MICROORGANISMS, SPICES AND FOOD SAFETY: THE WONDERFUL THREE-FOLD CORD

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UNIVERSITY OF IBADAN

MICROORGANISMS, SPICES AND FOOD SAFETY: THE WONDERFUL THREE-FOLD CORD

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By

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The Vice-Chancellor, Deputy Vice-Chancellor (Administration), Deputy Vice-Chancellor (Academic), The Registrar and other Principal Officers, Provost of the College of Medicine, Dean of the Faculty of Technology, Deans of other Faculties and Postgraduate School, Dean of Students, Distinguished Ladies and Gentlemen.

Preamble

I give God the unalloyed glory, thanks and adoration for making it possible for me to deliver this inaugural lecture on behalf of the Faculty of Technology. Were it not for God, I am one of the least of those who can be called to stand before this august audience doing what I consider as a great feat. I am particularly grateful to God and I am rest assured that one does not have to be great to start in life but one has to start to be great in life.

Mr.Vice-Chancellor, it is indeed gratifying to recall that I am delivering the fourth inaugural lecture from the Department of Food Technology, the first having been delivered by Professor A.O. Olorunda in 1986, the second by Professor J.O. Akingbala in 1993 and the third by Professor O.C. Aworh in 1994. Certainly it is not by might, power but the Spirit of God that empowered me to have got relevant experience encompassing the wonderful three-fold cord: Micro-organisms, Spices and Food Safety hence, the title of my lecture will center on the relationship within the cord particularly recalling the recent scourges of microbial infections in the country.

Microorganisms occupy a world of their own as they can be found associated with man, animals plants, soil, birds, insects, fishes, reptiles, atmosphere so it is safe to say "the good, the bad and the ugly" of microorganisms particularly when one thinks of the roles of microorganisms in food production, (the good) diseases and death (the bad), physical discomforts and ugly appearances (the ugly). A typical bacterial cell is illustrated in figure 1.

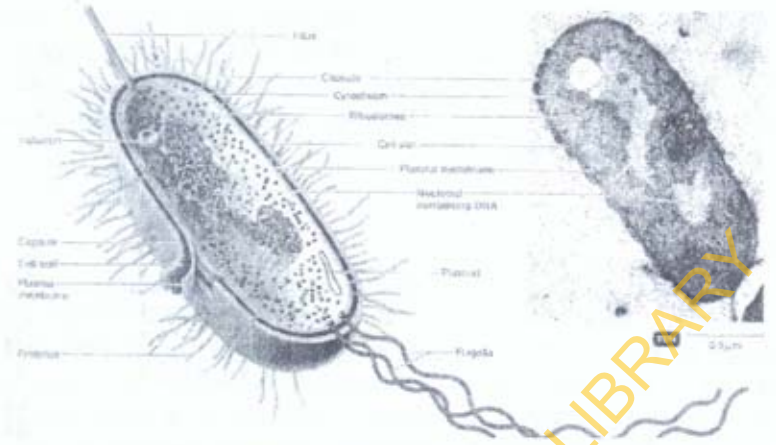

Fig. 1: An example of a bacterial cell.

There are several divisions within the subject, microbiology for example: there are:

(1) Medical Microbiology
(2) Veterinary Microbiology
(3) Food Microbiology
(4) Soil Microbiology
(5) Environmental Microbiology
(6) Industrial (Applied) Microbiology etc.

Spices are plants/materials (mostly aromatic) used for flavouring foods generally. Spices can be used to preserve foods and even treat some diseases of man and animals. Examples of spices are: Chile pepper, African cardamom, nutmeg, garlic, ginger, turmeric, clove (figs. 2, 3). Spices are also of economic importance nationally.

Fig. 2: Different types of spices.

Fig. 3: *Aframomum danielli* plant.

Food safety is an important branch of Public Health and Food Microbiology and it has been defined as all those hazards whether chronic or acute that may make food injurious to the health of the consumer (FAO/WHO 2003). Considerable attention is now being focused on this branch of food microbiology.

Mr. Vice-Chancellor, Sir, distinguished ladies and gentlemen, I am sincerely delighted to present today some of my research findings which encompass microorganisms of public health importance, spices particularly the under-utilized ones with potential bioactivities and food safety, a core health issue that affects all and sundry.

Introduction

Microorganisms are "very small organisms" which cannot easily be seen with the naked eyes, and they have been around from time immemorial. The science of microbiology can be traced to the development of a relatively crude microscope by an Englishman, Robert Hooke in 1665, who reported that life's smallest structural units as "little boxes" or

"cells". Thereafter, the development of microbiology was interesting for example; Pasteur in 1857 and 1864 has to this credit the phenomenon referred to today as fermentation and pasteurization respectively. The Germ theory (Koch's postulate) of disease is credited to Robert Koch. In 1920s, the first antibiotic was accidentally/fortunately discovered by Alexander Fleming. The Scottish physician and bacteriologist was almost throwing away some culture plates when he noted that a contaminating mould had produced some clearing zone around some normal bacterial colonies. The mould was later identified as *Penicillium notatum* and subsequently also renamed *Penicillium chrysogenum*. In 1928, Fleming named the antibiotic -penicillin. In 1953, Watson and Crick discovered DNA structure and thereafter there have been notable discoveries and Nobel Prize winners in Microbiology. It is my fervent prayer, Mr. Vice-Chancellor, Sir, that shortly Nigeria will produce another Nobel laureate, this time in Microbiology.

Beneficial and Non-beneficial Microorganisms

Microorganisms can be beneficial and non-beneficial to human beings.

Beneficial organisms:

1. Sources of food for example, fungi (Mushrooms)
2. Fermentation processes: epiphytic microflora essential for the production of wines, ogi and garri; Lactic acid bacteria essential for yoghurt and cheese; some yeasts are important in beer production.
3. Production of antimicrobial agents.

   Antimicrobial agents are associated with:

   (a) Microorganisms
   (b) Spices and plants
   (c) Birds, mammals and amphibians.

Microorganisms are not the only living cells producing antimicrobial agents. Antimicrobial agents (peptides) are found in plants, birds and mammals. With respect to birds, amphibians and mammals, their antimicrobial peptides have about 100 or less amino acids which carry positive electrical charge (cationic peptides) which enable them to disrupt microbial membranes that are rich in phospholipids which have a negative anionic charge (Tortora et al. 2010).

4. Production of Enzymes: With improved bio-technological processes, microorganisms are now, able to produce enzymes which were formerly produced by animals and often in an impure form. Some microorganisms producing useful enzymes are *Bacillus licheniformis*, *B. subtilis* (α – amylase), *Bacillus coagulans* (glucose isome-rase), *Aspergillus niger*; *A. oryzae* (lactase) etc. (Adegoke 2004).

5. Production of Acids and Alcohols: *Lactobacillus lactis* and *L. delbrueckii* are used for industrial production of lactic acid while *Aspergillus niger* is used for industrial production of lactic acid. Yeasts are important in the production of ethyl alcohol.

Non-beneficial Microorganisms

Pathogenic microorganisms are known to cause diseases in man, animals and plants. For example, Mr. Vice-Chancellor Sir, *Escherichia coli* strains can exchange genes with other strains or even other bacteria via bacteriophage (or bacterial virus), conjugation or naked uptake of DNA and this can lead to acquisition of new virulent strains. This was the case in *E.coli* 0104: H4 outbreak in Germany in 2011. It is on record that in this outbreak in Germany, a less harmful strain of *E.coli* acquired gene for the production of shiga toxin from a shiga-toxin producing *E.coli* leading to about 3,900 infections and 53 deaths (*wwwfoodsafety.com* accessed on 16/12/2014).

Mr. Vice-Chancellor, Sir, distinguished ladies and gentlemen, it is pertinent to note that while there are pathogens like *Escherichia coli*, *Staphylococcus aureus, Salmonella Typhi*, *Salmonella Enteriditis, Listeria monocytogenes* and others that are important in food matrices, human and animal health, *Escherichia coli* on its own has been associated with several foodborne outbreaks all over the world. *Escherichia* was named after Theodor Escherich, a German pediatrician who discovered the organism in 1885. It must be noted that not all strains of *E.coli* are harmful to humans as some strains are beneficial to humans in that they help in synthesizing vitamins B and K complex—thus assisting the gut. Mr. Vice-Chancellor, Sir, it takes only 40 hours for *E. coli* to completely colonize the gut of a new born baby. Interestingly, *E. coli* has been associated with eleven very prestigious Nobel prizes which involved:

(1) Understanding of DNA replication
(2) Genetic code
(3) Restriction enzymes
(4) Genetic engineering

The recent outbreaks of Ebola virus (EBV) infections and cholera in Nigeria and some West African countries are classical examples of the non-beneficial effects of microorganisms in addition to cases of tuberculosis, food poisoning and food intoxications. Mr. Vice-Chancellor, Sir, it is unfortunate to note that people with Parkinson's disease have been found to have different micro-biota in their intestines than their healthy counterparts (*www.nutringredients.com* accessed on 16/12/2014).

Spices and their Uses

The interactions between man and plants are very interesting. God in His infinite mercies has already given man divine control over animals, birds and plants (Genesis 1^{29}). In an age of advanced technology of nano particles, nano probes, biofortifications, development of nutritionally enhanced foods, development of transgenic 'golden rice', oils that have reduced levels of undesirable fatty acids and availability of

genetically engineered potato, are some examples of interactions between man, plants and microorganisms. The importance of plants to mankind cannot be overemphasized. Of considerable importance to my collaborators and me are spices. Spices are food supplements or food products which have been used not only as flavouring and colouring agents but also as food preservatives and herbs in folk medicines for thousands of years in Africa, Asia and other parts of the world (Srinivasan 2005). Furthermore, spices and herbs have been used as food and to treat ailments by humans. Thus spices like saffron, a food colorant; turmeric, a yellow-coloured spice; tea—either green or black—and flax seed do contain some components which provide significant protection against cancer (Lai and Roy 2004).

Mr. Vice-Chancellor, Sir, the usefulness of spices (whether indigenous or exotic) are *inter alia*:

(1) Potential contribution to national economy. India is a good example of a country where spices have contributed significantly to the national economic development (Vasundhara et al. 2008). It is therefore noteworthy when Fasoyiro (2014) opined that Africa must recognize the vast opportunities offered by spices and tap them for a better economic future by adding value to spices at the production, processing and marketing levels.
(2) Contribution to daily antioxidant intake in most diets especially in dietary culture where spices are used as whole meal (Carlsen et al. 2010).
(3) Prevention of oxidative stress both *in vivo* and *in vitro* (Wojdylo et al. 2007; Oboh et al. 2012).
(4) Good sources of phenolic compounds with superior antioxidant capacity (Pellegrini et al. 2006; Carlsen et al. 2010).
(5) Possession of good flavouring properties.

Mr. Vice-Chancellor, Sir, without spices, I am not sure if we could have got pepper soup, *usewu*, *suya*, *dodo Ikire* etc.! And we would not have been saying "*emi ti o je ata, emi yeepere ni*".

Whole spices may release flavour too slowly, but ground spices release their flavours more readily (Dziezak 1989). The contributions of essential oils and oleoresins to the flavouring characteristics of spices (Farrell 1990) cannot be over emphasized. At this juncture Mr. Vice-Chancellor, Sir, one may ask "why are spices having the aforementioned potentials? One clear way of answering is that spices have powerful phytochemicals. Phytochemicals are chemicals present in the leaves, stems, roots, flowers, fruits and seeds of plants' organs. Examples of phytochemicals are saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acid etc.

It is interesting to note that when phytochemicals with biological activities are consumed in foods some beneficial therapeutic effects can be noticed (Dorman et al. 2004; Shan et al. 2005).

Antioxidants in Biological Systems

Antioxidants are of interest to the food industry because antioxidants prevent rancidity (Dolliger 1991); antioxidants are also of interest to biologists and clinicians because they may help to protect the human body against damage by reactive oxygen species (ROS). Reactive oxygen species is a collective term which includes oxygen radicals and several non-radical oxidizing agents like hypoclorous acid and ozone (Halliwell et al. 1995). In biological systems, lipid peroxidation can occur mainly in bio-membranes where the content of unsaturated fatty acids is relatively high. Lipid peroxidation is a very complicated chemical and biological reaction involving free radicals, oxygen, metal ions and other factors (Jadhar et al. 1996).

