45th Inaugural Lecture

Unseen Forces: Microbes, Fermentation and Mankind
By
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Vice-Chancellor
Deputy Vice-Chancellor
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PREAMBLE:
It is with gratitude to God for His goodness, grace and mercies that I stand before you today as a Professor of Food and Industrial Microbiology of this great University.

While growing up, my career interest was to become a Medical Doctor but due to wrong subject combination during my Higher School Examinations, I ended up in studying Medical Microbiology and Parasitology for my first degree. Along the line, I interacted with Microbes and Nature and become a teacher of Microbiology.

My intention was to continue with Medical Microbiology; however, the knowledge I gained during my Youth Service in the Department of Botany and Microbiology, University of Ibadan exposed me to Food Microbiology and Fermentation. I then decided to do my second degree and Ph.D in Food Microbiology but with research interest in Lactic Acid Fermentation.
I stand before you this day 22nd August, 2012 to present the 45th Inaugural Lecture of this Great University, Lagos State University, the 2nd in the Department of Microbiology ***Entitled, Unseen Forces: Microbes, Fermentation and Mankind (MFM).***

INTRODUCTION
I will start the introduction by explaining some terms for ease of understanding.

What is Microbiology?
It is the study of microorganisms (microbes) which include bacteria, fungi (yeast and moulds) viruses, algae and protists. These are living creatures which cannot be seen with the naked eyes but with microscopes. They are heterogeneous in nature i.e. they can be plants or animals.
Microbiology covers Medical, Industrial, Food, Environment, Petroleum and Pharmaceutical aspects.
Food Microbiology is an aspect that deals with organisms responsible for fermentation and food processing and spoilage as well as food borne diseases. For this lecture, I shall concentrate on Food and Industrial Microbiology (Food Fermentation) i.e. the breakdown or conversion of carbohydrate substrates (cereal grains) by microorganisms through catabolic pathways to a reduced product that is more nutritious and that can sustain mankind.

What is Fermentation?
There are various schools of thought on fermentation.

George Emst -1697 gave the first definite theory concerning the nature of fermentation. He explained it as being a violent internal motion of particles of a fermenting substance giving a loosening end of the constituents of the substance with the formation of new particles which are carbon dioxide and alcohol; carbon dioxide was liberated and the alcohol remains in liquid.

Antoine – Laurent Lavoiser -1789 deduced that there is a chemical nature occurring in fermentation, not the splitting of sugar into carbon dioxide and ethanol, although he claimed that the analysis of sugar is in error.

Fraboni, A. -18th Century claimed that fermentation was the result of gluten derived from the grain starch and sugar.

Lious Jacques Thenard- 1803 observed that all fermentation produced materials resembling brewers yeast which was used to ferment pure sugar and it eventually changed living a white man (i.e. foaming).
Thomas Henry said carbon dioxide was not only a product of fermentation, but was its cause.
In physiological terms,fermentation is defined as the type of metabolism of a carbon source in which energy is generated by substrate level phosphorylation but to the biochemist, the term fermentation can be technically defined as the chemical transformation of organic compounds with the aid of enzymes particularly those made by microorganisms.

An Example of General Equation of Fermentation Reaction is:
Carbohydrate Substrate + Glycolytic Enzyme $\longrightarrow$ *Ethyl alcohol* (CH_2H_5oH) + Co_2 + *ATP*

Vice – chancellor Sir, to the Microbiologist, the term fermentation describes a form of energy yielding microbial metabolism in which an organic substance usually carbohydrate is incompletely oxidized and an organic carbohydrate acts as election acceptor (Adams 1960).

There are different types of fermentation: acidic, alcoholic and non alcoholic. This is referred to as *Lactic Acid Fermentation.*

Table 1: Examples of Alcoholic Fermentation

	Product name	**Area of production**	**Substrate**	**Starter**	**Menstrum**
1	*Burukutu*	Ethiopia Nigeria (north) Northern Ghana	Guinea corn and cassava	*Yeast and lactic bacteria*	Liquid
2	*Pito*	Nigeria (Bendel) Ghana	Guinea corn and maize	*Moulds, yeast and Lactobacillus spp.*	Liquid
3	*Kaffir beer*	South Africa	Kaffir corn or maize	*Lactobacillus spp. and yeasts*	Liquid
4	*Busaa* (maize beer)	East Africa	Maize	*Yeasts and Lactobacillus spp.*	Liquid
5	*Malawa beer*	Uganda	Maize	*Candida krusei*	Liquid
6	Zambian opaque	Zambia	Maize	*Yeasts*	Liquid

	maize beer		Sorghumq		
7	*Merissa*	Sudan	Sorghum	*Lactic acid bacteria, acetic acid bacteria*	Liquid
8	*Seketeh*	Nigeria (south)	Maize	*Unknown*	Liquid
9	*Bouza*	Egypt	Wheat or maize	*Unknown*	Liquid
10	*Talla*	Ethiopia	Sorghum	*Unknown*	Liquid
11	*Kishk*	Egypt	Wheat and milk	*Lactobacillus spp. and Bacillus spp.*	Liquid

Odunfa (Food and Agricultural Organisation) 2007

Table 2: Examples of Non-Alcoholic Fermentation

	Product name	**Area of production**	**Substrate**	**Microoganism involved**	**Textural characteristics of product**
1	*Ogi*	Nigeria, Benin	Maize, Sorghum or millet	*Lactobacillus sp. and yeasts*	Soft or stiff gel
2	*Bogobe*	Botswana	Sorghum	*Unknown*	Porridge
3	*Koko* and *kenkey*	Ghana	Maize, Sorghum or millet	*Lactobacillus spp. and yeasts*	Dough
4	*Mawe*	Dahomey	Maize	*L. fermentum, L. Cellobiosis, L. brevis, yeasts – Candida Krusei and S.cerevisae*	Dough
5	*Mahewu (magou*	South Africa	Maize, Sorghum or millet	*L. Delbrueckii, and L. bulgaricus*	Liquid
6.	*Uji*	East Africa	Maize, Sorghum or millet	*Lactobacillus sp.*	Liquid
7.	*Kisra*	Sudan	Sorghum	*Unknown*	Dough
8.	*Injera*	Ethiopia	Sorghum	*Candida guillermondii*	Dough

Odunfa (FAO) 2007

LACTIC ACID BACTERIA (LAB):
They belong to two main groups – The *homofermenters* and *heterofermenters*. The *homolactic* fermentation produces mainly lactic acid via the *glycolytic* pathway.

$C_6H_{12}O_6 \longrightarrow 2H_3CHOHCOOH$
glucose *Lactic acid*

1 mole of glucose yields two moles of lactic acid

In heterolactic fermentation: the end product is lactic acid, ethanol and carbondioxide.

$C_6H_{12}O_6 \longrightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$
glucose *lactic acid* *ethanol*

Lactic Acid Bacteria (LAB) consist of a number of bacteria genera within the Phyllum Firmicutes
The genera are *Carbonobacterium, Enterococcus Lactococcus*, *Lactobacillus, Lactosphaera, Leuconostoc, Milissococcus, Oenococcus Pediococcus, Streptococcus, Tetragenococcus. Vagococcus and Weisella* (Stiles and Holzaphel 1997, Jay 2000; Ercolini *et al.*, 2001; Holzapfel *et al.*, 2001.)

The members are gram positive bacteria that ferment carbohydrate into energy and acetic acid (Jay, 2000) In addition, they produce small organic compounds that give aroma and flavour to the fermented product (Caplice and Fitzgerald, 1999). The modern taxonomy of Lactic Acid Bacteria (LAB) is based on
16s ribosomal RNA (rRNA) sequential analysis.

