in all samples from the three habitats (Figure 28). However, *Pseudomonas* spp were the most dominant population in Rumuoparaeli. In contrast, the tail portion showed *Vibrio* spp as the dominant microorganism in all three ecosystems but four bacterial groups (*Salmonella*, *Shigella*, *Vibrio* and faecal coliforms) were found in PHMC and Ogbakiri while only *Vibrio* spp occurred in Rumuoparaeli (Figures 29a and 29b). We also found that faecal coliforms were highest in PHMC and lowest in Ogbakiri. Remarkably, positive correlations were found between the habitats and the microorganisms with higher correlation occurring between the cephalothorax and the habitats respectively (Rumuoparaeli: cephalothorax = 0.74596, tail =0.46058; PHMC: cephalothorax =0.65380,tail = 0.49725; Ogbakiri: cephalothorax =0.94419, tail = 0.25407). These variations in microbial composition as well as in the correlations clearly indicate that the microbial quality of the prawns is highly dependent on the ecosystems and the anthropogenic activities associated with the habitats. For example, the highest pH 7.51 was found in Ogbakiri and the lowest (5.90) in Rumuoparaeli with the concentrations of the macro-mineral varying with PHMC being dominated by calcium and magnesium (Figure 30) coupled with variations in the BOD and COD (pollution indicators) (Izuchukwu and Efiuvwevwere 2007). It is therefore evident from this work that the prawns harvested from PHMC are the least wholesome (i.e. most hazardous) and this reflects the ecosystem from which they were harvested. Consequently, necessary measures (including adequate washing and cooking) should be taken before their consumption.



Figure 27. A photograph of prawn from Port Harcourt Marine Creek

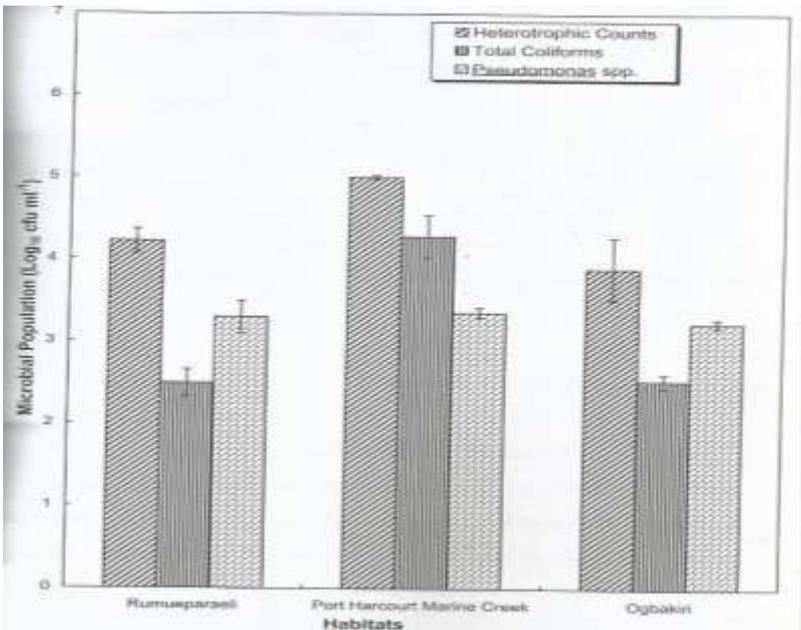


Figure 28. Microbial population of water samples from the different habitats of prawns.