Interestingly, in line with reactions that occur in foods, peroxidation can also cause changes in the human body. In the human body, where oxidation takes place, it is a metabolic process which leads to energy production necessary for essential cell activities. This normal metabolism of oxygen in living cells however leads to the unavoidable production of oxygen-derived free radicals and according to McCord (1994), production of these free radicals is approximately equivalent to a car's exhausting emissions that

result from incomplete combustion of fuel. Mr. Vice-Chancellor, Sir, some clinical conditions in which oxygen free radicals are thought to be involved (Aruoma 1994) are listed below:

Brain
- Parkinson's disease
- Neurotoxins
- Vitamin E deficiency
- Hypertensive cerebrovascular injury

Eye
- Photic retinopathy
- Ocular haemorrhage
- Cataractogenesis

Heart and cardiovascular system
- Atherosclerosis

Kidney
- Aminoglycoside nephrotoxicity
- Autoimmune nephrotic syndromes

Gastro-intestinal tract
- Oral iron poisoning
- Endotoxin liver injury
- FFA-induced pancreatis

Ageing

Iron overload
- Nutritional deficiencies (Kwashiorkor)
- Thalassaemia

Red blood cells
- Malaria
- Protophorphyrin photo-oxidation

Lungs
- Bronchopulmonary dysplasia
- Bleomycin toxicity
- Oxidant pollutants (ozone, SO_2, NO_2)

Ischaemia-reperfusion
- Stroke/myocardial infarction
- Organ transplantation

Source: Aruoma (1994)
FFA: Free fatty acids
(Skin injury due to solar radiation, porphyria, contact dermatitis and photosensitizers may also involve free radical mechanisms)

Mr. Vice-Chancellor, Sir, distinguished ladies and gentlemen, we have some relief with respect to these oxygen free radicals because when reactive oxygen species are generated in living systems, several natural antioxidant systems also come into play through up –regulating expression of genes encoding superoxide dismutase (SOD), catalase or glutathione peroxidase (Halliwell 1990; Aruoma 1994). Furthermore, phenolic compounds in spices can also inhibit formation of hydroxyl radicals (Pulla Reddy and Lokesh 1994).

Mycotoxins

In addition to oxygen free radicals, Mr. Vice-Chancellor, Sir, the human population worldwide still face the challenges posed by mycotoxins.The importance of mycotoxins cannot be overemphasized because food losses may be due to mycotoxins and mycotoxins have some adverse effects on human and [illegible] health with economic implications (Bhat and Vashanti 1999). According to the World Health Organization (WHO 1999a), up to 25% of the world's food crops were estimated to be significantly contaminated with mycotoxins.

Mycotoxins are acutely toxic, carcinogenic, mutagenic, teratogenic and estrogenic secondary metabolites of moulds and are produced on several commodities. Because of the toxic effects of mycotoxins and their high stability to heat treatment, the presence of mycotoxins in foods and feeds is potentially hazardous to the health of both humans and animals (Pittet 2001). Mr. Vice-Chancellor, Sir, ladies and gentlemen, it appears that as at today, mycotoxins have plagued mankind since the beginning of organized crop production. For example, according to Schoental (1984), ergotism was discussed in the Old Testament of the Bible and there are strong evidences that ergot alkaloids and *Fusarium* mycotoxins played a role in diseases from medieval Europe through to America's colonial times (Matossian 1989). The mysterious deaths of archaeologists after opening some Egyptian tombs have been linked to inhalation of mycotoxins particularly ochratoxin A which could have been responsible

for a series of acute renal failure incidents (Di Paolo et al. 1993). Mycotoxins are not controlled by geographical barriers for example in 1988 in Malaysia during the Chinese Festival of the Nine Emperor Gods, aflatoxins in a type of noodle were strongly implicated as the causative agent in the deaths of 13 children from the 45 victims affected (Chao et al. 1991).

According to Adegoke (2004), there are over 300 mycotoxins that have been isolated and characterized and examples are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin, trichothecenes, alternariol etc. In several African countries, aflatoxins have been given considerable attention. Kurtzman et al. (1987) associated the production of aflatoxins primarily to *Aspergillus flavus, A. parasiticus* and *A.nomius*. There are four main toxins produced by *Aspergillus* species and the toxins have been divided into B and G groups (B1, B2, G1 and G2). In addition to *A.flavus, A. parasiticus* and *A. nomius,* aflatoxins can be produced by *Aspergillus toxicarius* and *A. parvisclerotigens* on crops like corn, peanuts, cotton seeds and coffee beans. The importance of aflatoxins becomes real when it is known that aflatoxin contamination of cereals has been linked with cancer cases in Africa (Oettle 1964).

Aflatoxin contamination of crops like peanuts, corn and cottonseed does occur during active growth of the plant in the field (Moss 1989) although initially aflatoxin contamination was considered to be a post-harvest problem of poorly stored commodities. Contamination of agricultural commodities by aflatoxins is very difficult to control in the field because of climatic conditions like relative humidity and temperature. Moss (1991) also noted that soil moisture, drought stress, insect damage and mineral nutritive deficiencies as important factors that affect aflatoxin contamination of crops that may appear undamaged. However, the highest levels of aflatoxins contamination of crops are generally associated with postharvest growth of moulds of the genus *Aspergillus* on poorly stored commodities.

Mr. Vice-Chancellor, Sir, ladies and gentlemen it must be noted that a significant human exposure to aflatoxins can arise where undefined vegetable oils like groundnut oil are traditionally used for domestic consumption (Waghray and Reddy 1995). In the field, the following crops may be resistant or only moderately susceptible to aflatoxin contamination—soybeans, beans, cassava, sorghum, millet, wheat and rice. Cocoa beans, melon seeds, palm kernels, and sesame seed have also been reported to be contaminated by aflatoxins occasionally (Pittet 2001).

Ochratoxin A

Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin produced by *Asperigillus ochraceus, A. carbonarius, A.melleus, A. sclerotium* and *Penicillium verrucosum* (Benford et al. 2001). Ochratoxin A has been found in several commodities for example Aish et al. (2004) noted that OTA was found in wheat, corn and oats having fungal infection and in cheese and meat products of animal consuming ochatoxin (contaminated grains). Cassava flour, cereals, fish, peanuts, dried fruits, wine, eggs, milk, coffee, cocoa beans have been reported to be contaminated with OTA (Weidenborner 2001).

Mr. Vice-Chancellor, Sir, attention must be paid to ochratoxins as analyses of human serum samples from several European countries (IARC 1993; Breithottz-Emmanuelsson et al. 1994), North Africa (Maaroufi et al. 1996) and Canada (Scott et al. 1998) showed that blood from healthy humans frequently contained OTA—thus this could be a sign of continuous, widespread exposure to OTA.

In view of the importance of mycotoxins, attention must be given to the reduction of these toxic metabolites before they enter the food chains of human beings. Mycotoxin contamination of agricultural products can be prevented by using the following pre-harvest methods:

(1) Proper field management of agricultural commodities
(2) Use of biological and chemical agents
(3) Good harvest management.

After harvesting crops, the following methods can however be used to reduce mycotoxin contamination of agricultural commodities:

(a) Improved drying methods
(b) Good storage conditions
(c) Use of natural and chemical agents
(d) Irradiation (Kabak et al. 2006)

Mr. Vice-Chancellor, Sir, ladies and gentlemen, with respect to aflatoxins the following methods are useful for reducing aflatoxin contamination of agricultural commodities:

1. **Adequate drying of crops**
   Early harvesting and adequate drying of crops can help in reducing aflatoxin contamination. However, in several developing countries early harvesting, unpredictable weather, labour constraint, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Amyot 1983).

2. **Physical separation and processing**
   Physical separation of apparently contaminated cereals from bulk samples can be an effective way of reducing aflatoxin contamination. Thus, unit operations like sorting, winnowing, washing, crushing and dehulling are useful in reducing significantly the levels of aflatoxins and fumonisins in maize and maize products (Fandohan et al. 2005).

3. **Use of ozone**
   Alencar et al. (2012) reported that detoxification of aflatoxins with ozone in peanuts has been reported to be useful. Prudente and King (2002) even found a 92% reduction in aflatoxin when contaminated samples of corn were ozonized.

In addition to these methods, Mr. Vice-Chancellor, Sir, we have reported other strategies that can be adopted for preventing and reducing mycotoxins in agricultural commodities particularly in developing countries (Adegoke and Letuma 2013). For example, for ochratoxins, atmospheres greater than 30% (30-60% CO_2) can be used for preventing ochratoxin A (OTA) production during storage or transportation of cereals—a technique that can be adopted in developing countries (Paster et al. 1983). Roasting can also be used for reducing OTA levels in some agricultural commodities as van der Stegen et al. (2001) found reductions of OTA of up to 90% during coffee bean roasting. For preventing OTA from entering the food chain, the following post-harvest measures can be adopted particularly in developing countries:

(1) Regular and accurate moisture content measurements;
(2) Efficient and prompt drying of wet cereal grains for safe moisture levels (maize, 14%; rice, 13-14%);
(3) Provision of appropriate transport conditions;
(4) Appropriate storage condition of agricultural commodities at all stages with respect to moisture, temperature control, general maintenance and effective hygiene within storage facilities for prevention of pests (Magan and Aldred 2007).

Mr. Vice-Chancellor, Sir, with respect to fumonisins this group of mycotoxins are of considerable importance in maize producing and consuming nations, particularly in Africa as the mycotoxins have been linked with high incidence of human esophageal carcinoma in some parts of South Africa and even in China (IPCS 2000). Therefore, when is realized that fumonisins have been classified as possible human carcinogens (IARC 1993), the following strategies (Magan and Aldred 2007) are possible ways of controlling the group of mycotoxins:

1. **Pre-harvest measures**
   (a) Proper selection of maize hybrids, prevention of use of soft kernel hybrids;
   (b) Avoiding high cropping density and late dates for planting;
   (c) Good and balanced fertilization;
   (d) Avoiding late harvesting of crops;
   (e) Effective control of pests like corn borers.

2. **Post –harvest measures**
   (a) Effective cleaning of maize before storage
   (b) Minimizing periods between harvesting and drying
   (c) Efficient drying of maize to less than 14% moisture content
   (d) Effective hygiene and management of silos
   (e) Control of pests in stores/warehouses

Mr. Vice-Chancellor, Sir, distinguished ladies and gentlemen, it is interesting how nature has built in control mechanisms for fumonisins because insect damage of maize has been found to be a good sign of contamination by fumonisin (Avantaggio et al. 2002). As technological developments in our country Nigeria become more realizable, we can be planning now to use solar energy for controlling mycotoxin contamination of agricul-tural commodities. Afteralls in Pakistan, solar energy has been reported to be useful in reducing the incidence of corn ear rots, fumonisin and aflatoxins in the field and stored corns (Ahmad and Ghaffar 2007). The methods aforementioned for preventing and reducing mycotoxins before they enter the food chain must be given adequate attention because as Miller (1996) observed, 40% of the productivity lost to diseases in developing countries has been exacerbated by mycotoxins in particular aflatoxins.

Food Safety Issues

Mr. Vice-Chancellor, Sir, distinguished ladies and gentlemen, while it is very important to do everything possible to prevent

and reduce mycotoxins in the food chain, equally important is the issue of keeping our food safe. Then, what is food safety? Food is said to be safe if it will not cause any hazard to the consumer (Adegoke 2004) and a hazard is a physical, chemical or biological agent which if consumed can cause adverse effects. Unsafe food, according to the World Health Organization, causes many acute and life-long diseases to various forms of cancer (*http://who.int/food* safety, accessed on 20/03/2012). It is therefore noteworthy that in order to have more relevant knowledge on food safety globally, the World Health Organization in 2007 launched the Foodborne Disease Burden Epidemiology Reference Group (FERG) and Mr. Vice-Chancellor sir, distinguished ladies and gentlemen, my humble self was one of the three Africans chosen by WHO in 2007 to be a FERG member. The specific mandate of FERG included *inter alia* increasing the awareness of countries of and commitment to the implementation of food safety standards (*http://www.who.int/foodsafety/foodborne_disease*).

Ideally, the philosophy of food safety involves:

(1) Prevention
(2) Intervention and
(3) Response to reduce risks and contaminants in foods of animal and plant origins within the formal and informal food sectors

Generally, confidence in the safety and integrity of the food supply is an important requirement for consumers all over the world as foodborne disease outbreaks involving agents such as *Escherichia coli*, *Salmonella spp* and chemical contaminants give rise to problems with food safety and increase public anxiety that modern farming systems, food processing and marketing have not been able to provide adequate safeguards for public health (FAO/WHO 2003). I will now proceed to group the major research findings with my collaborators:

Group I: Microorganisms: isolation, identification and control
Group II: Spices and their bioactivities in food systems
Group III: Food safety

My Contributions to Microorganisms in Food Matrices

Mr. Vice-Chancellor, Sir, it may interest you that my first contact with microorganisms was in 1968 and it was not until 1980 that the first publication on microorganisms of public health importance appeared in literature. Akinboade, Adegoke, Ogunji and Dipeolu (1980) examined some parts of 60 African giant land snails (*Achachatina marginata*) and we isolated several microorganisms which were identified as *Escherichia coli*; *Klebsiella aerogenes*, *Aeromonas liquefaciens* and *Bacillus subtilis* (table 1).

Table 1: Bacteria isolated from Giant Snails

No of Snails	Bacteria Isolated
15	*E coli, K aerogenes*
15	*A liquefaciens, K aerogenes*
9	*K aerogenes, St albus*
6	*E coli, St albus*
3	*K aerogenes, Bacillus subtillis*
3	*A liquefaciens*
3	*B subtillis*

By 2014, Mr. Vice-Chancellor, Sir, ladies and gentlemen, we had processed and evaluated the quality of snail meat and found that the use of alum (hydrated potassium aluminum sulphate) and ginger and a combination thereof followed by brief boiling and oven-drying (80°C) gave snail meat with high microbiological and sensory profiles (Evbayiorvie 2014, unpublished data).