They were first isolated in milk (Sardine *et al.*, 1972, Metchokoff, 1908) and have since been isolated in food. In such food and fermented products as milk products, vegetables & meat (Harms *et al.*, 1992), they occur naturally.

Their anti microbial effect is due to lactic acid and organic acids produced causing the pH of the growth environment to decrease (Capilice and Fitzerald 1999). The low pH induces organic acid to become lipid soluble and which then diffuse through the cell membrane into the cytoplasm (Gottschalk 1988). Lactic acid bacteria have the ability to produce acetaldehyde, diacetyl, carbon dioxide, polysaccharide and bacteriocins (Caplice and Fitzerald 1999, Devugst and Degeest, 1999) some of which may act as antimicrobials. In addition to producing lactic acid, they produce hydrogen peroxide through oxidation of reduced nicotinamide, adenine, dinucleotide (NADH) by flavin nucleotide which reacts rapidly with gaseous oxygen. Apart from this, reaction, they produce an antibiotic effect on other organisms that might cause food spoilage; although they themselves are relatively resistant to hydrogen peroxide (Steinkraus, 1995)

Contributions of Microbial Fermentation to Mankind/Sustainability
Microorganisms have contributed immensely to the sustenance of mankind in various ways example of which is

- **Production of Fermented Foods.**

  It has been estimated by FAO (2004) that about 1/3rd of the diet of the whole world is fermented food. FAO (2007) corroborated this estimate at the World International Congress.

  There are various productions of fermented foods depending on the type of microorganism involved in the production, this could be:

A. Bacteria Fermentation

Table 3: Products of bacterial fermentation

Product	Substrate	Microorganisms involved
Sauerkraut	Cabbage	*Leuconostoc mesenteroides,Lactobacillus plantarum* *L. cucumeris, L. pentoaceticus*
Kimchi	Pickled Cabbage	*Lactobacillus plantarum, L. brevis, Streptococcus faecalis,* *Leuconostoc mesenteroides, Pediococcus pentosaceus*
Pickled cucumbers (e.g. *khalpi* in Nepal)	Cucumbers	*Leuconostoc mesenteroides* *Lactobacillus* species *for example L. cucumeris*
Sinki	Radish roots	*Lactobacillus fermentum, L. brevis, L. plantarum*
Gundruk	Mustard, radish and cauliflower leaves	*Pediococcus* and *Lactobacillus cellobiosus, L. plantarum* and *Pediococcus pentosaceus*
Pak – Gard-Dong (pickled leafy vegetable)	Mustard leaf (*Brassica juncea*)	*Lactobacillus brevis , Pediococcus cerevisiae, Lactobacillus plantarum*

Ref: Aidoo, (1986); Karki, (1986); Boon-Lung, (1986); Steinkraus, (1996).
Source: FAO (2007)

B. Yeast Fermentation.

Table 4: Products of yeast fermentation.

Product	Substrates	Organisms involved	Location of production
Red Grape wine (10-14%) alcohol, White Grape wine (10-14%) alcohol	Red Grape (*Vitis vinifera),* Pale yellow grape (*Vitis vinifera)*	*Saccharomyces cerevisiae*	Many African, Asian and Latin American countries
Banana Beer	Ripe banana (*Musa* spp.)	*Saccharomyces cerevisiae*	Throughout Africa
Cashew wine	Fruit of cashew tree (*Anacardium occidentale)*	*Saccharomyces cerevisiae* var. *ellipsoideus*	Countries in Asia and Latin America
Tepache	Maize, pineapple, apple and orange	*Torulopsis, Candida* spp. *Saccharomyces cerevisiae*	Mexico
Colonche	Prickly pear cacti (*Opuntia* species)	Yeasts and bacteria	Mexico
Fortified grape wines (20% alcohol)	Wines	Yeast	South and North Africa
Date wine	Dates	Yeasts	Sudan and North Africa
Sparkling grape wine	Grapes	*Saccharomyces cerevisiae* var. *ellipsoideus*	South Africa
Jack-fruit wine (7-8% alcohol)	Pulp of jack-fruit (*Artocarpus heterophyllus)*	Yeasts	India
Palm wine	Sugary palm saps. (*Raphia hookeri* and *Elaeis guineense)*	*Saccharomyces cerevisiae, Schizosaccharomyces pombe,* (and bacteria *Lactobacillus plantarum* and *L. mesenteroides*)	West Africa
Toddy	Sap from coconut palm	Yeasts	Throughout Asia, particularly India and Sri Lanka
Pulque	Juices of cacti (*Agave trovirens* and *A. americana*	*Saccharomyces carbajali* (and bacteria *Lactobacillus plantarum*)	Mexico

Ulanzi (Bamboo Wine)	Bamboo sap	Yeasts	East and South Africa
Basi (Sugar cane wine)	Sugar cane juice	Yeasts	Philippines
Muratina	Sugar cane	Yeasts	Kenya
Vinegar	Apples, grapes, beer, wine, cider	Yeasts (*S. cerevisiae ellipsoideus), Acetobacter aceti, A. xylinum* and *A. ascendens*	

Sources: Rose, (1961); Wimalsiri, *et al.*, (1971); Odunfa, (1985); Aidoo, (1986); Dirar, (1992); Steinkraus, (1992, 1996); Fellows, (1997); Ranken *et al.*, (1997); Fleet, (1998).

C. Mixed Fermentation

Table 5: Products of mixed (yeasts and bacterial) fermentations:

Product	Substrates	Location of production
Vinegars: Coconut water	Coconut water	Throughout Asia
Pineapple Peel	Peels from ripe pineapples	Latin America and Asia
Palm wine vinegar	Palm sap	West Africa
Coconut toddy vinegar	Coconut sap	Sri Lanka
Nipa Palm vinegar	Sap of Nipa palm (*Nipa fruitcans)*	Papua New Guinea
Cocoa powder	Cocoa beans (*Theobroma cacao*)	Africa, Asia and Latin America
Chocolate	Cocoa beans (*Theobroma cacao*)	Throughout Africa, Asia and Latin America
Coffee	Coffee beans (*Coffea arabica* and *Coffea canephora*	Brazil, Colombia, Indonesia, Mexico and Cote d'Ivoire
Vanilla	Pods of vanilla	Madagascar, Indonesia and South Pacific Islands
Tabasco	Chili pods	Mexico, Guatemala
Tea	Tea leaves	Latin America, Asia

Sources: Jayawardena (1977); Paivoke *et al.*, (1984), Carr (1985); Gates (1990); Glossop (1993); Steinkraus (1996); Fellows (1997).

Table 6: Examples of Fermented Foods in Nigeria

Substrate	Microorganisms involved	Product	Shelf life
Cassava	*Strept. lacticus, Geotrichum candidum, Lactobacillus* spp, *Corynebacterium manihot Leuconostoc* spp.	Gari granules Fufu paste Lafun powder	3 mths 1 wk 3 mths
Cereals: maize, sorghum, millet	*Saccharomyces cerevisiae, Lactobacillus* spp, *Fusarium* spp., *Candida mycoderma, Penicillium* spp.	*Ogi* *Agidi*	2 wk
	Rhizopus oryzae, Aspergillus flavus, P. funiculosum, Geotrichum candidum, Candida spp	*Pito* *Burukutu*	Days
Legumes: African locust beans, soya beans	Coryneform bacteria, *Bacillus subtilis, B. licheniformis, B. pumilus, S. saprophyticus, Pseudomonas aeruginosa*	*Iru* (*dawadawa*)	1 mth
Melon (*Citrullus vulgaris)*	*Bacillus spp, Escherichia spp. Proteus* spp, *Pediococcus* spp.	*Ogiri*	1 mth
Oil palm sap	*S. cerevisiae, Candida tropicalis, C. utilis, Lactobacillus brevis, B.* spp, *Streptococcus* spp.	Palm wine	Days
Milk	Lactic acid bacteria	*Wara*	Days

Source: Latunde-Dada (1995)

Advantages of Fermenting Foods:

Some of the advantages are

- ✓ **Extending the Shelf Life:**

  Food Fermentation represents one of the cheap and energy efficient means of preservation, for example, perishable raw materials (fruits and vegetables) as they usually undergo deterioration immediately after harvesting. The known options are freezing, canning and pickling; which are not economically viable in developing countries.