Error bars represent standard deviations

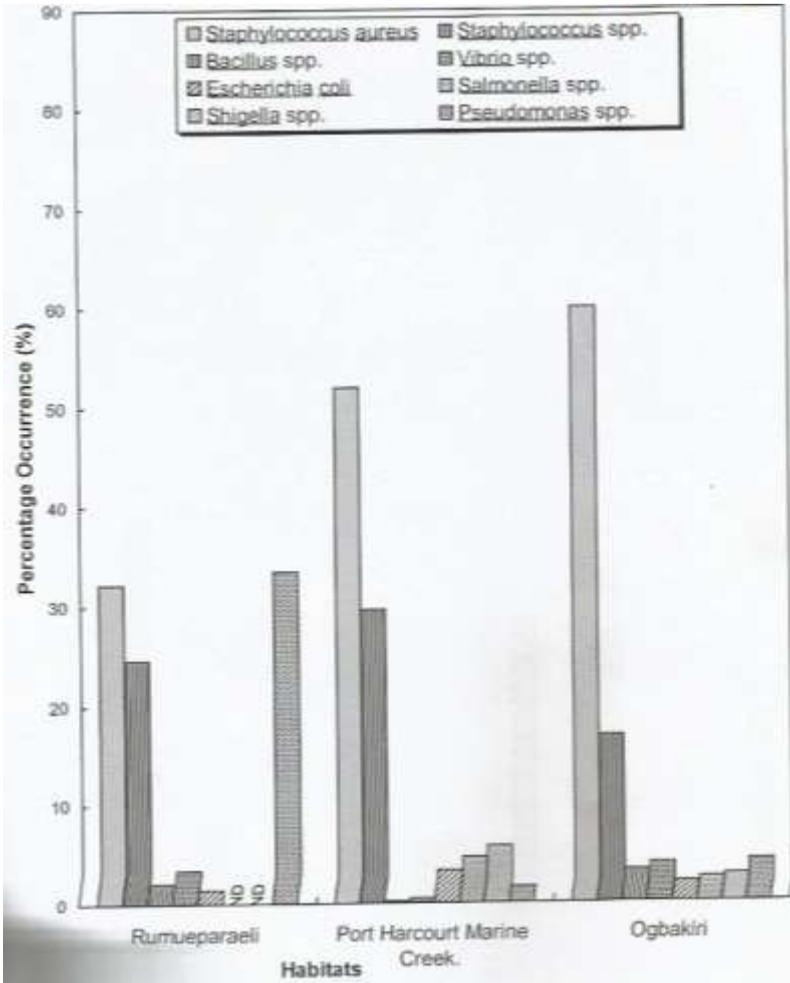


Figure 29a. Percentage occurrence of bacteria isolated from the tail portions of prawns from the different habitats. ND = Not detected