The usefulness of snail meat to human diet cannot be overemphasized. Smith and Ojofeitimi (1995) found that snail meat is high in protein but low in fat. Elmsilie (1982) even noted that land snail has more crude protein and less fat than chicken. Thus, snail meat is a healthy alternative food for

mankind particularly with respect to poverty alleviation and malnutrition.

Mr. Vice-Chancellor, Sir, following our publication on microbes of public health importance in snails in 1980, the impetus for academic works thereafter started with my M.Sc project on characterization of staphylococci from farm animals in 1981. Characterization of these microorganisms was done using: coagulase activities and antibiotic sceptibility patterns, Thus, out of 120 isolates of staphylococci isolated from clinically healthy and sick goats from University of Ibadan's Teaching and Research Farm, field samples from dairy farms in Oyo, Ondo, Ogun, Lagos and Anambra states, multiple antibiotic resistance was found among the isolates and three even showed resistance to methicillin. It is interesting to note that out of the 120 caprine isolates of staphylococci, 70.8% were identified as *Staphylococcus aureus* (Adegoke 1981).

My findings on staphylococci of caprine origin was in agreement with the findings of Love (1972) who noted that domestic animals may be a source of staphylococci which are pathogenic for man. To further differentiate staphylococci of caprine origin from human strains, Adegoke and Ojo (1981) used tests for production of beta-haemolysin and fibrinolysin and we found that of 120 caprine strains of staphylococci examined, 36 (30.0%) produced beta-haemolysin but no fibrinolysin, while 20 (16.7%) produced both. Interestingly, we had sixty-four isolates that produced either beta-haemolysin or a fibrinolysin alone or produced neither beta-haemolysin nor fibrinolysin and we grouped them as "unclassifiable" staphylococci (Adegoke and Ojo 1981). From these "unclassifiable" staphylococci, I used phenotypic, chemotaxonomic and genetic methods for the description of a novel staphylococcal strain – *Staphylococcus ovis*, ATCC 43624. Interestingly, some of my staphylococcal strains isolated different sources formed part of the collection used for the description of *Staphylococcus carnosus*. Furthermore, the methods used for the characterization of *Staphyloccus ovis* ATCC43624, were incorporated into the identification

scheme of Devriese, Schleifer and Adegoke (Devriese et al. 1995). The scheme of Devriese, Schleifer and Adegoke is useful up till now for classifying (mostly of animal origin) into novobiocin-sensitive species like *Staphylococcus hyicus, Staph. simulans, Staph. haemolyticus* and novobiocin-resistant species like *Staph. sciuri, Staph. lentus, Staph. saprophyticus* and *Staph. gallinarum*. As a follow-up to the scheme of Devriese, Schleifer and Adegoke, I developed a scheme to facilitate identification of *Staph. lentus, Staph.sciuri* and *Staph.gallinarum* (Adegoke 1988) and this scheme of Adegoke continues to be a reference point on staphylococci.

Mr. Vice-Chancellor, Sir, apart from staphylococci, my collaborators and I have also worked on other important microorganisms in food matrices. Adetunji and Adegoke (2007) screened some strains of lactic acid bacteria for their ability to produce bacteriocins and we found that *Lactobacillus plantarum* and *Lactobacillus brevis* produced bacteriocins which inhibited the growth of *Listeria monocytogenes* and *Escherichia coli* 0157:H7. Bacteriocins are peptides or proteins released extracellularly and they show bactericidal activities against species to the bacterocin-producing strain. With respect to lactic acid bacteria, Zottola et al. (1994) noted that this group of bacteria and their metabolites play an important role in improving micro-biological quality and shell life of many fermented food products and they provide a good example of bio-preservation. Furthermore, Rodriguez (1996) found that lactic acid bacteria notably *Lactococcus lactis* produced lisin, a bacteriocin which is active against several gram positive bacteria. Nisin is non-toxic, heat stable, does not contribute to off-flavours or off-odours and according to Jay (2000), nisin has some levels of antimicrobial activities. Nisin is a generally regarded as safe (GRAS) product and it has been approved as a food additive (Holzapfel et al. 1995). Adam and Moss (1995) noted that nisin is a polypeptide containing 34 amino acids and it is heat stable at acid pH. The inhibition of food pathogens- *Listeria monocytogenes* and *Escherichia*

coli by bacteriocins produced by lactic acid bacteria isolated by Adetunji and Adegoke (2007) is similar to the findings of other worker (Tarelli et al. 1994). Even Buyong et al. (1998) genetically modified *Lactobacillus lactis* to produce pediocin which in turn inhibited the growth of *Listeria monocytogenes.*

Listeria monocytogenes is a pathogen of man and animals. In man, people at risk with *Listeria monocytogenes* are pregnant women, aged people equal to or more than 65 years of age who have cancer, diabetes, kidney diseases, acquired immunodeficiency syndrome or who are on immunosuppressive medications. Infections with *L. monocytogenes* in pregnant women can lead to premature delivery, miscarriage and stillbirth. *Listeria monocytogenes* also causes abortions in cows and ewes (Adegoke 2004). Mr. Vice-Chancellor, Sir, if the name *Listeria monocytogenes* is dreadful, handling the pathogen is even more dangerous. However, my collaborator, Professor Brent Skura (University of British Columbia, Vancouver, Canada) and I did our best to handle this dangerous foodborne pathogen. We used the aqueous solution of the spice *Aframomum danielli* to control the growth of the pathogen and we found that after a 21-day growth at 4°C and at 35°C/24 hours, we found that the minimum inhibitory concentra-tion (MIC) of *A.danielli* for *Listeria monocytogenes* was 390 ppm (table 2) that is, at 390 ppm , we did not find any growth of *L. monocytogenes* while in the control sample we used we found luxuriant growth of the pathogen (Adegoke, Fasoyiro and Skura 2000). In their work with *Listeria monocytogenes* Pandit and Shelef (1994) found that the growth of the pathogen was suppressed at 5°C and 35°C when they used 5,000 ppm of rosemary. However, we found that the listericidal activities of the spice *A. danielli* both at 4°C and 35°C (Adegoke, Fasoyiro and Skura 2000) were more than those reported for cloves and oregano (Ting and Deibel 1992) and some Chinese plant extracts (Chung et al. 1990).

Table 2: Inhibition of *Listeria monocytogenes* a LCDC 81861 by different concentrations of aqueous solution of *Aframomum danielli*

A. danielli (ppm)	4°C/21 days	35°C/24h
195	+	+
390	+[c]	+
780	-	+
1560	-	+
3120	-	-
6250	-	-
12500	-	-
25000	-[b]	-
Control (no spice)	+	+

[a] Initial concentration 10^5cfu/ml
[b] No survivors
[c] When trypticase soy agar containing 4% NaCl was used, the minimum inhibitory concentration was also 390 ppm

In addition to the listericidal activities of the spice *Aframomum danielli*, we have found that the spice has potent antimicrobial effects on the foodborne pathogen, *Bacillus cereus* and some food spoilage yeasts. Using the aqueous solution of *A.danielli* (500 ppm), we did not find any viable cell of *Bacillus cereus* after a 4-hour exposure and the minimum inhibitory concentrations of *A.danielli* for the yeasts *Candida tropicalis, Hansenula anomala, Torulopsis candida* and *Kluveromyces thermotolerans* ranged from100 to 200 ppm (table 3).

Mr. Vice-Chancellor, distinguished ladies and gentlemen, it is at this point that I have to present more facts on the bioactivities and potential applications of the spice *A.danielli*.

Table 3: Minimum Inhibitory Concentration (MICs) and minimum Fungal Concentrations of terpenes for some food spoilage yeasts and aflatoxigenic moulds

	Terpenes							
	α-terpinene		(+)-limonene		α-pinene		1,8-cineole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Yeasts								
T. candida	4.9*(1)	9(2)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
K. fragilis	39(2)	312(5)	312(5)	312(5)	78(4)	312(5)	78(4)	312(5)
K. thermotolerans	78(4)	312(5)	39(2)	156(3)	78(4)	312(5)	156(3)	312(5)
C. tropicalis	156(3)	156(3)	312(5)	312(5)	156(3)	312(5)	156(3)	312(5)
P. pastoris	312(5)	625(6)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
H. anomala	312(5)	1250(7)	312(5)	312(5)	156(3)	625(6)	156(3)	625(6)
Moulds								
A. flavus	625(6)	1250(7)	312(5)	625(6)	ND**	ND	ND	ND
A. parasiticus	312(5)	312(5)	78(4)	312(5)	ND	ND	ND	ND

*μg/ml; () – mean of three replications: thus (1) – 4.1±1.4; (2) – 32.5±11.3; (3) – 130±45.0; (4) – 65±22.5; (5) – 260±90.1; (6) – 520±180.4; (7) – 1041±360.4.

**ND – not determined, as previous studies showed weak reactions with moulds.[11]

My Contributions to Uses of Spices

My collaborators and I have been working for more 20 years on some indigenous spices like *Xylopia aethiopica, Aframonum melegueta, Aframomum danielli, Monodora myristica, Piper guineense,* and *Congronema latifolia.* When we added 1% (w/w) of *X. aethiopica, A.melegueta, M. myristica, P. guineense* and *C. latifolia* (non-commercial spices) to tomato ketchup we prepared in the laboratory, there were reductions in microbial populations to different levels. For example, tomato ketchup spiced with *M. myristica* remained sterile after incubation at room temperature (26±2°C) while the control sample had 1.5×10^7 cfu/g (Adegoke and Sagua 1993).

Mr. Vice-Chancellor, Sir, ladies and gentlemen, of all the spices we have worked with, we have found that *Aframomum danielli* has got useful bioactivities and great unexplored economic potentials. *Aframomum danielli* belongs to the genus *Aframomum* of the family Zingiberaceae which is closely related to *Ammomum* Roxb from tropical Asia (Lock 1985). *Aframomum* has several species spread over West and East Africa and even *Elletaria cardomomum* (L). Mator, the source of commercial cardamons belongs to the family Zingiberaceae (Lock 1985). In our studies, the spice *A. danielli* on a wet basis with a moisture content of 10.5%, protein of 8.2% (dry matter basis) and calorific value of 469.7kg/100g has in varying amounts of minerals like calcium, magnesium, sodium, manganese, phosphorus, zinc and copper (table 4).

Table 4: Nutritional Composition of the Spice *A. danielli*

	B. danielli	Spice cardamon[a]	Garlic powder[a]
Mineral/Amino acid (m/100g)			
Calcium	70	383	80
Magnesium	110	229	58
Zinc	3.8	7	3
Phosphorous	190	178	417
Sodium	10		
Copper	0.8		
Manganese	33.5		
L-Aspartic acid	0.05		
L-Threonine	0.02		
L-Serine	0.02		
L-Glutamic	0.1		
L-Proline	0.02		
Glycine	0.03		
L-Alanine	0.03		
L-Valine	0.03		
L-Methionine	0.01		
L-Isoleucine	0.02		
L-Leucine	0.05		
L-Tyrosine	0.02		
L-Phenylalanine	0.02		
L-Histidine	0.02		
L-Lysine	0.02		
Ammonium chloride	0.03		
L-Arginine	0.10		

Protein=8.2%; calorific value =469.7 kcal/100g
[a]: from Adegoke and Skura (1994)

Our deep interest in the spice *A. danielli* arose from our findings with the broad spectrum antimicrobial profile of the spice as it inhibited the growth of foodborne pathogens like

Salmonella Enteriditis, Pseudomonas fragi, Streptococcus pyogenes (ATCC 19615); *Staphylococcus aureus*, (ATCC 25923); *Aspergillus flavus* (ATCC 36061); *Aspergillus parasiticus* (ATCC 34635) and *Aspergillus ochraceus* (Adegoke and Skura 1994). Furthermore, Adegoke, Fasoyiro and Skura (2006) found that the aqueous extract of the spice *A. danielli* was active against food spoilage yeasts like *Candida tropicalis, Hansenula aromala, Torulopsis candida* and *Kluveronyces thermotolerans*. The broad spectrum antimicrobial activities of the spice *Aframomum danielli* are interesting and are possibly due to any of the following—perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane-embedded enzymes (Cox et al. 2000), membrane disruption (Caccioni et al. 2000), or cell wall perturbation (Odhar et al. 2002).

On our part, Mr. Vice-Chancellor, Sir, we used nuclear magnetic resonance (NMR) spectroscopy to confirm the antimicrobial effect of the components of the spice *Aframomum danielli* on some yeasts and we found that α-terpinene and (+) – limonene caused membrane injury of *Candida tropicalis* – a food spoilage yeast (Adegoke, Iwahashi, Komatsu, Obuchi and Iwahashi 2000) thus we confirmed the effect of terpenoids on biological membrane of susceptible organism (Knoblock et al. 1989). Of interest to us working on the spice *Aframomum danielli* is the fact that prior to our publication of 1999 on *A.danielli*, there was a paucity of data on the spice in literature but presently, there are useful publications in literature. Kimbu et al. (1979) isolated aframodial from *A. danielli* and Odukoya et al. (1999) reported on the lipoxygenase inhibitors and the seeds of *Aframomum danielli*.