  Fermentation requires very little sophisticated equipment, a technique employed for generations, for example, in Sudan production of *kawal* (Arthur 1986). *Gundruk* is a fermented and dried vegetable in Nepal (Karki 1986*)* and *Ogi baba* (Adeyele and Odunfa 2006).

- ✓ **Detoxification**

  LAB fermentation has been used successfully for the detoxification of toxin (cyanogens) in cassava and this has contributed to the preservation, aroma and flavour improvement Although cooking has been used before, it leaves residual cyanogens in form of glucoside *cyanohydrin* or free cyanide that are themselves toxic (Capilace & Fitzgerald 1999), Castor oil bean toxic protein referred to as racin is also removed by fermentation.

- ✓ **Removal of Anti-nutritional Factors**

  Many fruits and vegetables contain naturally occurring toxins and anti – nutritional compounds. These are usually removed by microbes involved during fermentation, for example, Sudanese *kawal* removes the toxins in the leaves of *Cassia obtusifolia* removal of cyanide *glucoside* in cassava by combination of *Geotrichum candida* and *Corynebacterium lactii*, oligosaccharides in African locust bean is hydrolysed during fermentation to produce *iru*.

- ✓ **Improve Nutrition**

  Mr. Vice – Chancellor Sir, in developing countries there are usually dietary restrictions, taboos, misconceptions about availability of food and the most usually affected are women, children and weaning infants as it is believed in the village that mothers do not eat until after their husbands (the best part of any meal is for the husband). According to FAO (2007) approximately 30% of women consume less than their daily requirement of energy; 40% suffer from iron-deficiency anaemia. Fermentation has been found to improve nutritional value of food for example:

  - *Vitamins*- Fermentation results in increased levels, it has been found that *Saccharyomyces cerevisae* is able to concentrate thiamin, nicotinic acid and biotin to give enriched products For example, palm wine in West Africa has high vitamin B_{12} which is good for people with low meat in take.

    Sorghum Beer in South Africa has high riboflavin and nicotinic acid. It is recommended for people consuming high maize diet (as maize is in season) that are prone to Pallagra (a vitamin deficiency disease). Even children take it as it contains relatively little alcohol. *Idli* (lactic acid bacteria fermented food of India) is high in thiamin & riboflavin.

- ✓ ***Medicinal*: -** There are a lot of traditional beliefs that fermented foods are medicinal. For example, the Fur ethnic group in Sudan believe that fermented foods protect from diseases (Dirar 1992).
  - *Koumiss***: -** fermented milk in Russia is used to treat tuberculosis.
  - Lactic acid bacteria have beneficial health effect on intestinal flora (Ottogalli and Galli 1997; Bakre *et al.*, 1998).
  - Fermented foods have protective effect against cancer (Frohlich *et al.*, 1997).

The scientific basis for this is that; the lowering of pH inhibits the growth of food-poisoning organisms and pathogenic organism *(Hammes and Ticharczek, 1994)*

- ▪ Effect on immune system.

  LAB enhance the immune system function at the intestinal and systemic levels in human. They increase B-cells which recognize foreign matter.
- ▪ Ulcers:- LAB has been found to show some promises against stomach ulcers. Work with a specific strain of *L. acidophillus* demonstrated that it competes effectively (*in vitro* against *Helicobacter pylori* for attachment site, limiting the number of *H. pylori* that can attach to the cell wall (Brassart *et al.*, 1995).

- ✓ ***Digestibility***

  The enzymes of the microorganisms involved in fermentation have been found to contain celluloses which are incapable of being synthesized by humans. These enzymes hydrolyse cellulose into sugars which are then readily digestible by humans (Kovac 1997; Parddez – Lopez 1992).

- ✓ **Alternative Source of Income/Sustainability.**

  Fermented foods production has been found to provide a source of income to a lot of people around the world.

  Anon (1995) reported in the FAO of nation that there is value added through processing and marketing of raw products, for example, about 60% of workforces in sub-Sahara Africa are employed in small–scale food processing sector and between 1/3rd to 2/3rd in manufacturing of agricultural raw materials (World Bank 1989; Conrey *et al.*, 1995).

  In Asia for example, fermented food is a widespread traditional art, *Kimchi* fermented food accounts for about 10% of the gross economy.

Table 7: Contribution of Fermented Food to the Economy of Nations

Region	Product	Estimate
Korea	*Kimchi* (fermented cabbage)	> 2000 million litres/year
Indonesia – Japan	*Soysauce* (fermented legume)	> a billion litres
Taiwan	*Soysauce* (fermented legume)	> 150 million litres
Japan	*MISO Soysauce* (fermented legume)	> 560,000 tons /year

In Latin America, production of fermented cereal and alcohol drinks is the third most important sectors of the economy.

- ✓ **Making Inedible Foods Edible.**

  African locust beans and oil beans, for example are inedible in their unfermented state but according to Odunfa (1983) during fermentation, they become edible as a result of hydrolysis of the indigestible components which are removed by microorganisms. Statistically, about 200,000 tons of locust beans are produced annually in Northern Nigeria (Cobeley and Steel 1976)

- ✓ **Variety of Flavour.**

  For example, the acid flavour produced during lactic acid fermentation of cereals and cassava results in much different flavours from the unfermented food stuffs e.g *Ogi-baba* (Odunfa and Adeyele 1984)

✓**Other contributions of fermentation:**

Apart from aforementioned there are other benefits of fermentation which impact on the economy:

- **Waste to Wealth**

Fermentation helps to salvage waste food by changing the consistency or raw materials that are considered not usable. For example, in Sudan, bones, hides, locusts and fish bones are fermented into *doddery*; *Kaidu digla* is made from fermented backbone (Dirar 1992).

In Indonesia, waste products are used to produce nutritional foods, for example, *Tempe – Bonggrek*, is made from fermenting peanut and coconut. This is a mould fermentation.
In Latin America, the peels of fruits are usually fermented for about eight days For example, from pineapple peels, pineapple vinegar is produced.

- **Effect a balance in Nature**

It has been found out that microbes help in improving the amount of Nitrogen present in the soil by fixing Nitrogen to the root nodules. Examples of organisms involved include Bacteria- *Rhizobium, Clostridium, Nostoc, Klebsiella pneumonia*, *Bradyrhizodium japonicum* and *Anabena* spp. to mention but a few; Photosynthetic bactria: *Rhodospirillum rubrum, Rhodospsedomonas acidophilus*.
Apart from fixation of Nitrogen and Carbon others are involved in the breakdown of organic materials for easy assimilation in the soil. Examples are anaerobic organisms: *Clostridium Spp., Aeromonas spp.*, *Desul fomaculum.* Aerobic organism: All aerobic bacteria for example *Staphylococcus, Bacillus spp* and all aerobic fungi e.g *Penicillum sp.*

- ***Single Cell Protein***

Some examples of microorganisms used for single cell proteins productions.