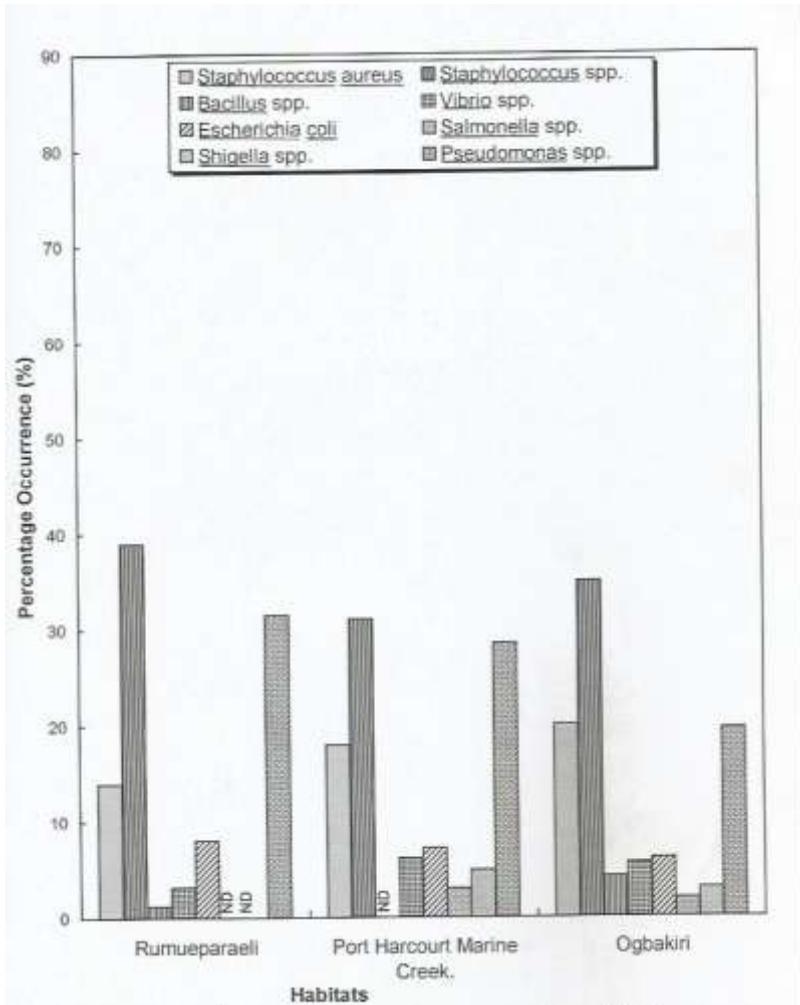


Figure 29b. Percentage occurrence of bacteria isolated from the cephalothorax portions of prawns from the different habitats. ND = Not detected

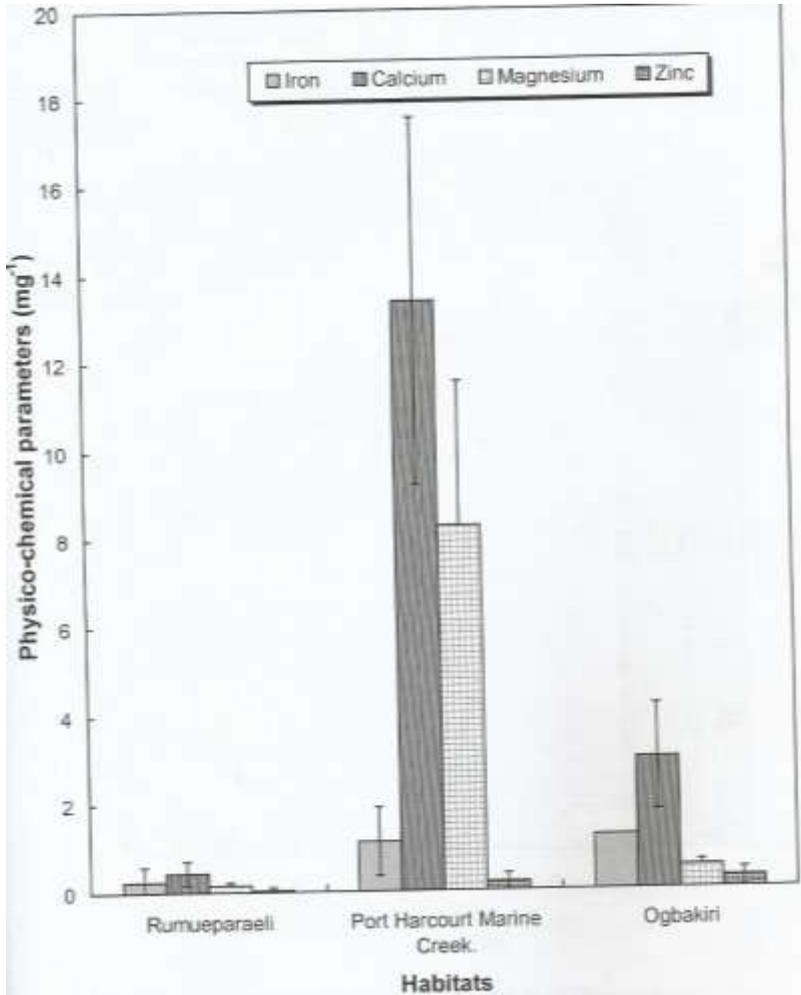


Figure 30. The concentrations of macro-minerals of the different eco-systems
 Error bars represent standard deviations.