Bankole and Somorin (2010) found that *A.danielli* aqueous extract was active against some rice moulds like *Aspergillus flavus, A. niger, Penicillium citrinum, P. oxalicum, Fusarium oxysporum* and *F. proliferatum*. Ashaye et al. (2013) processed roselle juices at 1:1 and 1:2 with or

without the spice *A. danielli* at 15% concentration and stored the samples for 2 weeks at room temperature. After one week of storage, Ashaye et al. (2013) found that roselle grape juice mixed with *A. danielli* (1:2) had acceptable nutrient and sensory qualities and the vitamin C content of samples examined by the authors were significantly higher ($p>0.01$) than the control samples used.

Using aqueous solution of *Aframomum danielli* at 6, 8 and 10%, Jatto and Adegoke (2010) found that with cashew juice nutrient stabilization particularly vitamin C was possible for 2 weeks at room temperature (25°C) during the 2 week-storage of cashew juice treated with *A. danielli,* there was reduction in the sugar content of the juice. Mr. Vice-Chancellor, Sir, ladies and gentlemen, the sugar-lowering capabilities of the spice *Aframomum danielli* is of considerable importance to us because of the link between sugar and diabetes. The World Health Organization (WHO 1999b) defined *diabetes mellitus* type 2 (T2D) as a metabolic disorder characterized by hyperglycemia, insulin resistance and beta-cell dysfunction. Furthermore, the World Health Organization (WHO 1999b) noted that diabetes is one of the leading threats to worldwide public health and a major cause of global death. As we know presently, two enzymes, α-amylase and α-glucosidase are very important in the management of diabetes type 2 (T2D). Inhibition of α-amylase and α-glucosidase which are involved in the digestion of carbohydrates (McCue et al. 2005; Ali et al. 2006), prolong overall carbohydrate digestion time causing a reduction in the rate of glucose absorption and consequently reduce postprandial plasma glucose rise (Bhadari et al. 2008). The control of postprandial hyperglycemia is an important step in the control of *diabetes mellitus* and concomitantly reduction of chronic complications associated with the disease (Ali et al. 2006).

Mr. Vice-Chancellor, Sir, when we added soy milk juice samples to powdered extract of the spice *Aframomum danielli* and determined experimentally the functionality of the spice in reducing glycaemic loads of treated samples, it is

interesting what we found out: while untreated (control) samples had glycaemic load (GL) of 10.26 (table 5) treated samples had 6.11 (Dauda and Adegoke 2014a). Our findings are similar to the reports of Pham et al. (2007) who used the spice cinnamon. Furthermore, Suryanarayana et al. (2005) found that curcumin and turmeric were effective against the development of diabetic complication in rats. There are also reports in literature that natural antioxidants have been used for the management of diabetes (Srinivasar 2005; Golbidi et al. 2011).

Table 5: Glycemic Load of treated Soymilk Juice Samples

Sample	Treatment and Glycemic Load of Samples		
A.danielli	**3g**	**2g**	**1g**
CWPS	7.20	7.09	6.11
R	10.26	10.26	10.26

CWPS: Blend of carrot, water melon, pawpaw and soymilk; R: Untreated samples

We are indeed very happy that the crude extract of the spice *Aframomum danielli* could produce low GL values and with we believe that with the consumption of the spice *A. danielli* and other spices, vegetables, legumes and fruits (fig. 4), management of T2D and its complications (Ramallah et al. 2010) is possible.

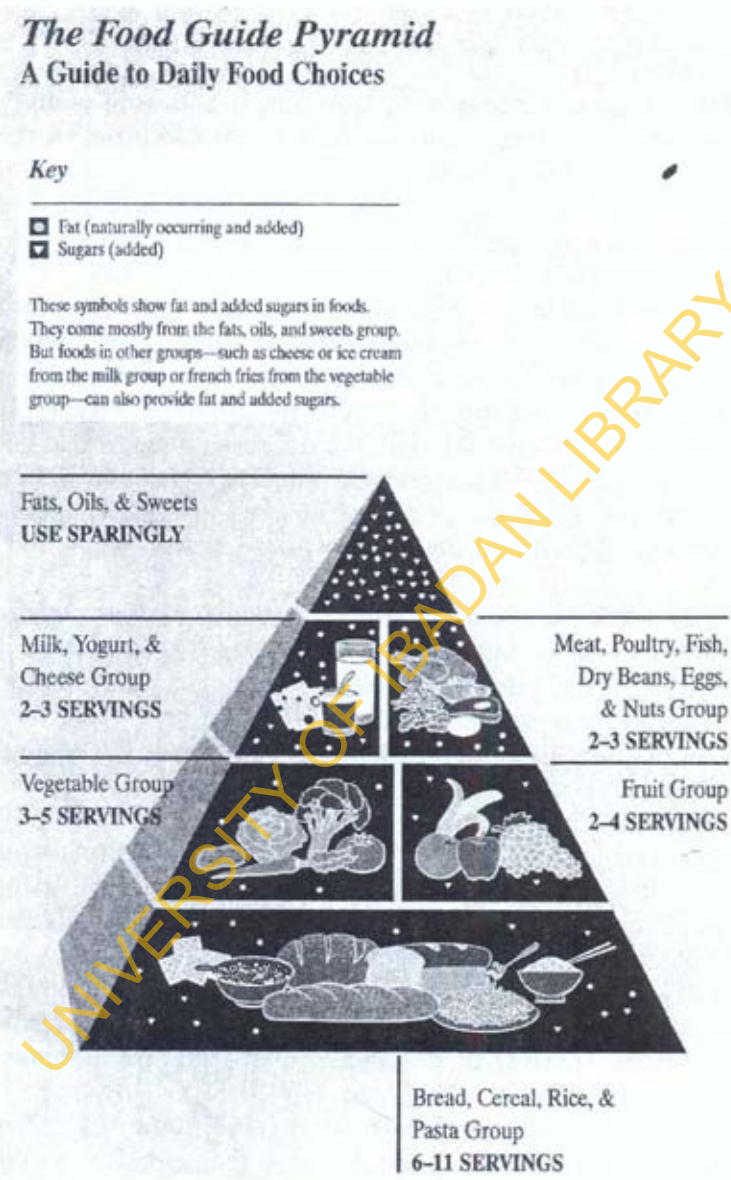

Fig. 4: The food guide pyramid.

Source: U.S. Department of Agriculture/U.S. Department of Health and Human Services

At this juncture it is essential to present 2 basic facts:

Fact 1: glycaemic response is how much and how quickly glucose enters the blood stream as a result of carbohydrate consumption. When high glycaemic response- inducing carbohydrates are consumed, there is an upsurge in glucose level and when this gets too high/much in the blood stream, insulin production by the pancreas sets in (insulin is a hormone charged with removing excess sugar (glucose) from the blood and it helps to stabilize blood sugar level). When the body does not use glucose (or energy) it converts to fat and has to store it – a process called "insulin trap". When this happens, it leaves the body with the impression that it had got more energy than it requires and this leads fat conversion being stopped. Thus, the more we consume insulin-producing carbohydrates, the more we have "insulin-traps".

Fact 2: when one realizes that a healthy adult's average normal total blood sugar is around 5 grams which is about 1 teaspoon and when this is compared to a single sip of 300 ml of soft drink, this amounts to about 40 gram or 8 times that amount of sugar. It is therefore nice to watch out for insulin-inducing carbohydrates in our diet.

Mr. Vice-Chancellor, Sir, my collaborators and I are not only excited because of the effects of the spice *Aframomum danielli* on glycaemic load, we are equally happy that using the powder of the spice, we have observed the liver cells can be protected. The crude extract of the spice *Aframomum* danielli has been found to exert no hepatotoxic effects on test albino rats, as serum enzyme levels of glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) and alkaline phosphate (ACP) were lowered by 67.0%, 86.3% and 49.7% respectively (table 6) when compared with control albino rats not fed with *A. danielli* powder in their meals (Adegoke, Gbadamosi et al. 2002).

Table 6: Toxicological Evaluation of *Aframomum danielli* on Albino Rats

Treatment	Enzymes			Total body weight (g)			Organ weight (g)		
	ALP	AST	ALT	Initial	After starving	Liver	Kidney	Heart	Lung
Control	98.0±1.0[a]	69.3±5.13	29.7±1.53	119±1.11	74.1±0.17	3.72±0.04	0.93±0.01	0.27±0.01	0.80±0.07
100 mg/kg	49.3±5.69	23.0±7.0	5.0±5.00	118.8±1.44	74.0±0.10	3.73±0.04	0.93±0.02	0.26±0.02	0.83±0.02
200 mg/kg	40.6±1.73	22.0±2.0	1.43±4.04	119.0±0.67	74.1±0.06	3.71±0.02	0.87±0.07	0.28±0.02	0.79±0.01

Values are the means of three replicates; ± values specify standard deviation. Treatments are in the order of low dose at 100 mg/kg (treatment 1) and high dose at 200 mg/kg (Treatment 2); ALP, AST and ALT were measured in international unit per litre (IU/L). Total protein was measured in g/100ml; Body and organ weights were measured in grams (g); ALP=alkaline phosphatase, AST (GOT)=aspartate aminotransaminase (glutamate oxaloacetate), ALT (GPT)=alanine amino transaminase (glutamate pyruvate transaminase)

The hepato-protective activities of the spice *A. danielli* are similar to the effects of herbal plants like *Tinospora cordifolia, Aloe Vera* and *Mangifera indica* (Singh et al. 2010; Saleh et al. 2008).

In addition to the hepato-protective capabilities of the seeds of *Aframomum danielli* we have noted that antioxidants produced from *A.danielli* were more potent than synthetic ones (Adegoke and Gopalakrishna 1998). According to Halliwell and Gutteridge (1989) and Halliwell (1990), an antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. The antioxidant extracts, my collaborator and I prepared from the spice *A. danielli* were generally more effective than butylated hydroxytoluene, α- tocopherol in stabilizing refined peanut oil (Adegoke and Gopalakrishna 1998). At 200 ppm, when the antioxidant extracts of *A. danielli* were added to peanut oil, the antioxidant effectiveness obtained were: DFADM (87.3%) > ADM (85.3%) > ADEE (83.4%) = *tert* butyl hydroquinone (83.4%) on day 20 of storage in an oven maintained at 65±1^{0}C. This formed the baseline information of the patent I obtained on natural antioxidants (Adegoke 2005). We have identified the antioxidant components from the seeds of *A. danielli* as phenolic compounds of trihydroxyl type with reducing properties (Adegoke and Gopalakrishna, 1998). Using ultraviolet, infrared and proton nuclear magnetic spectroscopy, we have now confirmed benzo-quinone as the active component of the antioxidant extract from the seeds of *Aframomum danielli* (Fasoyiro, Adegoke and Idowu 2006).

Mr. Vice-Chancellor, Sir, as soon as we found that the spice *Aframomum danielli* has some powerful antioxidative properties, we proceeded to add the spice to groundnuts and 'akara' in order to stabilize them against oxidation. Thus, when roasted peanuts (*Arachis hypogea*) were treated with 100, 200, 300 and 400 ppm of *A. danielli* followed by packaging in high density polyethylene and storage at 30^{0}C,

we found that the antioxidant effectiveness of *A. danielli* for treated peanut samples were 71.0%, 73.0%, 80.3% and 92.1% respectively (table 7). Furthermore, peanut samples treated respect with 200, 300 and 400 ppm of *A. danielli* antioxidant did not show any fungal growth within 21 days of storage (Adegoke, Falade and Babalola 2004).

Table 7: Fungal Counts of Control and *A. danielli* Antioxidant extract treated Roasted Peanuts

Sample treatment	Storage time (days)				
	0	7	14	21	28
Roasted (Control)	$2.3x10^2$	$4.5x10^2$	$6.2x10^2$	$9.0x10^2$	$4.1x10^2$
Roasted + 100ppm	$1.1x10^2$	$7.1x10^2$	$3.2x10^2$	$1.6x10^2$	$1.2x10^2$
Roasted +200 ppm	No growth	$8.0x10^2$			
Roasted 300ppm	No growth	No growth	No growth	No growth	$7.0x10^2$
Roasted +400ppm	No growth	No growth	No growth	No growth	$4.0x10^2$

Means with similar letters are not significantly ($p>0.05$) different using Duncan multiple range test. Data are means of 3 replicates, ±SD

The stabilization of peanut samples treated with antioxidant extract from *Aframomum danielli* is in agreement with the findings of Coulter (1988) who noted that traditional phenolic antioxidants are useful for extending shell life of foods. When we added antioxidant extract from *A. danielli* to 'akara' (a deep fat-fried product from cowpea) at 100, 200, 300 and 500 ppm, we obtained average antioxidant effectiveness of between 38.28% and 74.72% respectively (fig. 5) when we used groundnut oil (Falola, Okoro and Adegoke 2008). Antioxidant effectiveness was calculated from;

$$\frac{\text{Peroxide value (PV) of control} - \text{PV of test}}{\text{PV of control}} \times 100$$

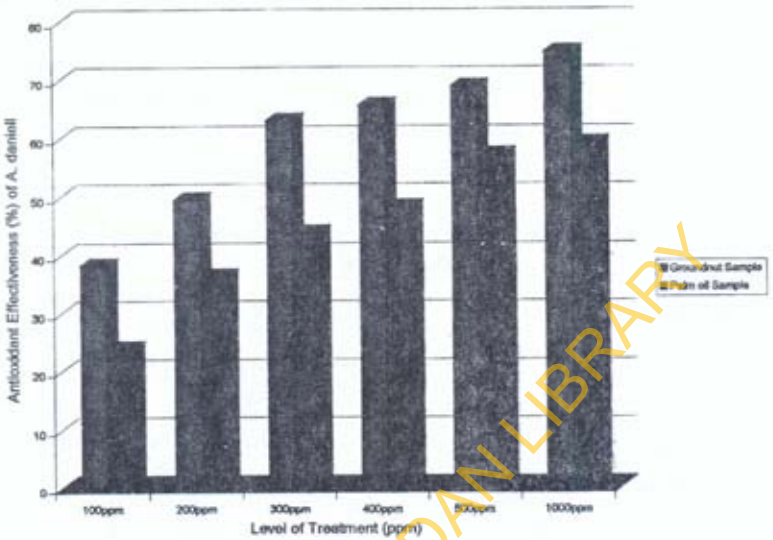

Fig. 5: Antioxidant effectiveness (%) of *A. danielli* on treated "akara" balls.