Yeast:

Saccharyonyces cerevisae: grown on molasses which is used as food.

Kluveromyces fragilis: is also grown on molasses and used as food in France (*le bel process*).

Algae:

Spirullina maxima: grown in the open using carbondioxide and sunlight is used as feed or food supplement. This is grown on commercial basis in Mexico.

Bacteria:

Methylophillus methylotrophus: is grown on methanol for food in United Kingdom on a commercial basis.

Fermented Food Classification:
Fermented food can be classified in different ways depending on the region. It could be acidic, alcoholic or non alcoholic or consistency.

Table 8: FERMENTED CEREAL BASED PRODUCTS IN AFRICA

Raw material		***Fermented product***	***Region***	***Reference***
<u>*Cereal-based product*</u>				
Gruels & Beverages				
a.	Maize	*Ogi (akamu)*[a.b.c]	Nigeria/West Africa	Onyekwere et al., 1985
b.	Sorghum	*Uji*[a], *Ogi* –	Kenya/EastAfrica/Nigeria	Mbugua 1981
c.	Millet	*baba*[b]	Ghana	Halm et al., 1993
d.	Rice	*Kenkey*[a]	Benin/West Africa	Hounhouigan. 1994
e.	Wheat	*Mawe*[a]	Nigeria	Gaffa & Jideani 2001 a.b
f.	*Tef*	*Kunnu zaki*[b.c]	Nigeria North	Maaji 2000
		Kwal kobo[b]		
g.	Green maize		Kenya	Kunyanga & Mbugua
		Kirario[b,c,g]	Nigeria	2009
		Fura[b.c]		

Table 8 shows fermented products that are produced from different raw materials. The superscripts indicate products from the same raw materials.

Another typical classification in South East Asia is according to the Microorganisms involved in the commodity (Odunfa 1988; Campell Platt 1987); Dirar (1993) classified them based on the function of food; i.e. typically Sudanese classification.

Table 9: **Different Classifications of Foods.**

Yokotsuka	**Campell-Platt (1987)**	**Odunfa (1988)**	**Sudanese (Dirar 1993)**
1. Alcoholic beverages (yeast)	1. Beverages	1. Starchy roots	1. Kisser – staples
2. Vinegars (Acetobacter)	2. Cereal products	2. Cereals	2. Millet – sauces and relishes for the staples.
3. Milk products (Lactobacilli)	3. Dairy products	3. Alcoholic Beverages	3. Marayiss – beers and other alcoholic drinks
4. Pickles (Lactobacilli)	4. Fish products	4. Vegetables proteins	4. Akil-munasabat – food for special occassions
5. Fish or meat (Enzymes and Lactobacilli))	5. Fruit and Vegetables product	5. Animal protein	
6. Plant protein (moulds with or without Lactobacilli and yeasts)	6. Legumes		
	7. Meat products		
	8. Starch crop products		
	9. Miscellenous products.		

Adapted from Dirar (1993)

The different classifications show the different input of authors.

Table 10: Classification of Fermented Food Based on Nutritional Value.

1. Ready for consumption, for example yogurt, salami, bread kunu, palm wine - *Ayib,* - *Zabadi* - *Kinrine*	1. Containing viable micro – organisms for example yogurt , cheese Nunu Ethiopia Nigeria	1. LAB – Fermentation 2. Mould – Fermentation Goat milk Cow milk Sheep milk 3. Yeast – Fermentation
2. Ready for consumption but mostly used as ingredient, for example *crème fraiche*	2. Not containing viable micro organisms, for example soy sauce bread, beer, wine	4. Other bacteria 5. Enzymatc
3. Only used as ingredient, for example *Dawadawa, Ogiri, Iru, Ugba*	3. Microorganisms used in an early steep of production, for example cocoa coffee, cassava products	

In Conclusion From the review it can be seen that microbes (Lactic and Acid bacteria) assisted mankind through fermentation to improve the nutritional status of foods, create variety of food and aid digestibility. It also helped in transformation of foods from one form to another. It is a means of sustaining of man, environment and economy.

My Contributions to Knowledge

The fifth most important cereal crop produced in the world is sorghum (Bicor/guinenese, Guinea corn in Nigeria) after wheat, maize, barley, and rice. It is African's second most important cereal (Taylor, 2003).

Cereals are processed into a wide range of fermented products in West Africa. They are fermented to produce thin gruels and alcoholic beverage which are known by various names *(Odunfa, 1985)*. In Yoruba land of Nigeria, maize gruel is known as '*Ogi'* or 'koko' Sorghum gruel is *'Ogi-baba'*, if the ogi is cooked to produce semi solid product, it is called 'agidi' in Ibo or 'eko' in Yoruba.

Vice – Chancellor Sir, of the cereals which are considered Guinea corn is one of the most important; 75% of the total number of calories from cereals in Zaria province of Nigeria is from Guinea corn. As a source of calorie it was found to be the third cheapest after millet and maize *Tuwo* of three foods compared by Simons (1976).

A survey of three Zaria villages in Nigeria showed that cereals constituted 1,588 calories out of a total of 2,641 daily per intake.

Sorghum is also the most widely cultivated cereals in Nigeria and other West African countries. FAO (1997) reported that Sorghum was the second most cultivated cereal in sub Saharan Africa after maize. It is 28.2% of world production (Table 11).

Table: 11 Production of Cereals (Thousand Metric Tons) in Sub-Saharan African

	1997	% of world production
Maize	24,798	4.2
Millet	10,950	38.9
Rice	11,321	2.0
Sorghum	17,400	28.2
Wheat	3,140	0.5

Source: FAO 1997

A comparison of chemical composition of Maize and Sorghum as in Table 12.

Table 12 Comparative Nutritive Value of Cereal Grains

When fully utilized for human consumption they would more than meet humanity's needs for essential amino acids.

Factor	**Wheat**	**Maize**	**Brown**	**Barley**	**Sorghum**	**Oat**	**Pearl Millet**	**Rye**
Available CHO (%)	69.7	63.7	64.3	55.8	62.9	62.9	63.4	71.8
Energy (kj/100g)	1570	1660	1610	1630	1610	1640	1650	1570
Digestible energy (%)	86.4	87.2	96.3	81.3	79.9	70.6	87.2	85.0
Vitamins								
Thiamin	0.45	.032	0.29	0.10	0.33	0.60	0.63	0.66
Riboflavin	0.10	0.10	0.04	0.04	0.13	0.14	0.33	0.25
Niacin	3.7	1.9	4.0	2.7	3.4	1.3	2.0	1.3
Amino acids (g/16gN)								
Lysine	2.3	2.5	3.8	3.2	2.7	4.0	2.7	3.7
Threonine	2.8	3.2	3.6	2.9	3.3	3.6	3.2	3.3
Met. And Cys	3.6	3.9	3.9	3.9	2.8	4.8	3.6	3.7
Tryptophan	1.0	0.6	1.1	1.7	1.0	0.9	1.3	1.0
Protein quality (%)								

True digestibility	96.0	95.0	99.7	88.0	84.8	84.1	93.0	77.0
Biological value	55.0	61.0	74.0	70.0	59.2	70.4	60.0	77.7
Net protein utilil.	53.0	58.0	73.8	62.0	50.0	59.1	56.0	59.0
Utilization protein	5.6	5.7	5.4	6.8	4.2	5.5	6.4	5.1

Adapted from Chavan and Kadan (1989)

Traditional Fermentation

Ogi – baba is widely used as a food for weaning infants and food for pre – school or school children and adults. It is also used as a source of lactation for nursing mothers. It is a popular breakfast meal and mostly consumed by the Muslims during Ramadan to break their fast with 87.9% of it being home produced.

