

# Use of Biotechnology in Traditionally Fermented African Foods



## 1.1 Introduction

The relationship between biotechnology and traditionally fermented foods has not been discussed widely in the literature hence the writer's choice of the topic. It should be noted however that biotechnology can be of help in the improvement of fermented foods at three levels:

Firstly - raw materials. Fermented foods are produced from either animal or plant starting materials, and the availability of these substrates will aid in the production of fermented foods. Biotechnological methods to improve animal and plant production have been dealt with by experts in those fields on many occasions. It should be noted that certain wild plants and marginalised crops (the so called lost crops of Africa should not be neglected e. g., sorrel and okra) Attempts to restore the forest cover should give some attention to trees that bear fruits used during famines or even trees that host caterpillars.

The second level is fermentation engineering. Recent developments in biotechnology have given rise to great innovations in bioreactor designs. Most of these designs deal with liquid reaction media, but it should not be forgotten that a great number of fermented foods are produced through a solid-substrate fermentation in which the fermenting paste is frequently hand mixed. Bioreactors to simulate such a process are needed for the modernization of such traditional fermented foods.

The third level is microbiology and enzymology. There are many opportunities for biotechnological innovations in the microbiology of fermented foods. First, all the microorganisms involved should be isolated, characterized, and preserved as a germplasm collection. Second, the metabolic role of each of the strains involved should be clearly identified, and their full potential, even in

other fields of biotechnology, should be studied. The powerful technique of monoclonal antibodies for the characterization of different strains of the same species can be of great help in this area.

Many of these organisms have the enzyme complement to produce vitamins and amino acids in fermented foods. This potential can be improved through the technique of recombinant DNA technology to produce strains that are capable of producing and releasing the required amino acid or vitamin into the food. To avoid food losses due to spoilage-causing organisms and to avoid possible development of food-poisoning microbes, it is possible to genetically engineer a strain required for a process as a pure culture. Such a strain may bring about all the changes required in the food and grow at a convenient temperature.

The general aims of food technology are to exploit natural food resources as efficiently and profitably as possible. Adequate and economically sound processing, prolongation of shelf life by preservation and optimization of storage and handling, improvement of safety and nutritive value, adequate and appropriate packaging, and maximum consumer appeal are key prerequisites to achieving these aims. Fermentation is one of the oldest methods of food processing. The history of fermented foods has early records in Southeast Asia, where China is regarded as the cradle of mould-fermented foods, and in Africa where the Egyptians developed the concept of the combined brewery bakery. The early Egyptian beers were probably quite similar to some of the traditional opaque sorghum, maize, or millet beers found in various African countries today (Hesseltine, C. W; 1981) In technologically developed regions, the crafts of baking, brewing, wine making, and dairying have evolved into the large-scale industrial production of fermented consumer goods, including cheeses, cultured milks, pickles, wines, beers, spirits, fermented meat products, and soy sauces. The introduction of such foreign "high-tech" fermented products to tropical

countries by early travellers, clergymen, and colonists was followed by an accelerated demand during the early post independence period. Their high price ensured status, and their refined quality guaranteed continued and increasing consumption. In contrast, many of the traditional indigenous foods lack this image; some may even be regarded as backward or poor people's food. Factors contributing to such lack of appeal include inadequate grading and cleaning of raw materials, crude handling and processing techniques, and insufficient product protection due to lack of packaging. Such unhygienic practices are easily translated into a fear of food borne diseases. From a nutritionist's point of view, many traditional starchy staples are deficient in energy, protein, and vitamins. Variable sensory characteristics (quality) and lack of durability (shelf life) reduce convenience to the consumer: time needs to be spent selecting products of adequate quality, whereas perishable products require frequent purchasing and result in increased wastage. In addition, ungraded heterogeneous products, inconvenient unpacked bulk foods, or unattractive presentation inhibit consumers to develop regular purchasing attitudes.

Below is a table of current information available on African Foods and their methods of inoculation.

**Table 1.1** African fermented foods and their methods of inoculation - compiled from information obtained from Odunfa and Oyewole (1997).