Generally for stabilizing mayonnaise against oxidation, synthetic chemicals are added to the egg-based product. When we added antioxidant extract of *A. danielli* at 200 ppm and compared the treatment with 200 ppm of butylated hydroxyl anisole (BHA)—a synthetic antioxidant—the ability to retard lipid oxidation (antioxidant effectiveness) of *A. danielli* was more than that of BHA. We have also found that antioxidant effectiveness of *A. danielli* is solvent – dependent as we noted, that on the 60th day of storage of treated mayonnaise samples, we had antioxidant effectiveness of 83.3%, 80.3% and 78.7% when we used diethyl ether, ethanol and α-hexane respectively (Etti, Adegoke and Etti 2012).

In vitro models have been used to assay acetyl-cholinestrase inhibitory activity and antioxidant properties of phenolic extracts from *A. danielli* (Adefegha and Oboh 2012). For the determination of the phenolic composition of the seed extracts of *A. danielli*, Adefegha and Oboh (2012) used reversed phase high performance liquid chromatography (RP – HPLC) and gas chromatography coupled with flame

ionization detector (GC – FID). The authors noted that *Aframomum danielli* extracts exhibited acetylcholinestrase inhibitory activity in a dose-dependent manner (125 – 1000 μg/ml). The seeds of *A. danielli* were found to have chlorogenic acid and p-hydroxybenzoic acid in abundance. The presence of phenolic compounds in *A. danielli*, antioxidant property of the spice and inhibitory effects on acetylcholinestrase were linked by Adefegha and Oboh (2012) to the protective effect of *A. danielli* against oxidative stress in the brain.

Mr. Vice-Chancellor, Sir, ladies and gentlemen, God has endowed human beings with many beneficial plant foods that have antioxidants. According to Chao et al. (1991) garlic, spinach, Brussels sprouts, alfalfa, broccoli and beets have excellent antioxidant capacities. Interestingly, garlic and the spice *A. danielli* belong to the family Zingiberaceae. Good antioxidant activities have also been associated with grapes and wine and they are known to reduce the incidence of cardiovascular diseases (Kinsella et al. 1993; Yi et al. 1997).

Mr. Vice-Chancellor, attempts to reduce problems associated with microbial pathogens, browning and lipid oxidation in foods have centered on the use of synthetic chemicals (Monsalve – Gonzales et al. 1993; Sofos and Busta 1983; Eckert 1979). With the loss of registration of benlate (Roberts and Reynold 1994), report on the harmful effects of sulphiting agents on sensitive consumers particularly asthmatics (Taylor et al. 1986) and the adverse reports in literature on synthetic antioxidants (Adegoke et al. 1998), it is the right time we considered useful alternatives to synthetic chemicals particularly in our food matrices.

The spice *Aframomum danielli* is non-toxic but hepato-protective (Adegoke et al. 2002) and we have found that the spice can be used to control browning, microbial growth and lipid oxidation (Adegoke, Fasoyiro and Skura 2002). The problems associated with browning and lipid oxidation in the food industry are enormous. According to McEvily et al. (1992), the shelf life of maximally processed fruits and vegetables can be limited by enzymatic browning during

storage. Considering the adverse reports on synthetic antioxidants (Branen 1975; Ito et al. 1986), we found that antioxidant extracts from *A. danielli* are effective in stabilizing oils against oxidation. When we used the antioxidant extract from the spice *A. danielli* and compared it with rosemary and α-tocopherol in soybean oil at 200 ppm and 300 ppm respectively, we found that *A. danielli* antioxidant extract was as active as rosemary and better than α-tocopherol (Fasoyiro, Adegoke, Obatolu, Ashaye and Aroyeun 2001). Adegoke and Gopalakrishna (1998) had earlier found that antioxidant extract from *A. danielli* was more effective than synthetic antioxidants like butylated hydroxytoluene (BHT) and α-tocopherol in stabilizing refined peanut oil.

Nutrient Stabilization by *A. danielli*

Mr. Vice-Chancellor, Sir, one of the healthiest fruits is tomato because it is packed with antioxidants like vitamin E, ascorbic acid, phenolic compounds and carotenoids. Carotenoids are an interesting group of compounds because the human body cannot synthesize them and they must be acquired from fruits and vegetables and once acquired, they provide strong protection against free radicals. Thus, the need to stabilize the useful nutrients in tomato. An important aspect of the bioactivities of the spice *Aframomum danielli* is its ability to stabilize nutrients of foods. When tomatoes (freshly harvested) were treated with 4 to 5% of *A. danielli* aqueous solution followed by storage under ambient condition (26 ± 2^{0}C), the ascorbic acid level was well maintained. Furthermore, while progressive reductions were noticed in the phenolic contents of tomatoes treated with $NaHCO_3$ and untreated (control), samples treated with 5% ($^{w}/_{w}$) *A. danielli* had higher phenolic contents than other samples examined (Babarinde, Adegoke and Akinoso 2013).

Table 8: Impact of *Afromomum danielli* aqueous extract on Ascorbic Acid (mg 100/g) of tomato stored at 26±2°C Storage Period

Treatment	Storage period (days)			
	0	5	10	15
1%	25.17±1.04^{a}	20.33±0.58^{b}	12.50±3.69^{c}	5.37±0.06^{b}
2%	25.17±1.04^{a}	20.67±0.58^{b}	14.33±0.57^{c}	2.27±0.06^{b}
3%	25.17±1.04^{a}	20.67±0.58^{b}	14.17±0.29^{c}	5.17±0.06^{a}
4%	25.17±1.04^{a}	20.50±3.50^{b}	13.67±0.00^{d}	5.13±0.05^{a}
5%	25.17±1.04^{a}	20.17±0.29^{b}	13.33±0.00^{d}	6.00±0.00^{a}
$NaHCO_3$	25.17±1.04^{a}	20.17±3.86^{b}	9.00±0.00^{c}	
Control	25.17±1.04	20.00±3.69^{c}	9.63±0.00^{b}	

Means with similar letters are not significantly ($p>0.05$) different using Duncan multiple range test. Data are means of 3 replicates, ±SD

When *Aframomum danielli* powder was added to blends of soymilk-based juice, followed by storage for 12 weeks, vitamin C losses were between 7.62% to 12.75% compared to vitamin C loss of 38.41% which was found with untreated (control) samples (Dauda and Adegoke 2014b). Our findings with *A. danielli* treated tomatoes have shown that the spice is capable of preventing the degradation of vitamin C and thus, *A. danielli* can be used for the retention of this important nutrient. Ozkan et al. (2004) noted that ascorbic acid is an index of nutrients quality of fruits and vegetable and it is very sensitive to degradation arising from food processing and storage conditions.

Flavouring and Preservative Profiles of *Aframomum danielli*

Mr. Vice-Chancellor, Sir, with my collaborators in Kenya, when we added very low concentrations of *A. danielli* as a flavouring to stirred yoghurt, we found that the organoleptic properties of the flavoured samples of yoghurt were not different from those yoghurt samples flavoured with strawberry and vanilla (Adegoke et al. 2013). We also identified flavour components like cycloheptane and 3-

methylbut-2-enyl (figs. 6, 7) which are known to provide desirable aroma and taste in yoghurt (Ott et al. 1997; Hubbers et al. 2004).

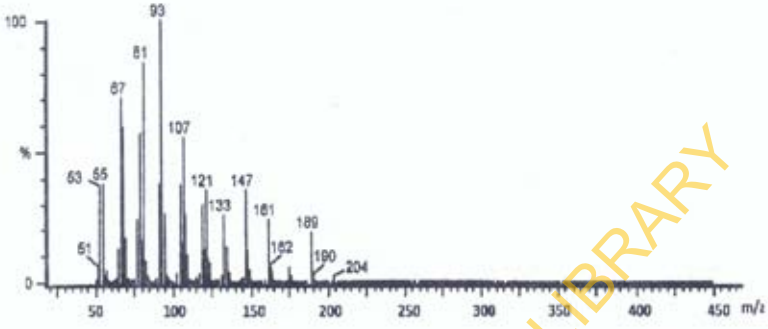

Fig. 6: Cycloheptane-flavour component of *A. danielli.*

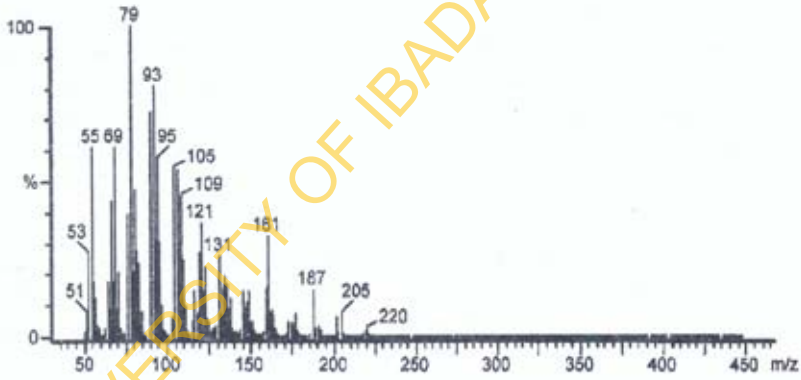

Fig. 7: 3-methylbut-2-enyl flavour component of *A. danielli.*

Mr. Vice-Chancellor, Sir, not much is known about probiotic yoghurt in several parts of the developing world. Consumption of probiotic yoghurt and indeed probiotic – containing foods is known to confer benefits like anti hypertension properties (Lye et al. 2009) reduction of LDL-cholesterol levels (Sindhu and Khetarpane 2003) and disruption of pathogenesis of hepatic encephalopathy (Solga 2003).

In addition to flavouring probiotic yoghurt with *Aframomum danielli*, we have also flavoured bread with the spice's extracts. We have used alveograph and consistograph to evaluate the physical properties of dough like water absorption capacity, tenacity, extensibility, strength of flour and peak-time of the flavoured bread. We have found that as the concentration of *A.danielli* in the dough increased from 0 to 4%, alveograph tenacity increased from 96 to 193 mm H_2O, extensibility decreased from 92 to 27mm, gluten decreased from 12.21 to 10.56mm, flour strength decreased from 365 to 255 while consistograph water absorption capacity increased from 56.8 to 58.9%. Using 2% *A. danielli* in bread, we found that from the results of sensory evaluation the spiced bread had colour, texture and uniform crumb grain similar to those of control bread samples (Adegoke, Awoyele, Lawal and Olapade 2008). So, Mr. Vice-Chancellor, Sir, bread may be bread but bread spiced with *A.danielli* is a better bread health-wise!

We have also examined the flavouring effects of *Aframomum danielli* on biscuits. When we compared *A. danielli*-flavoured biscuits with biscuits flavoured with 500 ppm vanilla, synthetic antioxidants (BHT/BHT, 200 ppm), control (unflavoured) samples and biscuits with 200 ppm *Aframomum danielli*, we obtained sensory scores (overall acceptability) of 4.3, 2.5 and 3.6 respectively. We did not use any synthetic antioxidant with the biscuit samples spiced with *A. danielli* thus, the spice has exhibited potential anti-oxidative and flavouring properties within the storage period of 10 weeks at 25°C (Afolabi and Adegoke 2014). With respect to the preservative capabilities of *Aframomum danielli*, Mr. Vice-Chancellor, Sir, ladies and gentlemen, when we added the powder of the spice to maize and soybeans and stored them under ambient conditions (26 ± 1^0C/RH 75±5%), we found that there were no mouldiness and insect infestation for 15 months. We also found that after eight weeks of storage, the chemical compositions of maize and soybeans (moisture content, 10%) treated with 4.0%, 6.0% and 8.0% of *A. danielli* did not differ significantly (P=0.05) from those of untreated (control) samples (Adegoke et al. 2002).