Ogi – baba, as earlier mentioned, is produced traditionally in various homes. A typical way of production is as shown in the photograph.

Figure 1: Photograph of a woman processing *Ogi baba*.

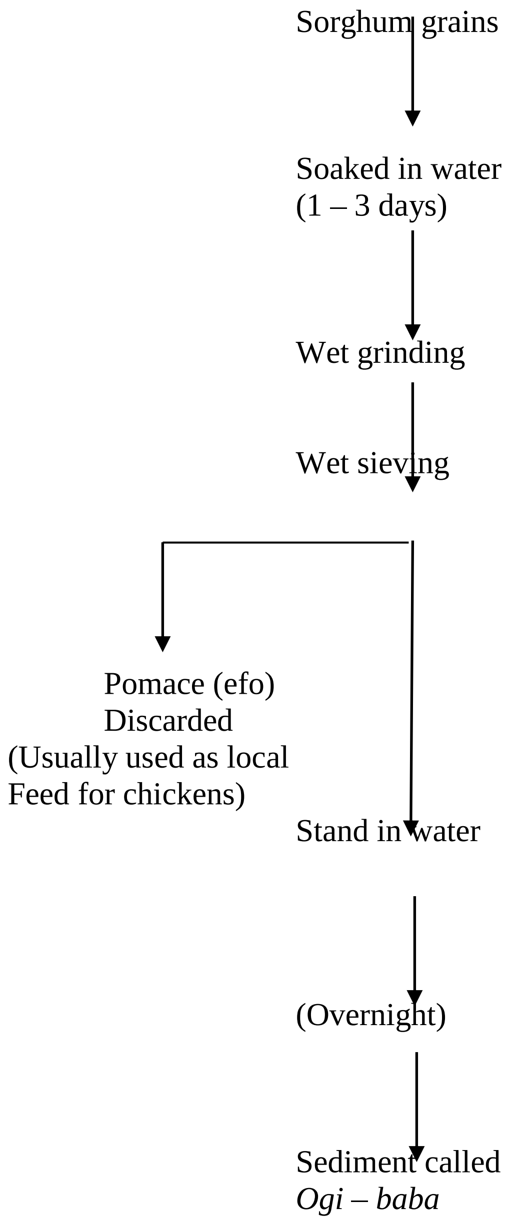

Fig.2. Flow Chart of process for producing *Ogi – baba* in the traditional way.

The Findings of the Study.

Microbial analysis shows that the microbial population was mainly bacteria and yeasts. Fungi occurred only on the fermented grains. During steeping, *Lactobacillus plantarum* and *Streptococcus lactis* appeared initially and a yeast. *Debaromyces hanseii* appeared after 48hours and persisted until the end of fermentation. During souring, the major organism in addition to the steeping organisms was yeast *Candida Krusei*. The yeast was identified by a culture collection centre in UK. Further study was made to know the origin of the organisms and it was found out that the unfermented grains contains *Bacillus*, *Lactobacillus*, *Streptococcus* and two fungi *Aspergillus niger* and *Penicilluim* sp.

Physicochemical Changes

The physicochemical studies showed that there were changes in physicochemical parameters i.e. the pH and acidity. As the lactic acid increased, the acidity increased. The fermentation of the sugars and the acidity that developed favoured the growth of the yeast which subsequently multiplied rapidly. As fermentation increased, the dissolved oxygen decreased making the environment more anaerobic as shown in Table 12

Table 13: Changes in pH and Acidity during the steeping stage of Sorghum Fermentation

Time (h)	pH	Titratable Acidity (% lactic acid)	Dissolved oxygen (% saturation)
0	6.4	0.30 $\pm$ 0.04	28.0
24	6.0	0.45 $\pm$ 0.03	12.0
48	5.6	0.51 $\pm$ 0.05	2.1
72	5.0	0.53 $\pm$ 0.06	2.0

Table: 14 Changes in pH and Acidity during the souring stage of Sorghum Fermentation

Time (h)	pH	Titratable Acidity (% lactic acid)	Dissolved Oxygen
0	5.0	0.54 $\pm$ 0.01	8.0
24	4.8	0.62 $\pm$ 0.04	6.12
48	3.8	0.78 $\pm$ 0.05	2.46

The pH became more acidic during the souring period as can be seen in the % lactic acid in the medium. The dissolved oxygen was not as low as during steeping because the environment was not as anaerobic.

It was then assumed that invariably, the yeast and lactic acid bacteria were in symbiotic association; lactic acid provides acid environment for yeast and yeast provides vitamins for lactic acid bacteria growth in the medium.

Carbohydrate Changes

Mr. Vice - Chancellor Sir, I gave one of the definitions of fermentation as the conversion of carbohydrate from one form to a simpler form. In my research findings, it was observed that the unfermented grains contained some reducing sugars (fructose, glucose and maltose).

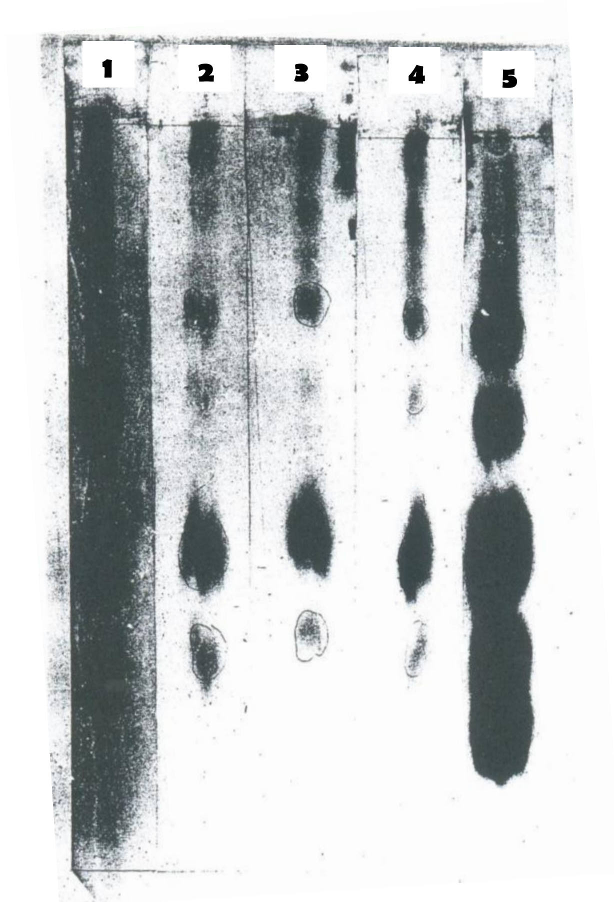

Figure 3:. Example of Chromatogram showing changes in the sugar content during Souring Stage.

A. Raffinose
B. Maltose
C. Sucrose
D. Glucose
E. Fructose
F. Xylose

Key for 1, 2, 3, 4, 5

1. – 0 hr Souring
2. – 24 " "
3. – 48 " "
4. – 72 " "
5. – Reference Standard sugars.

During steeping stachyose and raffinose decreased while sucrose was the major fermentable sugar as a result of galactosidase and invertase enzyme activities. Colorimetric assay also showed a decrease in the sugars during souring.

Further work on the sugar utilization by the organisms in relation to the enzymatic activities revealed that amylolytic activity was stable for the first 24 hours while the alpha glucosidase activity increased to a peak at 36hours.

The two yeasts produced the highest amylase and were able to hydrolyle starch on starch agar plate and also had the highest invertase activity. This could further be linked to their ability to utilize sucrose, xylose and galactose.

Titratable acidity showed that the growth of *Streptococcus lactis* was highest in glucose whereas the growth of all the other microorganisms was significantly higher in galactose and maltose.