13.6.3 Exposure of shellfish “Ngolo” to simulated crude oil polluted microcosms

Crude oil contamination of seafoods is wide-spread in the Niger Delta Region of the country. However, little or no research attention has been paid to such occurrence with respect to the impacts on “Ngolo”, a popular local shellfish with international trade potential. We therefore carried out simulated study by exposing unshucked “Ngolo” samples to different levels (0%, 1%, 5% and 10%) of crude oil pollution (Efiuvwevwere and Oruwari, 2014). We found that total heterotrophic counts (THCs) (microbial population), sanitary indicators (coliforms) and hydrocarbon degraders increased with pollution level and exposure time resulting in THCs of \log_{10} 7.97 cfu/g on day 12 (Figures 31-33). Concentrations of heavy metals (Nickel, Copper and Cadmium), BOD, and COD increased with pollution level. Remarkably, higher contents of these parameters occurred in “Ngolo” samples compared with the microcosms thereby indicating bioaccumulation. Shucked “Ngolo” samples exposed to 0% or 1% pollution level for 9 days were acceptable organoleptically but those subjected to 5% or 10% pollution after 6 or 3 days were rejected. This work has shown that the safety and acceptability of “Ngolo” samples are pollution level/exposure time dependent (Efiuvwevwere and Oruwari, 2014). Thus, it is highly recommended that clean-up operations are carried out within 3 days of crude oil pollution to recover such shellfish as “Ngolo”.

Figure 34 shows the mortality rate following exposure to the crude oil polluted microcosms.

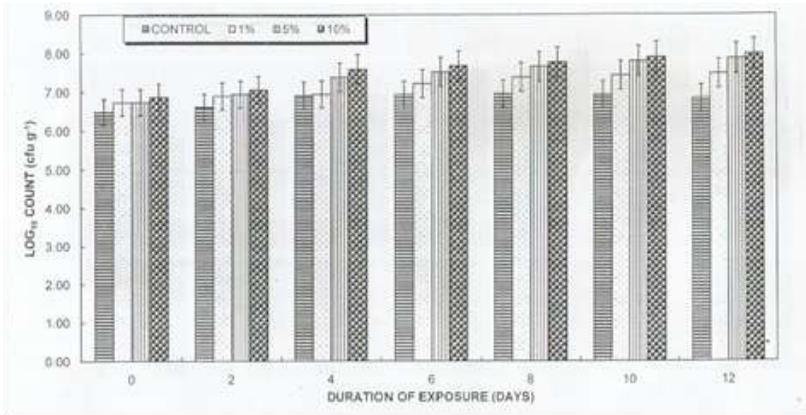


Figure 31. Changes in Total Heterotrophic counts of “Ngolo” samples subjected to different simulated crude oil polluted Microcosms Values represent the mean of six determinations. Bars indicate standard errors

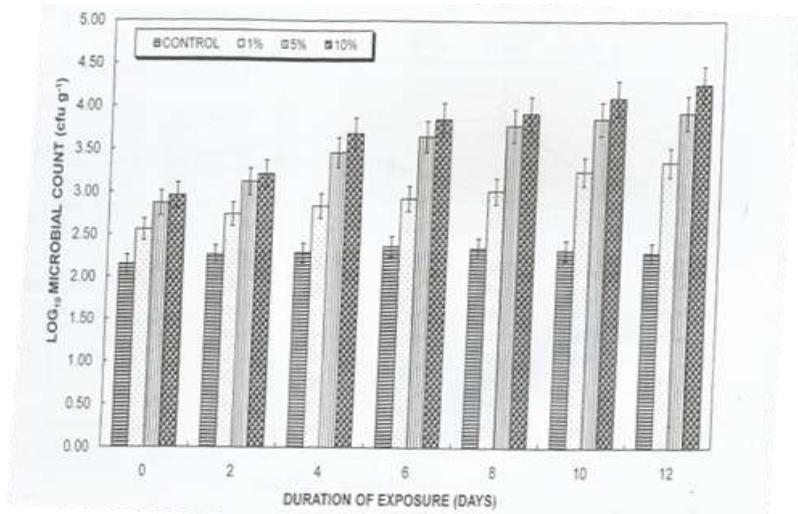


Figure 32. Changes in hydrocarbon degraders in “Ngolo” samples subjected to different crude oil simulated polluted Microcosms. Values represent the mean of six determinations. Bars indicate standard errors