When we prepared 'wara' from fresh cow milk and added 1 – 3% (w/v) of *A. danielli* followed by storage at 27±2^{0}C for 6 days, we found that lipid oxidation was considerably retarded and antioxidant effectiveness of *A. danielli* was more pronounced at ambient condition (Ashaye, Taiwo and Adegoke 2006). Our findings with the preservation of 'wara' using *A. danielli* were similar to the observations of Aworh and Egounlety (1984) in their work with the preservation of West African Soft cheese using chemical treatment.

Daniellin ™

Mr. Vice-Chancellor, Sir, the name Daniellin™ was given by me to the patented dietary antioxidant produced from *Aframomum danielli* (Adegoke 2005; 2006). Daniellin™ with a moisture content of 2.1%, pH 7.0 is a powerful antioxidant because when it was added to fresh dairy cow milk at 50, 150 and 200 ppm, it gave some interesting levels of antioxidant effectiveness (table 9).

Table 9: Peroxide values and antioxidant effectiveness of Daniellin™ in dairy milk

	Time (h)			
Daniellin™ (ppm)	0	3	6	9
50	2.4	1.4(30)*	1.0(58.3)	0.8 (75)
150	2	1.2 (40)	0.7 (70.8)	0.6 (83.3)
200	1.8	1.1 (45)	0.5 (79.2)	0.4 (91.7)
Control	2	2	2.4	2.6

*% antioxidant effectiveness: (Peroxide value (PV) of control-PV of test)/PV of control

Mr. Vice-Chancellor, Sir, we are excited on the potential application of Daniellin™ because when we added it to the raw materials used for the production of 'kunu-zaki' (Adegoke, Odeyemi, Hussein and Ikheorah 2007), the concentration of ochratoxin A (OTA) a mycotoxin that can damage the kidney and also cause cancer (Vrabecheva et al. 2004), was reduced from 50 mg/kg to 1.5 mg/kg when Daniellin™ was used at 1.5%, 2.0% and 2.5% respectively during processing (table 10).

Table 10: Percentage reduction of OTA in kunu-zaki using Daniellin™

Sample	Ota level (μg/kg)	% Reduction
Untreated kunu-zaki	10	-
Kunu+0.5% Daniellin™	5	50
Kunu+1.0% Daniellin™	<2.5	75
Kunu+1.5% Daniellin™	<1.5	100
Kunu+2.0% Daniellin™	<1.5	100
Kunu+2.5% Daniellin™	<1.5	100

Sorghum used for kunu-zaki production had 50 μg/kg of OTA

Mycotoxins and *Aframonum danielli*

Mr. Vice-Chancellor, Sir, ladies and gentlemen, our works did not stop with the patented dietary antioxidant Daniellin™ from *A. danielli* rather with our interest in cocoa and cocoa products we found that the essential oil and aqueous extracts of the spice also reduced ochratoxin A (OTA) levels in cocoa products. When we used 0, 500, 1000, 2000, and 3,000 ppm of the essential oil and 0, 500, 700 and 750 ppm aqueous extract of *A. danielli*, we found that OTA was to our surprise reduced to zero by 2,000 ppm of the essential oil of *A. danielli* while the aqueous extract gave OTA reduction rates of 5.6 to 80% (Aroyeun and Adegoke, 2007). The importance of cocoa and cocoa products to our nation's economy before now and even presently cannot be overemphasized. Reduction of more than 90% of OTA has also been reported by Romani et al. (2003) but the authors used a final coffee temperature of 204^{0}C (dark roast). We have also found that at a maximum concentration of 60,000ppm, the powder of *Aframomum danielli* can be used as a bio- preservative agent for cocoa powder (Aroyeun et al. 2011).

Using response surface methodology (RSM) with dependent variables as water activity, *aw* (0.94 – 0.98), pH (5-9), temperature, (15-35^{0}C) and essential oil of *A. danielli* (500-2,500 ppm), we noticed that at water activity of 0.94-

0.98, pH 5-7, temperature of 20-25^{0}C and essential oil of *A. danielli* at 1500 ppm, the growth of *Aspergillus flavus* (a mycotoxin-producing mould) and aflatoxin B_1 production respectively were controlled (Aroyeun et al. 2013). Thus, with careful processes of growing, harvesting and storing cocoa beans, mould growth and mycotoxin formation can be controlled or reduced (Aroyeun, Ogunbayo and Olaiya 2006).

Hitherto, detoxification methods have been used to reduce the toxicity of mycotoxins but the issues pertaining to safety of reaction products and nutritional characteristics of foods so treated have been of concern (Piva et al. 1995) therefore, we explored alternative methods like using the essential oils of some spices to detoxify some mycotoxins. We used aflatoxigenic and ochratoxigenic moulds to contaminate cocoa beans and we treated the infected cocoa beans with *A. danielli* essential oil at 500, 100, 1500 and 2000 ppm. We found that after using reverse phase high performance liquid chromatography, 94.3% reduction efficiency was found particularly with aflatoxin B_1 (Aroyeun, Adegoke, Varga and Teren 2009). We have also used other methods to reduce effectively the levels of mycotoxins in foods. After washing 13 month-old cassava tubers followed by peeling, grating and fermentation (3-7 days, 28±2^{0}C) and with the addition of 1%, 0.75% and 0.25% sodium metabisulphite followed by other relevant unit operations for the production of gari, we found aflatoxin degradation (table 11) of 66%, 61% and 42.5% respectively (Adegoke, Babalola and Akanni 1991). Our using sodium metabisulphite for reducing aflatoxin contamination in cassava was because when 1% sodium metabisulphite was used to degrade aflatoxin in combination with heat (45-65^{0}C), degradation levels of aflatoxin B_1 in dried figs was reported to be 48-68 % (Altug et al. 1990).

We also have used processing to reduce mycotoxins in food matrices. During the processing of cassava to obtain cassava bread, we found that the bread had a final aflatoxin level of 0.03mg/kg but the initial level of aflatoxin in the raw cassava was 1.91 mg/kg (Adegoke, Akinnuoye and Akanni 1993).

Table 11: Degradation of Aflatoxin B1 (AFB1) in Gari and Lafun using some Chemicals

Sample	Gari					Lafun			
Sample code	CBCG	1B	2B	3B	4B	CS	3S	6S	9S
Control sample	348*								
Sodium metabisulphite:									
-- 1.0 %		131 (66 %)							
-- 0.75 %			150 (61 %)						
-- 0.50 %				225 (42 %)					
-- 0.25 %					244 (37 %)				
Control sample						150			
Hydrogen peroxide:									
-- 3 %							131 (13 %)		
-- 6 %								113 (25 %)	
-- 9 %									75.0 (50 %)

*AFB1 concentration (µg/kg); ( ): % degradation

Mr. Vice-Chancellor, Sir, we did not stop with cassava bread because when we produced 'tuwo' a cereal-based snack from sorghum, we found that heating at $\geq 100^0$C for 30 and 60 minutes produced aflatoxin reductions of 68% and 80.8% respectively. During the production of ogi, unit operations like steeping, fermentation and boiling gave a 100% aflatoxin reduction (Adegoke, Otumu and Akanni 1994). In the production of 'soyogi', Ogunsanwo et al. (1989) reported a 51.4% reduction in the aflatoxin level of the cereal-based product. Elsewhere, steam-cooking and washing of maize have been found to be responsible for 39% aflatoxin destruction (Celino and Madamba 1985). Thus, with the adoption of hazard analysis critical control point (HACCP) system involving effective handling, processing and storage of cereal and cereal-based foods, the problems associated with toxicants in foods (Fenwick et al. 1990) can be reduced.

Essential Oil Components of *A. danielli*

Mr. Vice-Chancellor, Sir, with my collaborators at the Central Food Technological Research Institute, Mysore, India

we analyzed the essential oil obtained from the seeds of *Aframomum danielli* by Gas Chromatography (GC) and GC-MS and we found that the oil contained 41 constituents of which 29 were identified for the first time. We also found that the major constituents of the oil of *A. danielli* from Nigeria were 1, 8-cineole (59, 8%), β-pinene (13.2%), α-terpineol (9.3%) α-pinene (4.3%) and α-terpinyl acetate (3.2%) (Adegoke, Rao and Shankaracharya 1998). α- terpinyl acetate is known to improve the flavour quality of cardamom oils (Guruduth et al. 1996).

When we used (+)- limonene, α-pinene and 1, 8-cineole (essential oil components of *A. danielli*) against some food spoilage yeasts and aflatoxigenic moulds, we found that with α-terpinene, the mean inhibitory concentrations (MICs) for some food spoilage yeasts like *Torulopsis candida*, *Kluveromyces fragilis*, *K.thermotolerans* and *Candida tropicalis* were 4.9, 39, 78 and 156 μg/ml respectively. Furthermore, with my collaborators in NIBHT, Tsukuba, Japan we found that within 60 minutes of exposure of *Candida tropicalis* to α-terpinene (312 μg/ml), the population of the spoilage yeast (table 12) was reduced from 10^5 to 10^3 cells (fig. 8). We also found that the MIC of (+) – limonene of the spice *A. danielli* for *Aspergillus parasiticus* – a mycotoxigenic mould was 78 μg/ml (Adegoke, Iwahashi, Komatsu, Obuchi and Iwahashi 2000).

We have used α-terpinene of *A. danielli* in combination with hydrostatic pressure synergistically. With a pressure of 1,800 kg/cm and 150 mg/ml of α-terpinene at 25^0C for 1 hour, we noticed a growth reduction of 3 log cycles with *Saccharomyces cerevisiae* (Adegoke et al. 1997). Without using hydrostatic pressure, we found that *S. cerevisiae* was resistant to 300 and 600 μg/ml of terpinene respectively (table 13). Thus, the application of low pressure (1800 kg/cm^2) in the presence of α-terpinene of *A. danielli* confirmed the findings of Popper and Knorr (1990) and Papineau et al. (1991) with respect to pressure on microorganisms being more effective in the presence of spice extracts.

Table 12: Minimum inhibitory Concentration (MICs) and Minimum Fungal Concentrations of Terpenes for some Food Spoilage Yeasts and Aflatoxigenic Moulds

	Terpenes							
	α-terpinene		(+)-limonene		α-pinene		1,8-cineole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Yeasts								
T. candida	4.9*(1)	9(2)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
K. fragilis	39(2)	312(5)	312(5)	312(5)	78(4)	312(5)	78(4)	312(5)
K. thermotolerans	78(4)	312(5)	39(2)	156(3)	78(4)	312(5)	156(3)	312(5)
C. tropicalis	156(3)	156(3)	312(5)	312(5)	156(3)	312(5)	156(3)	312(5)
P. pastoris	312(5)	625(6)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
H. anomala	312(5)	1250(7)	312(5)	312(5)	156(3)	625(6)	156(3)	625(6)
Moulds								
A. flavus	625(6)	1250(7)	312(5)	625(6)	ND**	ND	ND	ND
A. parasiticus	312(5)	312(5)	78(4)	312(5)	ND	ND	ND	ND

*μg/ml; () = mean of three replications: thus (1) = 4.1±1.4; (2) = 32.5±11.3; (3) = 130±45.0; (4) = 65±22.5; (5) = 260±90.1; (6) = 520±180.4; (7) = 1041±360.4.
**ND = not determined, as previous studies showed weak reactions with moulds.[11]

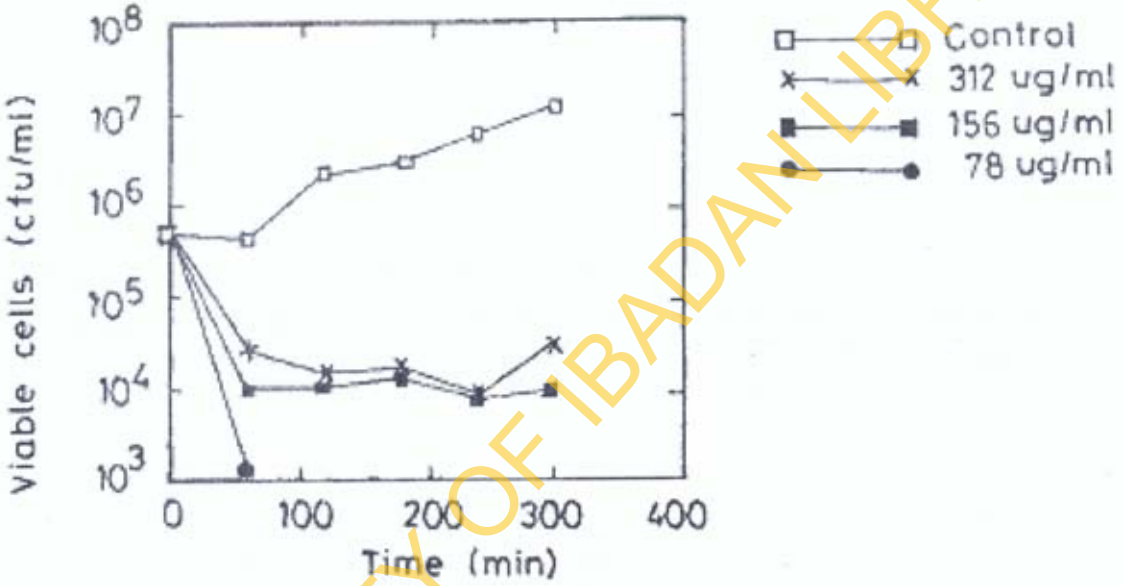

Fig. 8: Kinetics of inhibition of *C. tropicalis* by terpinine.