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development <sup>2</sup>
cassava	Gari	West and Central Africa	Solid state	<i>Corynebacterium mannihot</i> , <i>Geotrichum</i> species, <i>Lactobacillus plantarium</i> , <i>Lactobacillus buchneri</i> , <i>Leuconostoc</i> species, <i>Streptococcus</i> species.	Natural/chance	1, 2, 5, 7, 8
	Fufu	West Africa	Submerged	<i>Bacillus</i> species, <i>Lactobacillus</i> species such as <i>Lactobacillus plantarium</i> ; <i>Leuconostoc</i>	Natural / chance	1, 2, 5, 6

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development <sup>2</sup>
(B)	Lafun / Konkote	West Africa	Submerged	<i>mesenteroides</i> ; <i>Lactobacillus cellobiosus</i> ; <i>Lactobacillus brevis</i> ; <i>Lactobacillus coprophilus</i> ; <i>Lactobacillus lactis</i> ; <i>Leuconostoc lactis</i> and <i>Lactobacillus bulgaricus</i> , <i>Klebsiella</i> species, <i>Leuconostoc</i> species, <i>Corynebacterium</i> species and a yeast of the <i>Candida</i> species. <i>Bacillus</i> species, <i>Klebsiella</i> species, <i>Candida</i> species, <i>Aspergillus</i> species; <i>Leuconostoc</i>	Spontaneous	1, 2, 5, 6
				<i>mesenteroides</i> , <i>Corynebacterium manihot</i> , <i>Lactobacillus plantarum</i> , <i>Micrococcus luteus</i> and <i>Geotrichum candidum</i>		
				<i>Corynebacterium</i> , <i>Bacillus</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> ,		
	Chikwangue	Africa / Zaire	Solid state		Spontaneous	1, 2, 7
		Central Africa / Zaire	Solid state		Spontaneous	1, 2, 7
	Cingwada	East and Central Africa	Solid state	<i>Pseudomonas</i> , <i>Acinetobacter</i> and <i>Moraxella</i> <i>Corynebacterium</i> , <i>Bacillus</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> ,	Spontaneous	1, 2
(B) Gruels and Beverages						
maize	Ogi	West Africa / Nigeria	Submerged	<i>Lactobacillus plantarum</i> , <i>Corynebacterium specie</i> , <i>Aerobacter</i> , yeasts <i>Candida mycoderma</i> , <i>Saccharomyces cerevisiae</i> and <i>Rhodotorula</i> and molds <i>Cephalosporium</i> , <i>Fusarium</i> , <i>Aspergillus</i> and <i>Penicillium</i>	Appropriate starters produced by back-slopping	1, 2, 3, 4, 5, 7
Sorghum	Abreh	Sudan	Solid state and Submerged	<i>Lactobacillus plantarum</i> .	Appropriate starters produced by back-slopping	1, 2
Millet	Uji	East Africa/	Submerged	<i>Leuconostoc mesenteroides</i> ,	Appropriate starters	1, 2

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development <sup>2</sup>
maize	Kenkey/ Koko/ Akasa	Kenya	Solid state	<i>Lactobacillus plantarum</i> .	produced by back-slopping/inoculation belt	1, 2
		West Africa / Ghana		<i>Enterobacter cloacae</i> , <i>Acinetobacter sp.</i> , <i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida mycoderma</i>	Spontaneous	
(C) Alcoholic Beverages						
Palm	Palm wine / Emu	West Africa	Submerged	<i>Saccharomyces cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i> .	Spontaneous	1, 2, 7
Various types of African cereal grains (Maize, Sorghum, Millet-used)	Busa	East Africa / Kenya	Submerged	<i>Saccharomyces. Cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i> .	Spontaneous	1, 2, 7
	Mbege	Tanzania	Submerged	<i>Saccharomyces cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i> .	Spontaneous	1, 2
	Burukutu	West Africa	Submerged	<i>Saccharomyces cerevisiae</i> , <i>S. chavelieri</i> , <i>Candida sp</i> and, <i>Leuconostoc meseteroides</i> . <i>Acetobacter sp.</i>	Spontaneous	1, 2
	Pito	West Africa	Submerged	<i>Geotrichum candidum</i> , <i>Lactobacillus sp.</i> and <i>Candida sp.</i>	Natural / chance Inoculation belt	1, 2,
	(C)	Acid Leavened Bread / Pancakes				
Various types of African cereals grains	Kisra	Sudan	Submerged		Appropriate starters produced by back-slopping	
	Enjera / Tef Injera	Ethiopia	Submerged		Appropriate starters produced by back-slopping	