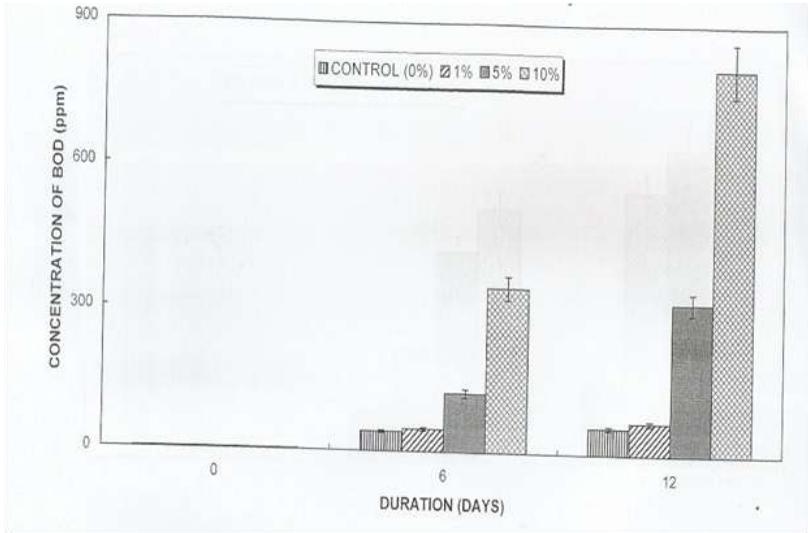


Figure 33. Changes in BOD of “Ngolo” habitat (water) subjected to different simulated crude oil polluted microcosms
 Values represent the mean of four determinations. Bars indicate standard errors

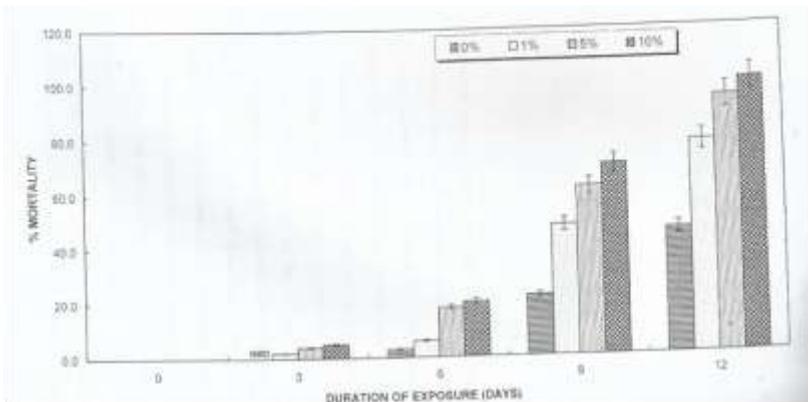


Figure 34. Percentage Mortality rate of “Ngolo” exposed to different concentrations of crude oil simulated polluted Microcosms
 Values represent the mean of six determinations. Bars indicate standard errors

Figure 34 Mortality rate of ‘Ngolo’ exposed to different crude oil pollution levels is shown in Figure 34.

13.6.4 Fresh Water Snail From Different Habitats

Fresh water snails are highly contaminated and their microbial profiles vary with habitats. We established that of the four habitats (locations) all in Ebonyi State) from which these sea foods were harvested, Akpoha was the most contaminated both in terms of total microbial population and types (including faecal coliforms). The intestine had higher microbial (total anaerobic) counts than the meat (Ezeama and Efiuvwevwere, 2007). On the contrary, Oziza showed the best microbial profiles. These variations are related to the various anthropogenic (human) activities and the ecological variables (salinity, pH, DO) prevalent in such habitats. It is therefore apparent that consumers are constantly exposed to hazardous sea foods since they are harvested from the wild and no sanitary control is put in place to regulate their cultivation and harvest in spite of their safety risks and economic importance.

13.6.5 Microbial profiles of different sites of the new Calabar River

Various studies have highlighted the microbial distribution of ecosystems. Our quest was therefore to embark on investigation with focus on three sites (Choba, Iwofe and Ogbakiri) of the New Calabar River being a major aquatic life resource. We found predominantly eight Gram-negative bacterial genera from the samples with variations in the bacterial profiles of the three different sites with the highest total heterotrophic counts and total coliform load occurring in Iwofe but the lowest at Ogbakiri. Overall maximum pH was observed in Iwofe and the lowest at Choba but DO values did not vary appreciably among the sites (Edun and Efiuvwevwere, 2012). These findings underscore the impacts of human and industrial activities on microbial distribution/profiles as well as the implications on the aquatic life of the habitats.