When cocoa powder and cocoa beverage were spiked with standard ochratoxin A (OTA) solution at 100, 120, 140, 160, 180 and 200 ppb (levels above acceptable European Union regulatory limit of 2 ppb for cocoa and cocoa products) followed by treatment with essential oil of *A. danielli* (0, 500, 1000, 2000 and 3000 ppm) and using standard detection methods for OTA, Aroyeun and Adegoke (2007) found that the oil of *A. danielli* at 2,000 ppm reduced OTA to zero (table 14). Ochratoxin A (OTA) is a very dangerous mycotoxin as it is nephrotoxic and carcinogenic (Vrabcheva et al. 2004) and the mycotoxin has been reported in cocoa powder in Ivory Coast, Guinea, Nigeria and Cameroon at levels of up to 4.4 mg/kg which are higher than the European Union regulatory level (Bonhevi 2004).

Table 13: Combination effects of Hydrostatic Pressure (1800 kg/cm^2) and Monoterpenes on Survival of *S. cerevisiae*

	S. cerevisiae (log cfu/mL)[a]	
Terpene (μg/ml)	**Pressure**	**No pressure**
-(+)- limonene		
0	6.8	8.3
220	5.9	8.7
440	5.6	8.0
2,200	3.9	6.3
α-terpinene		
0	7.0	9.0
37	6.4	9.0
75	4.1	9.0
150	2.5	8.6

[a] Mean of three viable counts

Table 14: Effectiveness of *A. danielli* Spice on OTA reduction in Cocoa Powder

Spike Levels (ppb)	Essentials oils (ppm)					
	5000	%(RE)	1000	%(RE)	2000	3000
100	30	70	10	90	nd	nd
120	40	66.7	10	91.7	nd	nd
140	40	71.1	10	92.9	nd	nd
160	50	68.8	10	93.8	nd	nd
180	40	77.8	10	94.4	nd	nd
200	50	75	10	95	nd	nd

RE: reduction efficiency; nd: not detected

Flower, Leaf and Stem of *Aframomum danielli*

Mr. Vice-Chancellor, Sir, after we found that the seeds of the spice *Aframomum danielli* possess antioxidative properties that are better than synthetic antioxidants like butylated hydroxyl anisole (Adegoke and Gopalakrishna 1998), we have now found that the flower, leaf, stem and root of the spice also possess potent bioactivities. Enzymatic and non-enzymatic mechanisms are known to be responsible for lipid oxidation in foods and according to Kanner et al. (1987), singlet oxygen which can be generated by the interaction of light, photosensitizers and molecular oxygen can initiate lipid oxidation by reacting with linoleic acid-containing foods. Furthermore, polysaturated fatty acids like linoleic, linolenic and arachidonic acids can be oxygenated by lipoxygenases and these enzymes have been associated with oxidative damage to foods during storage. In our work with 15-lipoxygenase (15-LO), we found that both the leaf and flower fractions of *Aframomum danielli* at 750 µg/ml gave 87% and 77% inhibition of 15-LO respectively confirming the potentials of the spice in stabilizing foods against oxidative damage during storage. Furthermore, we have found that extracts from the flower, leaf, root and stem of *A. danielli* inhibited the growths of foodborne pathogens like

Staphylococcus aureus, Escherichia coli, Salmonella Enteriditis and *Aspergillus flavus* (Afolabi, Adegoke and Mathooko 2011).

When we used 1-1, dipheny1-2-picrylhydrazyl (DPPH) for antioxidant determination, we found that at 1000 ppm, the flower and leaf of *Aframomum danielli* possess considerable antioxidant activities as the flower gave 80.55% and the leaf 67.18% (Adegoke, Afolabi, Fasoyiro, Babarinde, Ntakatsane and Skura 2013).

Mohdaly et al. (2011) noted that DPPH is a stable organic -free radical having an absorption band at 517 nm and it is usually used as a reagent to evaluate free radical – scavenging activity of antioxidants.

Table 15: Inhibition of 15-lipoxygenase (15-LO) Enzyme by Flower, Leaf, Stem and Root Fractions from *A. danielli*

	Enzyme inhition acitivity % of sample tractions		
Sample	**250µg/ml**	**500µg/ml**	**750µg/ml**
FF1	25.11±1.11	33.11±0.16	40.55±1.12
FF°	21.79±1.09	29.12±1.10	47.36±1.26
FF3	17.11±1.18	48.11±2.16	52.15±1.19
FF4	63.76±2.17	76.12±2.51	82.33±2.26
FF5	75.11±3.42	79.11±3.11	87.11±1.13
LFI	44.10±1.61	45.12±1.17	47.88±2.29
LF°	37.35±1.51	51.11±1.01	55.34±2.21
LF3	53.13±1.49	53.22±2.00	60.11±1.78
LF4	61.35±2.11	77.11±3.52	82.51±2.62
FL5	62.55±3.12	70.05±2.99	77.30±2.51
RF1	45.19±2.10	44.17±1.76	46.55±1.79
RF°	42.11±3.42	46.12±3.11	51.16±2.11
SF1	37.35±101	45.13±2.81	43.26±0.70
SF°	38.11±1.82	39.23±1.76	40.11±0.30

± -standard deviation, FF-flower fractions, LF-Leaf fractions, RF-Stem Fraction, BF- Stem fraction

Mr. Vice-Chancellor, Sir, we have found that the leaf and flower extracts of the spice *Aframomum danielli* can prevent browning of food products. When apple mashes were exposed to 1000 ppm of flower extract of *A.danielli* for 3 hours, we noticed that control samples produced 100% browning, ascorbic acid (antibrowning agent) and samples treated with *A. danielli* flower extract gave 34% and 32% browning effects respectively (Adegoke et al. 2013). Saper and Miller (1992) have associated the browning effect of ascorbic acid to its ability to reduce quinones to hydrophenols. We have thus associated the antibrowning effect of the flower of *A. danielli* to the antioxidant effectiveness of the spice and its reducing power which facilitate its ability to donate electrons.

Food Safety: My Contributions

Historically, it is only in the last 200 years that concepts involving foodborne germs, antiseptics and refrigeration became popular. Safety of foods is not only important to man, for example, while conline, a poison from the leaves of the plant hemlock, (*Conium maculatum*) is very poisonous to man, it is however unaffected by the bird called skylark (*Alanda arvensis*). Thus, some plants that are eaten by animals are very toxic to humans (Shaw 2013). Mr. Vice-Chancellor, Sir, we must thank God for giving us cells with internal food safety mechanisms which enable us to eat several plants- however cassava must be fermented to reduce or eliminate cyanide toxicity.

When one considers the pioneering roles of the US National Aeronautical Space Agency (NASA) in Hazard Analysis Critical Control Point (HACCP) system, the significance of food safety from the standpoint of astronauts' safety will be understood. For space travels, NASA generally sourced foods from reliable producers and the foods are cooked properly under ultra-hygienic conditions in a way to prevent post-contamination. This is how we came about the multi-faceted system called HACCP which is useful for addressing issues of pathogens, chemical contaminants, natural toxicants, additive safety and allergens (Shaw 2013).

Mr. Vice-Chancellor, Sir, what then is food safety? Food is said to be safe if it will not cause any hazard to the consumer (Adegoke 2004). According to the World Health Organization (WHO), unsafe food causes many acute and life-long diseases ranging from diarrhoeal diseases to various forms of cancer (*/http:who.int/foodsafety*).

Food safety can be well understood from the standpoints of food preservation and foodborne diseases. That is, unpreserved food is likely to get spoilt or damaged. Foods can be preserved generally by:

(1) addition of some specific inhibitory compounds like organic acids, sulphur dioxide and other food-grade chemicals that are safe for human consumption.
(2) removal of water as with lyophilization, freeze-drying or use of spray dryers; decreasing water availability by the addition of salt or sugar
(3) pasteurization and use of high temperature
(4) refrigeration, freezing – use of low temperatures
(5) use of ionizing (gamma rays) and non-ionizing (UV) radiation-irradiation
(6) addition of substances like bacteriocins to foods to control food borne pathogens
(7) avoidance of microbial contamination-physical filtration (removal of oxygen)

Mr. Vice-Chancellor, Sir, microorganisms causing foodborne diseases are not limited to developing countries or to children (Tauxe et al. 2010). In the United States of America (USA) alone, foodborne diseases have been known to result in 76 million diseases, 325,000 hospitalizations and 5,000 deaths each year according to the estimates provided by Mead et al. (1999). On a worldwide basis, and from the standpoint of food safety, the following are very important disease-causing microorganisms: *Salmonella enterica*, *Escherichia coli* 0517:H7, *Clostridium botulinum*, *Campylobacter coli*, Norwalk virus and hepatitis A, *Listeria monocytogenes* and *Yersinia enterolitica*.

In recognition of the global health importance of foodborne diseases and in order to promote economic growth

and development, the World Health Organization (WHO) commissioned the Foodborne Disease Burden Epidemiology Reference Group (FERG) to undertake the systematic reviews of some chemicals and toxins like cyanide in cassava, aflatoxins, foodborne pathogens and peanut allergens (Hird et al. 2009). In line with the works of FERG and based on 10 food safety performance indicators like pesticide use, total diet studies, foodborne illness rates, national food dietary consumption studies for risk assessment, risk management (national food safety response capacity, food recalls, food traceability and radionuclides standards) and risk communication (food allergies and public trust), Canada and Ireland were adjudged to be the top two countries in food safety performance in the world. Other countries that followed Canada and Ireland were France, the UK, Norway and USA (*www.foodsafetytech.com-accessed* on 16/12/2014). Mr. Vice-Chancellor, Sir, ladies and gentlemen, I could not find the name of any African country listed among food safety performance ranking.

The "food safety box" (fig. 9) can however facilitate localization of food borne problems, suggest solutions along the dimensions of the pathogen, food vehicle of transmission and level of food processing (Tauxe et al. 2010).

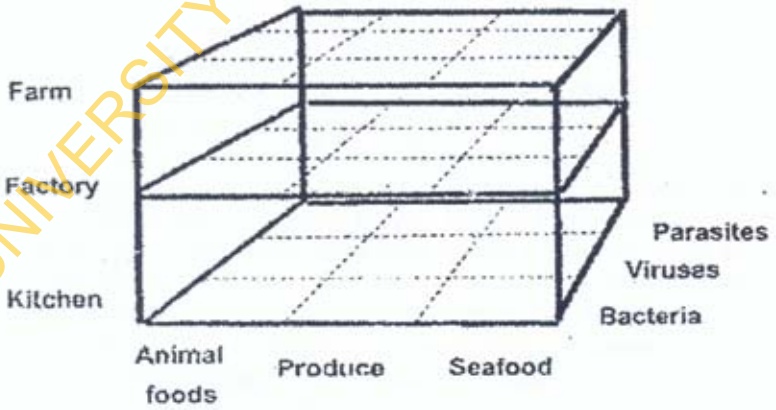

Fig. 9: Food safety box in which problems and solutions can be localized, along with the dimensions of pathogen, food vehicle of transmission and level of processing.

Mr. Vice-Chancellor, Sir, it is hard to believe that Nigeria was not listed in the food safety performance ranking when the country has the following legislature on food safety:

(1) Public Health Law (1917) referred to as Public Health Ordinances Cap 164 of 1958
(2) Standard Organization of Nigeria Decree 56 of 1971
(3) Food and Drugs Decree No 35 of 1974.

As at 2015, it is again difficult to believe that Nigeria has no policy on food safety when other developing countries like Uganda, Lesotho and Bangladesh have functional National Policies on Food Security/Food Safety backed up by appropriate implementing agencies. That Uganda has a Policy on Food Safety made it possible for the country to be the ONLY African country to have obtained financial and human resources from the World Health Organization (WHO) on Country Burden of Disease Pilot Study. I can say this with all sense of truth and responsibility as a member of Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization (WHO).

With my experience as a member of the World Health Organization's FERG, the critical control points (CCPs) for the processing of some street foods consumed in Nigeria have been established. For example we have found that the CCPs for '*akara*' are cowpea, water, onion, salt, unit operations like dehulling and wet milling (Adegoke, Egunjobi, Agbola, Olatuberu and Moy 2008). We have also found that the CCPs during the processing of soy-cheese are washing, filtering, boiling, straining and frying (Fasoyiro, Obatolu, Ashaye, Adegoke and Farinde 2010). Adhering to these CCPs during the processing of the afore-mentioned street foods can reduce the occurrences of food borne infections or illnesses. Mr. Vice-Chancellor, it is very interesting that my contribution to food safety was the maiden paper published in 1980 wherein we found that of 60 giant snails we examined, 54 of the snails were contaminated with organisms like *Escherichia coli* a pathogen of public health importance (Akinboade, Adegoke,

Ogunji and Dipeolu 1980). Mr. Vice-chancellor, Sir, several foods we consume in Nigeria come from the informal food processing sector. Foodborne diseases do however occur within the formal and informal food sectors with the latter having more risks or hazards. A case in point is the disease outbreaks in the European Union (EU) in 2011. Over 4,000 cases of *Escherichia coli* 0104: H4 infections with 900 cases of haemolytic ureamic syndrome (HUS) and 53 deaths occurred in Germany and some EU countries as a result of contaminated fenugreek sprouts imported from Egypt (*http://www.nature.com*, assessed on 21/093/2012). While no one is hoping for such outbreaks to be traced to our agricultural products in Nigeria, one cannot but ponder on what could have happened if such magnitude of outbreaks in EU in 2011 were to occur in a developing country like ours.