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development <sup>2</sup>
(D)	Legumes					
Locus bean / Soybeans	Iru, Dawadawa/ Etchum, Kal Soumbara, Chu	West Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> and <i>Staphylococcus saprophyticus</i>	Spontaneous	1, 2, 3, 6, 7
African oil bean	Ugba			<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> and <i>Staphylococcus saprophyticus</i>	Spontaneous	
Melon Seeds, castor oil seeds, pumpkin bean, sesame	Ogiri / Ogili	West, East and Central Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> , <i>Staphylococcus saprophyticus</i> , <i>Lactobacillus plantarum</i>	Spontaneous	1, 2
Cotton seed	Owoh	West Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> , <i>Staphylococcus saprophyticus</i>	Spontaneous	1
(E)	Animal Products					
Goat milk	Ayib	East and Central Africa		<i>Candida</i> spp., <i>Saccharomyces</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp.,	Spontaneous	1, 2
Cow milk	Leben / Lben	North, East Central Africa Afi		<i>Candida</i> spp., <i>Saccharomyces</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp.,	Spontaneous	1, 2, 3

**\*\*Key to codes for the 'state of development'**

- 1: Micro-organisms involved known
- 2: Roles of individual micro-organisms known
- 3: Genetic improvement carried on organisms.
- 4: Starter cultures available for the fermentation
- 5: Varieties of raw materials that are best suited for the product known
- 6: Improved technology available and adopted
- 7: Pilot Plant production
- 8: Industrial Plant production

If blank, then information is not available

<sup>2</sup>State of development of each fermented product. It is the personal assessment of data, literature, internet search and other information by O. B. Oyewole as at March 2009.



## 1.2 Description

The fermentation bioprocess is the major biotechnological application in food processing. It is often one step in a sequence of food-processing operations, which may include cleaning, size reduction, soaking, and cooking. Fermentation bioprocessing makes use of microbial inoculants for enhancing properties such as the taste, aroma, shelf-life, safety, texture, and nutritional value of foods. Microbes associated with the raw food material and the processing environment serve as inoculants in spontaneous fermentations, while inoculants containing high concentrations of live micro-organisms, referred to as starter cultures, are used to initiate and accelerate the rate of fermentation processes in non-spontaneous or controlled fermentation processes. Microbial starter cultures vary widely in quality and purity.

Starter culture development and improvement is the subject of much research both in developed and in developing countries. While considerable work on GM starter culture development is on-going at the laboratory level in developed countries, relatively few GM micro-organisms have been permitted in the food and beverage industry globally. In 1990, the United Kingdom became the first country to permit the use of a live genetically modified organism (GMO) in food. It was a baker's yeast, engineered to improve the rate at which bread dough rises by increasing the efficiency with which maltose is broken down. This modification was done by using genes from yeast and placing them under a strong constitutive promoter. The United Kingdom has also approved a GM brewer's yeast for beer production. By introducing a gene encoding glucoamylase from yeast, better utilization of carbohydrate present in conventional feedstock can be obtained, resulting in increased yields of alcohol and the ability to produce a full-strength, low-carbohydrate beer. More recently,

two genetically modified yeast strains were authorized for use in the North American wine industry (Bauer *et al.*, 2007).

Current literature documents volumes of research reports on the characterization of microbes associated with the production of traditional fermented foods in developing countries. Relatively few of these studies document the application of the diagnostic tools of modern biotechnology in developing and designing starter cultures. The development and improvement of microbial starters has been a driving force for the transformation of traditional food fermentations in developing countries from an “art” to a science. Microbial starter culture development has also been a driving force for innovation in the design of equipment suited to the hygienic processing of traditional fermented foods under controlled conditions in many developing countries.

Starter culture improvement, together with the improvement and development of bioreactor technology for the control of fermentation processes in developed countries, has played a pivotal role in the production of high-value products such as enzymes, microbial cultures, and functional food ingredients. These products are increasingly produced in more advanced developing economies, and are increasingly imported by less advanced developing countries, as inputs for their food processing operations.