Starter Culture

Since the fermentation is a chance inoculation using a technique referred to as Multiple regression analysis for forecasting for the measurements of outcome of the interactions.

The total plate count in all the inocula increased; for example *Streptococcus lactis* increased from 8 x 10^2cfu/ml to 23 x 10^6cfu/ml accompanied by high acid production and a pH drop to 3.56. The end of the 5^{th} day the pH of the mixed culture inoculation decreased from 6.7 – 3.25. This sample was similar to locally fermented *Ogi-baba* (Fig 1).

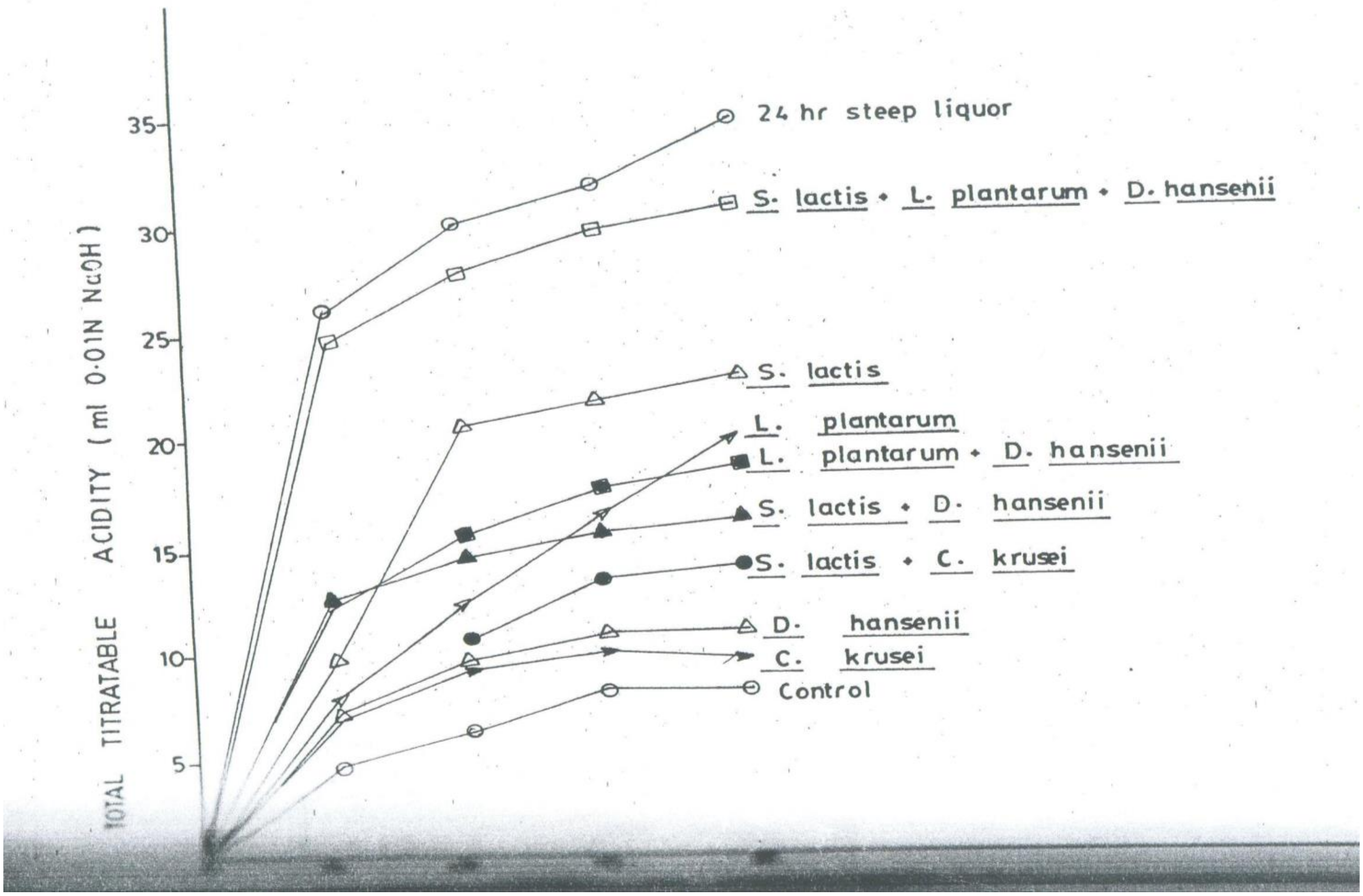

Figure 4. Starter culture inoculation

Spoilage of *Ogi-baba*

With the various research works, there was the need to look into the spoilage of *Ogi-baba* as stated earlier. In Microbiology, there is nothing like spoilage but it is just a conversion to another form, though traditionally spoilage in *Ogi –baba* is noticed with off – odour. In the study, it was observed that the yeasts which were beneficial later turned out to be dominant during spoilage as the pH increases. *Sacchamyonyces cerevisae* and *Candida krusei* were dominant. The titrable acid was checked regularly in order to ascertain the acidic nature and changes. Acidity increased from 0.37 to 1.35.

However, lactic acid bacteria survived throughout the storage 14days (2wks) (this might be due to production of hydrogen-peroxide) as it increased until 5th day and later started decreasing.

Micrococcus, *Corynebacterium*, *Aeromonas Saccharomyces cerevisae*, *Candida* sp, *Hansenuela anomala* were isolated after the 10th day of unrefrigerated *Ogi – baba*. The colour changed and odour was unacceptable, pH dropped to 2.70. However it was concluded that changing the sour water daily, the shelf life without refrigeration was extended from 7 days to 10 days *(Bakre 2004)*

Health Benefits

Further study was done on the survival of diarrhagenic agents in two weaning foods, *Ogi* and *Koko*. *Ogi* as we know is a weaning food for infants and breakfast for adults in many homes. *Koko*, a Ghanaian fermented cereal, is prepared in the same way as ogi and is a breakfast cereal hawked with akara (beans cake) and sold to many peasants or as dough (mbar). *Uji* is similar except that it is prepared due to the time involved in food preparation. Large amounts of *ogi* are usually prepared for babies and usually kept for many hours before consumption in a plastic container of a warmer or flask and the practice of diluting weaning foods with unwholesome water before feeding babies.
This, amongst other unhygienic practice, increases the exposure of local weaning foods to pathogens. Another major factor is the instability in electricity; the hot climate may allow the growth of pathogenic bacteria.

The study showed that *ogi* had a slightly lower pH value than *koko* before and after cooking, (Bakre, *et al.*, 1998). The pH value of *Ogi* ranged from 3.0 to 3.6 and *koko* from 3.5 to 3.9. The survival experiment showed that the inoculated enteric pathogens (*Enteroinvasive Esherichia coli*, *Salmonella typhi*, *Shigella flexneii* and *Vibrio cholera*) were inhibited in cooked *ogi* and *koko* during storage for 24 to 48 hours. The antibacterial effect of cooked *Koko* was more pronounced on the four enteric pathogens than of cooked *Ogi.*
It is noticeable that except for *Shigella flexnerii* and *Escherichia coli* in *Ogi*, none of the other bacteria studied was recovered after 48 hours. The sensitivity testing showed that *Koko* inhibited the organisms more than *Ogi*, when the seeded medium of 24 hours culture was flooded with the food samples in which pH was adjusted to 7.5 values of different samples of uncooked *Ogi* and *Koko* compared with cooked samples.