14.0 Prospects and Challenges

14.1 Prospects

It is evident from the lecture that the prospects for microbiologists in general and food microbiologists in particular are good and diverse both locally and globally.

These may be summarized as follows:

1. Employment in government regulatory establishments/agencies such as NAFDAC with focus on formulation of food safety policies, food legislation and enforcement of approved policies.
2. Employment by Universities and other institutions of higher learning where teaching and research as well as raising public awareness concerning food borne illnesses and prevention should be emphasized.
3. Employment by food manufacturing industries to ensure that Good Manufacturing Practices (GMPs) and HACCP concept on production processes are carried out effectively in order to comply with government and international food standards and regulations.
4. Employment by international organizations such as FAO, WHO and WTO to help formulate food safety standards and monitor global trends about food-borne illness to ensure compliance with international food standards to minimize the incidence of food borne disease outbreaks.
5. Establishment of small and medium scale enterprises (SMEs)/cottage food industries is very promising. In fact, such industries are already thriving but they need to be better organized, encouraged and supported technically by food microbiologists and government. Examples include:
 - i. Production of kunun–zaki and other fermented products using known starters to obtain products of consistent quality and safety to enhance their commercialization.
 - ii. Production of several non-fermented foods using some of the protocols/regimes discussed in this lecture to improve their shelf-life and commercial potentials.

- iii. Use of MAP to provide supply of fresh produce with extended shelf-life to help distribute such produce (e.g. plantain) to various parts of the country. Moreso, MAP is a simple preservation technique and the raw materials for production of the desired packages for adequate O₂/CO₂ levels are available locally (Eleme Petro-Chemical Company).

Whereas the prospects are numerous and diverse, research activities especially in the government establishments are very challenging and particularly evident is the absence of state-of-the-art facilities.

14.2 Challenges

Several challenges with respect to research activities and associated entrepreneurial outputs in food microbiology are apparent and they include:

- (i) Lack of regular power supply which impedes effective research activities and outputs. For instance, some of our research works were carried out using personal power generating sets (generators).
- (ii) Absence of microbial culture collection centres and improved microbial strains both locally and nationally is a major drawback to research and advancement in food microbiology both for academic and industrial purposes.
- (iii) Lack of the state-of-the-art facilities such as HPLC, Polymerase Chain Reaction (PCR) equipment etc is a serious challenge to research activities to facilitate major contributions to food microbiology and national development.
- (iv) Lack of harmonization (among regulatory agencies) of food safety standards thereby resulting in conflicts and lack of commitment.
- (v) Inadequate enforcement of existing food safety standards thereby leading to more prevalence of food borne illnesses.

15.0 Recommendations and Conclusion

Vice-Chancellor Sir, distinguished ladies and gentlemen, based on the foregoing, I wish to make the following recommendations:

1) That a Centre for Food Quality and Safety (CFQS) be established to carry out research activities on all foods both popular and peculiar to this region as well as to collate necessary data and become the reference point for national and international organizations. The centre will become the data base/bank for accessibility of the much inaccessible existing scientific information due to dearth of statistics on food borne illness in the country such as pathogens of greatest concern and their associated foods.

2) The need to construct and equip a microbiological culture collection centre to help preserve most needed isolated microorganisms and to exploit their applications for research activities (e.g. fermentation, bioremediation) in the various sub-disciplines of microbiology and related disciplines such as Biochemistry. This will lead to enhanced small-medium scale enterprises/improved University-Private-Partnership and production of foods with consistent and improved safety attributes.

3) That more commitment should be given to food safety issues by the three tiers of government with the Federal Ministry of Health (FMOH) and NAFDAC taking the lead and greater attention being given to food-related issues by NAFDAC rather than to drug-related issues as currently practised.

4) That a food microbiology course be introduced into the curriculum of the medical and paramedical programmes for acquisition of more knowledge to help reduce the incidence of food borne disease outbreaks in the country.