In order to highlight the importance of food safety to the generality of Nigerians particularly with regards to agricultural commodities coming from the informal food sector, my collaborators and I made concerted moves for legislation on food safety in Nigeria. With a non-governmental organization (NGO) in 2010, I led the team that presented at the National Assembly, Abuja, "A bill* or an Act to Establish the Nigeria Food Safety Service Commission charged with the responsibility for among other Things, Preventing of, and Fighting Against Food and Waterborne Diseases: and for Related Matters".

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CONTENT

INDEX TO LEGISLATIVE INSTRUMENTS

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***Bill Number HB 407 (2010). National Assembly Journal 7 (20), C1341-C1356**

Mr. Vice-Chancellor, Sir, the Bill which was sponsored by Honourables Mayor C.L. Eze, Daniel Reyeseju, Godfrey Ali Gaiya and George E. Daika passed through the first and second readings at the National Assembly (House of Representatives) but unfortunately the Bill could not scale through the final reading. Thus, there is no National Policy on Food Safety as at 2015.

Mr. Vice-Chancellor, Sir, the National Food Safety Bill when invariably passed will encompass the following:

(1) Collaboration with relevant agencies to ensure food safety standards
(2) Set food standards from production to consumption in order to protect the health of food consumers and ensure fair practices in food trade.
(3) Ensure healthy food markets
(4) Ensure food handlers, food vendors food traders, food processors and food service centres in Nigeria adopt and follow HACCP and HARP (hazard analysis risk preventive) systems.

At this juncture, with or without a National Food Policy, and should we still want to continue with the present Single Agency System or choose Multiple Agency Food Control System (based on multiple agencies' responsible for food control) or Integrated System—a system based on a national integrated approach (FAO/WHO 2003), we must know that all these systems are dependent on changing global factors (fig. 10) like:

(1) Increasing volume of international trade;
(2) Expanding international and regional bodies which can result into some legal obligations;
(3) Increasing complexity of food types and geographical sources;

(4) Intensification and industrialization of agriculture and animal production;
(5) Increasing travels and tourism;
(6) Changing food handling patterns;
(7) Changing dietary patterns and food preparation preferences;
(8) New food processing methods;
(9) Increasing resistance of bacteria to antibiotics;
(10) Changing human/animal interactions with potential for disease transmission (WHO 2006).

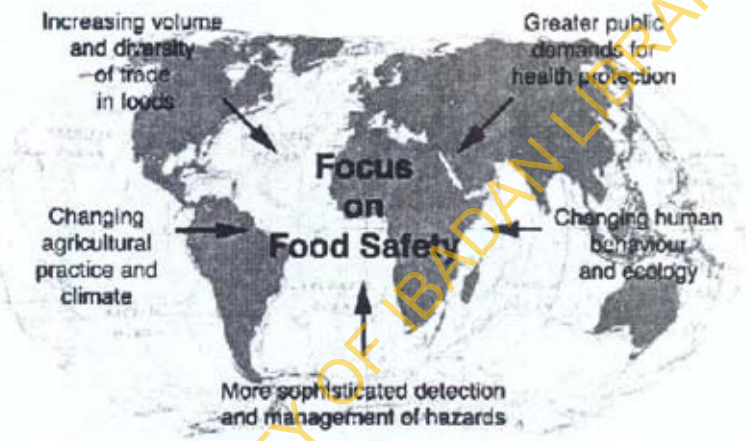

Fig. 10: Factors driving changes in food safety systems.

Conclusions and Recommendations

From 1968 when I had any initial contact with pathogenic microorganisms up till now, I can confirm that these small living cells can be subdued or controlled and with my collaborators and me, we have provided a platform for controlling the survival of some foodborne pathogens. We have produced and identified essential oil compounds in the spice *Aframomum danielli* and we have found that these bioactive compounds can penetrate the cell walls of some pathogens, disrupt and damage their cell walls and make the pathogens useless/inactive.

- We have identified a plant product that has bioactive components that can protect the liver and kidney against damage by mycotoxigenic moulds.
- We have used the components of *Aframomum danielli* in a plant- derived food preservation technology to keep maize and soybeans for 15 months without spoilage and loss of nutrients.
- We have provided a solid platform on which to build a National Policy on Food Safety.

Before we start the move to obtain a National Policy on Food Safety, the following are useful recommendations:

1. We URGENTLY need sustainable education in food safety at all levels (fig. 11)

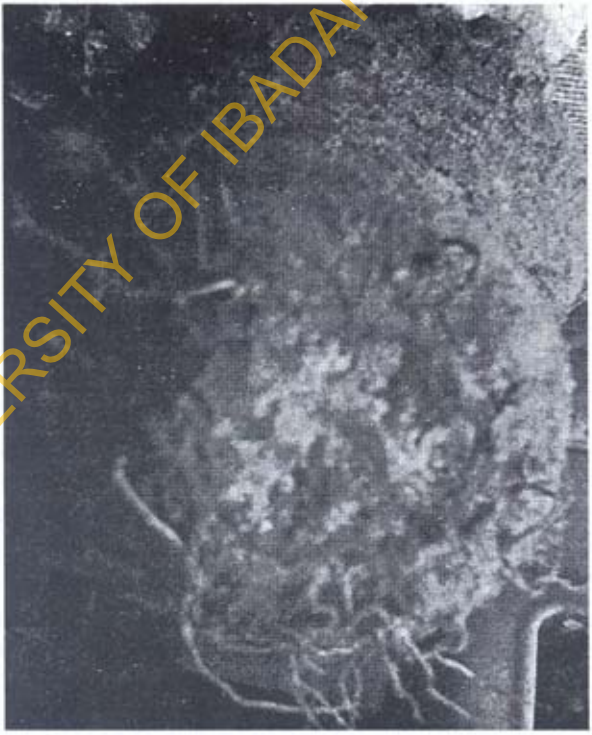

Fig. 11: Yam contaminated with a dangerous mould.

2. We cannot continue to handle meat and foods prone to microbial contamination as we are presently doing (fig. 12)

Fig. 12: Faulty meat handling practices.

3. We cannot continue to produce street foods without care of the environment (fig. 13)

Fig. 13: An unhealthy food market environment.

4. We have to start the process of building healthy markets all over our country, Nigeria (fig. 14)

Fig. 14: Unhealthy markets in one of the towns in Nigeria.

Building healthy or safe food markets is a process but it has to start now as part of ensuring a National Policy on Food Safety.

5. We need to establish a center of excellence for food research in Nigeria—***National Food Research Institute***, that will be charged with early warning systems and control protocols for foodborne diseases or outbreaks,

food surveillance-particularly for the informal food sector, healthy markets and abattoir settings, food safety education, risk assessment and risk communication.

6. To avert food borne illnesses/outbreaks in the near future, we need to study sincerely and carefully the statement of the Director-General of the World Health Organization (WHO), Dr. Margaret Chan, when she said *inter alia*, "governments need to give ***food safety*** just as much attention as they devote to the quality and safety of pharmaceutical products, ***not everyone*** needs medicine every day ***but all people need food each and every day***".

   In the light of this statement, I ask:

   (1) Shall we continue to winnow groundnuts with air from the mouth?
   (2) Shall we continue to watch tea vendor/seller 'cool' his hot tea with "cold air" from his mouth?
   (3) Shall we continue to consume fruits and vegetables without first washing them?
   (4) Shall we continue to buy those meat and dried fish hawked around our markets or streets?
   (5) Shall we continue to take stale/expired loaves of bread that have been carefully scrubbed with foam?
   (6) Shall we continue to take loaves of bread with no expiry date, place of production?
   (7) Shall we continue to compete for space with stray animals, heaps of refuse and waste materials in our markets?

Once we say no to all these, we have started to take the statement of the Director-General of the World Health Organization seriously and we are indirectly and directly urging governments at all levels in Nigeria to do something concrete with respect to food safety as we do not want to have any food safety challenges because we have not forgotten issues relating to the last episode of Ebola virus outbreaks in

Nigeria and some West African countries. We must note that Ebola virus is one of the numerous microorganisms that can cause diseases of great proportions.

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BIODATA OF
PROFESSOR GABRIEL OLANIRAN ADEGOKE

Gabriel Olaniran Adegoke was born in Ondo, Ondo State on 22nd November, 1949 to late Mr. Daniel Adegoke and late Mrs. Adeyela Adegoke (Nee Adegbie). He attended Saint Anne's Primary School, Ondo and Gboluji Grammar School, Ile-Oluji and passed out in Division 1. Between 1968 and 1984, he attended the School of Medical Laboratory Sciences, University College Hospital Ibadan, British Columbia Institute of Technology, Vancouver, Canada and the University of Ibadan. He obtained his Ph.D. degree (Veterinary Microbiology) in 1984 from the University of Ibadan. He was appointed as Lecturer I in the Department of Food Technology in 1987, promoted to the grade of Senior Lecturer on October 1, 1991, Reader in 1996 and to the grade of full Professor on October 1, 1999.

Professor Adegoke has 108 papers in international journals with high impact factors and he is the author of 2 books and 6 chapters in books. He is the Editor-in-Chief of a US-based journal, Current Research in Bacteriology. He is the Regional Editor of 5 international journals and he is an online reviewer for over 15 journals. Professor Adegoke is a Fellow of the United Nations University, Tokyo and the Nigerian Institute of Medical Laboratory Sciences. He is a member of the Nigerian Institute of Food Science and Technology and Institute of Public Analysts of Nigeria.

Professor Adegoke was twice the Sub-Dean (Postgraduate) in the Faculty of Technology 1998-2000; twice Hall Master, Mellanby Hall, U.I. (2006-2011); Director, Equipment Maintenance Centre, U.I. (2006-2008); Head, Department of Food Technology (2007-2011) and he has served on the Senate Curriculum Committee and the Pioneering Committee on Multidisciplinary Research Laboratory, U.I. Professor Adegoke has successfully supervised 13 Ph.D. students and 4 others are currently at advanced stages in their Ph.D. research works. Professor

Adegoke was a Visiting Professor at the University of Zululand, South Africa in 2006 and Jomo Kenyatta University of Agriculture and Technology, Kenya in 2010.

Professor Adegoke served as a Member, Nigeria's Codex Technical Committee and was appointed Consultant Food Safety and Chairman, Committee for the Review of Nigeria's Food Safety Policy, Federal Ministry of Health, in 2010. He was Nigeria's Representative, African Codex Experts Technical Codex Committee on Hygiene (CCFA) at the meeting held in Nairobi, Kenya in November, 2010. He was invited to chair the Joint FAO/WHO Expert Meeting on Benefits and Risks on the Use of Chlorine – containing Disinfectants in Food Products and Food Processing, Ann Arbor, USA in 2008.

Professor Adegoke was invited as a Consultant by the World Health Organization (WHO) in 1996, 1999, 2002 and 2007 on issues relating to Healthy Food Market, acrylamide and food safety (Foodborne Disease Burden Epidemiology Reference Group, FERG) respectively. He was on the WHO's Mission that introduced Hazard Analysis Critical Control Point (HACCP) system to Nigeria in 1996. He has been a consultant on Food Safety with the Food and Agriculture Organization (FAO) from 2006 to date.

Professor Adegoke was a recipient of University of Ibadan's Senate research grants; scholarship awards for undergraduate and postgraduate studies by the Federal Government of Nigeria. He has received several international fellowships, travel grants and scholarships for example, ABOS, Belgium; DAAD, Germany; United Nations University Fellowship, Japan; Finnish Academia, Finland; CIDA/NSERC, Canada and British Council, UK. Professor Adegoke is happily married to Mrs. Comfort Adegoke and they are blessed with the gifts of five children and grand-children.

NATIONAL ANTHEM

Arise, O compatriots
Nigeria's call obey
To serve our fatherland
With love and strength and faith
The labour of our heroes' past
Shall never be in vain
To serve with heart and might
One nation bound in freedom
Peace and unity

O God of creation
Direct our noble cause
Guide thou our leaders right
Help our youths the truth to know
In love and honesty to grow
And living just and true
Great lofty heights attain
To build a nation where peace
And justice shall reign

UNIVERSITY OF IBADAN ANTHEM

Unibadan, Fountainhead
Of true learning, deep and sound
Soothing spring for all who thirst
Bounds of knowledge to advance
Pledge to serve our cherished goals!
Self-reliance, unity
That our nation may with pride
Help to build a world that is truly free

Unibadan, first and best
Raise true minds for a noble cause
Social justice, equal chance
Greatness won with honest toil
Guide our people this to know
Wisdom's best to service turned
Help enshrine the right to learn
For a mind that knows is a mind that's free