Table 15: **pH Values**

Sample	**Uncooked Ogi**	**Cooked Ogi**	**Uncooked Koko**	**Cooked Koko**
A	3.0	2.6	3.55	3.1
B	3.2	3.0	3.5	3.75
C	3.4	3.3	3.7	3.65
D	3.6	3.5	3.9	3.7

A - *Escherichia coli;* **B** - *Salmonella typhi;* **C** - *Shigella flexneri,* **D** - *Vibrio cholera*

Ogi and *Koko* with low pH values (acidic) i.e. *Ogi – kikan* may have a possible role in reducing the role of contamination and may not be vehicles for transmitting of diarrheal disease among infants in developing countries and thus recommended as weaning foods for babies against diarrhea.
From previous studies, it has been established that the *Lactobacillus* was responsible for production of lactic acid which is the major component of *Ogi* flour.

Acetaldehyde determination
This was determined by titration method (acid/base) and used as an index of ethyl alcohol. It increased as souring increased but was stable and started dropping after 96 hours which might be as result of decline in lactic acid bacterial growth. Acetablehyde increased from 0.13 at the 0hr of souring to 0.36 by 48hrs.

Utilization of Souring Water
The *Ogi* water referred to as *Omidun/Omikan* contains acetaldehyde (ethyl alcohol) which is the pleasant flavour. It is the starter ingredient for *Adoyo*. *Adoyo* originated from Republic of Benin some decades ago, it was then transferred to Badagry Nigeria and Togo around 1978).
It is believed that Adeyo reduce stress and give vitality, a medicinal value as a non alcoholic beverage. It is used as a local drink with the addition of lemon grass and other herbs depending on taste.

Omidun

↓

Omidun + Lemon grass (boiled for 30 minutes)

↓

Omidun + Lemon grass allowed to cool and sieved

↓

Solution of burnt sugar added + Sliced grape fruit

↓

Ice block added

↓

Adoyo / Final Product

Figure 5. Flow Chart for Adoyo Drink

Table 16: Nutritional Values of *Adoyo*

Parameter	Values
Acidity	0.45
pH	3.5
Ash	0.087
Sugar	5.5

The low pH was attributed to the production of lactic acid by the *Lactobacillus* species present in the waste water during souring since pH decreased from 3.3 to 3.5; the combustible material being as low as 0.087% showing that the bulk is water, hence a good source of water for the body during hot weather.
Some enter pathogenic organisms were seeded into fresh *Adoyo* at various concentrations. *Adoyo* was able to hinder some of the food-borne pathogens at different concentrations which show that the drink actually has some antibacterial effect.

Table 17: Antibacterial effect of Adoyo after 7 days

1 - Inhibition of growth of food borne pathogens
N1 - No Inhibition
L1 - Limited Inhibition

Test Organism	Concentration of Samples					
	0.5	1.0	2.0	3.0	4.0	5.0
Escherichia coli	1	1	1	1	1	1
Salmonella sp	1	1	1	1	1	1
Shigelli sp	N1	1	1	1	1	
Pseudominas sp	N1	N1	L1	L1	L1	1

Waste to Wealth

Ogi waste for enzyme production

During the processing of sorghum for *ogi – baba,* considerable waste is usually generated. The waste contains the pericarp germ as well as some residual starch. It is estimated that the pomace constitutes about 30% of the original weight of guinea corn. Traditionally, the waste is known as '*Efo' or 'Eri'* and is discarded or may be used for poultry reared around the compound.
Mr. Vice – Chancellor Sir, since in future it is anticipated that *Ogi-baba* will be produced on an industrial scale, processing waste that will be generated may constitute a disposal problem. Previous study *(Odunfa and Adeyele 1984)* showed that the processing waste has high starch content. The proximate composition of *ogi-baba* waste as determined in the Laboratory is as follows:

Table 18:

Carbohydrate	26.6%
Protein	8.18%
Ether	17.8%
Total ash	1.35%

From the analysis of waste, there is the possibility of utilizing it as substrate for amylase enzyme production. Amylase has a wide range of industrial applications. The following organisms isolated from Cassava were utilized; *B, subtilis* 1,2 (dry surfaced), *B subtilis* 3 (slightly moist) and *Corynebacterium manihot (Bakre et al., 1997)*. The *B, subtilis* was improved on a minimal medium and had the highest amylase enzyme production on the waste.

Other Sources of *Lactobacillus*

Attempt was made for other sources of Lactobacillus for use as starter cultures. It has been established that lactobacilli account for 1% of the total microbes in the healthy human mouth (Laden 1976), constitute < 0.1% of population of either cheek / tongue (Marsh and Martin 1984). Lactobacilli are indifference in stomach of rodents, pigs and the crop of chickens. They are found to be natural commensals

The following organisms were isolated from 50 samples of pig faeces. Ten different Lactobacillus species were isolated. The predominant specie was *L. acidophillus* (21.44%). All the isolates were resistant to the eight antimicrobials tested for this study *Staphylococcus aureus* ATCC 25293 was used as positive control and this was the 1st time in Nigeria that an attempt was made to isolate *Lactobacillus* from chicken droppings. The commonly isolated organisms in this study were *L. acidophillus, L. cellobiosus* and *L. salivarus.*

Although, Lactobacilli have not been proven to be pathogenic in pigs there is the need for further work to identify their role in the case of epidemic diseases.

Other Research Works

Microbial safety of snacks sold to primary school children since diarrhea has long been recognized as a major cause of mortality among children in developing countries, we tried to look into the microbial safety of snacks sold to school children in Lagos metropolis, in was established during study on *ogi – baba* that it is not a source of diarrhea in school children.

Ten (10) schools were visited and samples of home made snacks common to all the schools were collected.

Table: 19 Common Snacks Collected from Schools.

Items	Constituents
Kokoro	Local biscuits (maize + sugar)
Kulikuli	Friend groundnut balls
Lollypop	H_2O, Sugar, Flavouring + Colouring
Donkwa	Groundnut, sugar, oil + water
Sisi pelebe	Coconut, sugar + water
Balewa	Local sweet (sugarcane/honey)
Tarfinrin	Ground dry maize with groundnut sugar
Coconut	Groundnut chips, Lemon + sugar

Ten different types of bacteria were isolated

Escherichia coli, Streptococcus faecalis, Bacillus sublilis, Staphylococcus aureus, Shigella flexneri and *Staphylococcus aureus*, a casual agent of boils and carbuncles was common and constantly associated with these foods. The presence of *Escherichia coli* and *Staphylococcus faecalis* is an indication of level of personal hygiene. Although Bacillus species are not *enteropatogenic*, *Bacillus cereus* has been implicated in food poisoning *(Krammer et al., 1982*).

It is therefore important that there is the need for careful monitoring of snacks sold to primary school children especially finger snacks.

Another attempt

In Nigeria the bacterial agent of diarrhea has been extensively documented, However, in spite of the high frequency of isolation of diarrheagenic agent among school children there is no microbiological information on their food consumption and habits especially in Ibadan.

In the investigation on the microbiological evaluation of snacks sold to primary school children in Ibadan metropolis, the following items, *Tanfinrin, Kulikuli, Kokoro, Balewa*, *Donkwa* and coconut were the most commonly displayed. Six different types of organisms were isolated; *Bacillus cereus,*

Bacillus subtilis, *Streptococcus sp*, *Esherischia coli*, *Candida specie* and *Pseudomonas sp*.

The organisms were similar to those isolated in Lagos except the presence of *Candida* spp.

This study underlines the poor microbiological quality of finger snacks sold to Ibadan school children and the attendant health hazard.

Another research was carried out on the utilization or beneficial uses of sugar cane peels which forms heaps at Alaba Sugarcane whole sale site opposite LASU gate was studied. Sugarcane (*Saccharum officinarum*) is a raw material in the production of sugar (mainly sucrose) used as a sweetening agent in foods and pharmaceuticals.