5) That Food Safety Day be introduced to the University to raise awareness level among students and the public to help minimize the incidence of foodborne diseases e.g. lecture to be given to the food service outlets on campus and its environs.

6) That better articulated food safety regulations and standards (especially with production of new products) should be put in place with legislative arm of government (National Assembly) to enact laws to protect the public from foodborne illnesses.

7) That a centralized national foodborne illness surveillance system (with well-defined organogram) where state and local government ministries and departments of health report incidences of foodborne illness/outbreaks to such a centre through NAFDAC to FMOH to help provide accurate and up-to-date statistics.

8) That regulations of the areas for cultivation and/or harvest of fresh water/brackish/marine foods be articulated for implementation to minimize highly contaminated seafoods as practised in developed countries.

Conclusion

Vice-Chancellor Sir, distinguished ladies and gentlemen, I wish to conclude this lecture by stating that the various implications associated with the roles of microorganisms in foods and food preservation as well as microbial food safety are globally recognized and have been highlighted. But we have a duty to play our part. I therefore wish to emphasize that greater awareness and provision of state-of-the-art facilities for food microbiological research be given a priority to enhance food safety and minimize incidences of foodborne diseases. It is therefore my prayer that we do not become victims of food borne illnesses.

Thank you for listening

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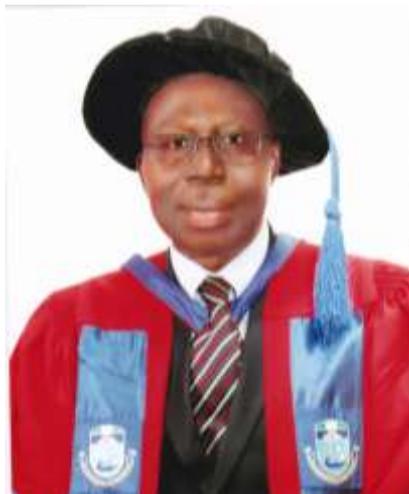
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**CITATION ON
PROFESSOR BERNARD JOHNSON OKPAKO EFIUVWEVWERE
B.Sc. (Detroit Institute of Technology), M.Sc. (Pennsylvania
State University) Ph.D (University of London), *fns***



BIRTH

Prof. Bernard Johnson Okpako Efiuvwevwere was born on 21st June, 1950 in Urhodo-Ovu, Ethiope-East Local Government Area of Delta State, to the family of Chief & Mrs. Efiuvwevwere Atoke Idivwrikesi, and he was the second child of the family. At birth, he was given the name, ‘Okpako’, which means senior, a prophetic name, loaded with great responsibilities which has been playing out in the course of his life time. He is fondly called ‘BJO’.

EDUCATION

He started his academic voyage at St. John Primary School, Urhodo - Ovu from where he proceeded to the renowned St. Peter Claver’s College, Aghalokpe-Sapele for his secondary education in January, 1964. He finished his secondary education in flying colours in 1968. With this excellent performance and determination to achieve his academic dream, he proceeded to the United States of America to advance his academic mission. In 1976, he graduated from the

Detroit Institute of Technology with a B.Sc. in Biology (Microbiology), Magna Cum Laude (equivalent of Second Class Upper Division).

To further prepare himself for his chosen career, he enrolled for his M.Sc. programme in the Pennsylvania State University, Pennsylvania, U.S.A in 1977 and graduated with M.Sc. in Food Microbiology/Food Processing in 1979. To completely colour his academic cap, he enrolled for his Ph.D programme in Food Microbiology and Biodeterioration at the University of London (King's College, London), and completed the programme in December, 1986.

To become a professional in Food Safety and Drug Administration, he obtained a certificate in Aseptic/Thermal Processing and Packaging Operations in acidified and low-acid foods from the United States Food and Drug Administration/Better Process Control School, University Park, Pennsylvania, in April, 1979.

WORK EXPERIENCE

Prof. B. J. O. Efiuvwevwere work experience cuts across local, national and international domains. His first career exposure (a special privilege) was as a Teaching Assistant/Demonstrator (Microbiology) to Professor J. Ehrlich of Detroit Institute of Technology, U. S. A (1975-1976). He acquired industrial experiences at the well-known Pharmaceutical Company, Parke-Davis & Co, Detroit, as a Microbial Quality Control Analyst (1977) and in Guinness Nigeria Limited, Benin City as Quality Control Manager Trainee (1980 – 1981) during his NYSC programme.

He joined the University of Port Harcourt as an Assistant Lecturer in 1981, and rose through the rank and file to emerge an Academic General, Professor of Microbiology (specializing in Food and Industrial Microbiology) in the Millennium year 2000. He is an Academic Adviser to many students, and a Role Model to many of his colleagues with the philosophy of diligence, discipline, and excellence. He has supervised numerous undergraduate students, 11

M.Sc. and 6 Ph.D students, among whom are Professors, Directors, Managers as well as Administrators. Currently, he supervises 3 Ph.D, 2 M.Sc. and several undergraduate research projects.

PUBLIC SERVICE

He had served as a member, and Chairman in various NUC Accreditation Teams to several Universities. Furthermore, he is an External Examiner for undergraduates and postgraduates and External Assessor for Professorship to several Universities.

AWARDS AND APPOINTMENTS

Prof. Efiuvwevwere has received several prestigious national and international awards, and notable among them are:

- i. University of Bologna (Prof. M. E. Guerzoni Grant), Bologna, Italy.
- ii. Netherlands Organization for Scientific Research (NOSR/NWO) Fellowship, The Hague;
- iii. International Foundation for Science (IFS), Sweden.
- iv. Research Grant, Federal Ministry of Education;
- v. Postgraduate Overseas Scholarship;
- vi. Readers' Digest Tuition Grant.

He has served meritoriously in several dignified positions in both public and professional services. He was the Editor-in-Chief, Nigerian Journal of Microbiology. He also served as Editor, Scientia Africana, as Business Manager, Nigerian Society for Microbiology, as Head of Department, Microbiology (1999-2001), University of Port Harcourt. The Department was ranked number one in academic content by NUC under his leadership as Head of Department.

Furthermore, he served as member, Board of Governors, College of Continuing Education, Chairman, University of Port Harcourt Convocation Committee, and above all, he is the immediate past Deputy Vice-Chancellor (Academic), University of Port Harcourt.

PROFESSIONAL AFFILIATION AND PUBLICATIONS

Prof. B. J. O. Efiuvwevwere is an active member of several professional societies, among which are:

- i. Member, American Society for Microbiology;
- ii. Fellow, Nigerian Society for Microbiology;
- iii. Member, International Association of Milk, Food and Environmental Sanitation, USA;
- iv. Member, New York Academy of Science ;
- v. Member, Science Association of Nigeria;
- vi. Member, American Association for the Advancement of Science, USA

He is also recognized/listed in Marquis WHO's WHO in Science and Engineering (2000), New Jersey, USA.

He has authored one textbook, and co-authored three text books respectively. He has published fifty five (55) articles in reputable and indexed International Journals. He has presented a number of researched and technical papers in several Local and International Conferences, Symposia, and Workshops.

His consultancy services cut across a number of organisations including the National Agency for Food and Drug Administration and Control (NAFDAC), Standard Organization of Nigeria (SON), Non-Governmental Organizations (NGOs) on Food Safety, Standards, Processing and Quality Control, Federal Ministry of Science and Technology/Raw Material Development and Research Council, Abuja, and Nigerian National Petroleum Corporation (NNPC).

FAMILY AND SOCIAL LIFE

Prof. Efiuvwevwere is happily married to Mrs. Roselyn Asawa Efiuvwevwere, and they are blessed with 6 children and grand children that are of good standing in the society. He is a good philanthropist and also a member of the Urhobo Solidarity Club, Nigeria.

CONCLUSION

Mr. Vice Chancellor Sir, I present to you, a Sound Academic, a Good Administrator, a Quiet Listener, a Scientist of great repute, a meticulous Researcher, an Achiever, and a Great Philanthropist, to present the 114th Inaugural Lecture in the Inaugural Lecture Series of the University of Port Harcourt.

Thank you.

Professor Gregory O. Awwiri