Isolation of yeasts was carried out and the yeasts associated were identified using 26s ribosomal DNA (r DNA) sequencing. A total of 6 different yeasts were identified comprising *Candida tropicals* (which has been recovered from a variety of foods) *Candida pseudointermedia*, *Henseniosporia quilliemondii*, *Trichosporon asachii* and *Saccharyonyces cerevisae*. This was the first time yeast was being isolated from sugarcane dumpsite in Nigeria. The importance of this study was that the characterization of the yeasts was done by 26s r DNA D1/D2 sequence with the assistance of a colleague in the Department and *FIIRO*. This is because previous identification of yeast from Nigeria was by chemotaxonomic methods.

Further study was done on the use of these yeasts for the production of ethanol using sugarcane juice as substrate. It was observed that the Red sugar cane had more juice than the green variety. After inoculation/fermentation and distillation, the alcohol content was determined by specific gravity measurement. The ethanol content was found to be high.

Interdiscplinary Research

Various researches were carried out with the Department of Fisheries. Among which were the study on Bacteria flora in *Oreochromis niloticus* from Lagoon, frozen depot and open market (Bakare and Akintola 2004) in which *Escherichia coli* and *Staphylococcus aureus* were reported as the common organisms isolated from the open markets and Lagoons of Lagos State. In another research, Akintola and Bakare (2011) established that the shelf life for prawn under ice storage is 7 days and noted that the population of psychrophillic *Pseduomonas sp.* festered with increasing days of ice storage. The common practice of subjecting prawns to Direct Contact with Ice (WCI) and Without Direct Contact with ice (WCI) were investigated with the results showing that values of TVB-N (mgN/100g) in prawn stored with DCI had inverse relationship with storage days but increased with storage days in the WCI. Changes in the values of ash and crude protein were relatively constant as the storage days increased in both treatments (Akintola and Bakare, 2012). The contribution to knowledge on this research with fisheries is that icing of prawns affects the proximate values hence better to prevent prolong storage of prawn by means of ice.

In conjunction with Department of Biochemistry on comparative sensitivity to zinc sulphate of *Shigella* isolates recovered from Nigerian children with low and marginal plasma zinc concentration (Iwalokun and Bakare, 2008). We determined the ability of zinc sulphate to inhibit growth of *Shigella* (in vitro). It was concluded that zinc sulphate may have potential to be used as a *Shigellocidal* and anti-virulent agent in the management of Shigellosis in children. This can be treated with *Ogi-baba*.

SUMMARY

1. The unseen forces (microbes) have contributed through fermentation to the Nutritional value of the food consumed by man; for example there is an increase in the soluble fraction (sugar) during fermentation. This has resulted in varieties.

2. The total amount of sugar at the end of fermentation can be easily assimulated

3. The vitamin content increased as certain microorganism (yeasts) produce vitamins for the use of Lactic acid bacteria (synergistic relationship) and lactic acid produced by the lactic acid bacteria for yeast growth.

4. The cooking on fire further increases the starch digestibility with the gelatinzation of the starch.

5. Microbes help to promote the function of human digestive system positively by probiotic effect and help the immune system.
6. Economically, it is an alternative source of income (waste to wealth). The waste generated can be utilized for other purpose.

7. Vice-Chancellor Sir, I think I have been able to show to some extent the importance of microbes meeting the sustenance of mankind and the therapeutic values associated with *Lactobacillus* and harnessing it. These unseen agents are beneficial in curbing malnutrition and infant mortality.

8. As I conclude finally that Government should increase the budget on Education and encourage young scientists to improve on our local or traditional foods that are usually produced as a traditional home made art.

Relationship between Gown and Town

1. There should be a meeting point between academia and the public such that research findings are sponsored for adaptation in small or medium biotechnology centres

2. There is the need for Government to fund and equip research laboratories in Universities or centres. Stable electricity or source of power supply is a key factor for research; not alternative source of power supply.

3. There is a need for a culture collection centre in Nigeria to service Africa as most microbiologists depend on the culture collection centres abroad for final identification of local cultures.

4. Nigeria should put up a food policy in place in which there will be an increased productivity in local cereals in the agricultural sector.

5. Education of the populace about the benefits of consuming fermented foods is important therefore the need to include the health benefit of fermented foods in health education in school curriculum.

6. Development of starter culture and processing methods adaptable to the local people is necessary for them to benefit from fermented foods daily intakes.

7. Need for more modern methods that are readapted from the local processessing.

ACKNOWLEDGMENTS

I acknowledge the grace of God my Maker, my Creator, my Helper.

To my father, Late Deacon Moses Jivoh Oladele Adeyele, who was more than a father to me. You were always proud of me, but you didn't live long to see your handiwork, I thank God for your life ,my sweet mother Olabisi Adeyele, (*ayounge* mummy/grandma) who could not be here because of old age @ 94 I thank God for you that you are alive hail and healthy.

My mentors that nurtured me from the cradle of Microbiology, Prof. Mrs. Ibe and Dr. Mrs. Ogunsanya, I always admire you as my role models. My undergraduate supervisor in the Department of Microbiology and Parasitory LUTH, Prof. Tolu Odugbemi you believed so much in Sena Adeyele that you handed me over to Mr. Abu the Lab Technician to assist me all the time; this is the result of your foundation.

When I was sent to the Department of Botany and Microbiology, University of Ibadan, Botany, for my Youth Service, it was not chance meeting with Prof. Sunny Odunfa (grandfather, micro). Little did I know that God was going to use him to re-orientate and nurture me in the area of Food Microbiology with interest in Africa fermented foods, I thank you. I would like to acknowledge others Prof. B. A. Oso (Grandfather micro), Prof. Adisa (though we met again in LASU) and Prof. Fagade (Uncle Fag). Prof. Esther Aderibigbe of Ekiti State University I thank you for all your assistance.

My colleagues in Science Faculty, I say God bless you all for your love towards me. Special thanks to Microbiology Department family, we believe so much in ourselves that other Departments call us microbes, the cord of love among us shall wax stronger.

I wish to specially acknowledge all my Postgraduate students, especially Mr. Adebajo and Sidquat Abdulah and my undergraduate students, thank you. I would also like to thank all those that assisted in this manuscript.

I appreciate Dr. Mrs. Bosede Amoo (my sister) and the staff of Multimedia Centre for their assistance today and all the time I needed assistance.

To Prof. Dapo Asaju you have been an inspiration to me for several years now, I say thank you.

I also want to acknowledge my siblings (Dele Adeleye clan) Seyi, Nunayon, Mawume (late) Sewede, Semetan, Whenayan and Pedetin. They have been so good and wonderful. My Anties - sister Lanre, Mrs Kufeji and Mrs Soetan, My in-laws, Engr. And Prof. Mrs. Ijaya, Pharmacist Lara Adeyemi. To my Dear Pastor of Stone Church, Lagos, I appreciate you for the word of God which you taught.

Finally to my brother, my friend, the one and only Dr. Olabode Bakare, 'BB' you were always there as an encourager; indeed you call me 'darling' you're a darling in a million, you made today possible when I thought of no material to use you kept on saying you can make it.

To my beautiful children, Olabode, Olatubosun, Olubusayo and Olufunmilayo, I bless God because you have always been my joy.

Finally, to my Lord, my Creator and Maker, I say Eyin ni Baba to to Baba, Eyin ni Baba to ju Baba lo.

Vice - Chancellor Sir, I say thank you for making today possible. Thank you Ladies and Gentlemen for your attention.

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