### **1.2.1 Spontaneous Inoculation of Fermentation Processes**

In many developing countries, fermented foods are produced primarily at the household and village level, using spontaneous methods of inoculation. Spontaneous fermentations are largely uncontrolled. A natural selection process, however, evolves in many of these processes which eventually results in the predominance of a particular type or group of micro-organisms in the

fermentation medium. A majority of African food-fermentation processes make use of spontaneous inoculation (Table 1). Major limitations of spontaneous fermentation processes include their inefficiency, low yields of product and variable product quality. While spontaneous fermentations generally enhance the safety of foods owing to a reduction of pH, and through detoxification, in some cases there are safety concerns relating to the bacterial pathogens associated with the raw material or unhygienic practices during processing.

### 1.2.2 “Appropriate” Starter Cultures as Inoculants of Fermentation Processes

“Appropriate” starter cultures are widely applied as inoculants across the fermented food sector, from the household to industrial level in low-income and lower-middle-income economies. These starter cultures are generally produced using a back slopping process which makes use of samples of a previous batch of a fermented product as inoculants (Holzapfel, 2002). The inoculation belt (Holzapfel, 2002) used in traditional fermentations in West Africa serves as a carrier of undefined fermenting micro-organisms, and is one example of an appropriate starter culture. It generally consists of a woven fibre or mat or a piece of wood or woven sponge, saturated with “high”- quality product of a previously fermented batch. It is immersed into a new batch, in order to serve as an inoculant. The inoculation belt is used in the production of the indigenous fermented porridges, “*uji*” and “*mawe*,” as well as in the production of the Ghanaian beer, “*pito*” (Table 1).

*Iku*, also referred to as *iru*, is yet another example of an “appropriate” starter culture produced by back slopping. This starter culture is produced from concentrated fermented *dawadawa* (a fermented legume product), mixed with ground unfermented legumes, vegetables such as pepper, and cereals, such as

ground maize. It is stored in a dried form and is used as an inoculant in *dawadawa* fermentations in West Africa (Holzapfel, 2002). A range of appropriate starter cultures, either in a granular form or in the form of a pressed cake is used across Asian countries as fermentation inoculants. These traditional mould starters are generally referred to by various names such as *marcha* or *murcha* in India, *ragi* in Indonesia, *bubod* in the Philippines, *nuruk* in Korea, *koji* in Japan, *ragi* in Malaysia and *Loog-pang* in Thailand. They generally consist of a mixture of moulds grown under non-sterile conditions.

### **1.2.3 Defined Starter Cultures as Inoculants of Fermentation Processes**

Few defined starter cultures have been developed for use as inoculants in commercial fermentation processes in developing countries. Nevertheless, the past ten years have witnessed the development and application of laboratory-selected and pre-cultured starter cultures in food fermentations in a few developing countries. “Defined starter cultures” consist of single or mixed strains of micro-organisms (Holzapfel 2002). They may incorporate adjunct culture preparations that serve a food-safety and preservative function. Adjunct cultures do not necessarily produce fermentation acids or modify texture or flavour, but are included in the defined culture owing to their ability to inhibit pathogenic or spoilage organisms. Their inhibitory activity is due to the production of one or several substances such as hydrogen peroxide, organic acids, diacetyl and bacteriocins (Hutkins, 2006). By and large, defined cultures are produced by pure culture maintenance and propagation under aseptic conditions. They are generally marketed in a liquid or powdered form or else as a pressed cake.

Defined starter cultures are also widely imported by developing countries for use in the commercial production of dairy products such as yogurt, kefir,

cheeses, and alcoholic beverages. Many of these cultures are tailored to produce specific textures and flavours. In response to growing consumer interest in attaining wellness through diet, many yogurt cultures also include probiotic strains. Probiotics are currently produced in India for use as food additives, dietary supplements and for use in animal feed (e.g. [www.prnewswire.co.uk/cgi/news/release?id=262320](http://www.prnewswire.co.uk/cgi/news/release?id=262320)). Methodologies used in the development and tailoring of these starters are largely proprietary to the suppliers of these starters. Monosodium glutamate and lactic acid, both of which are used as ingredients in the food industry, are produced in less-advanced developing countries using defined starter cultures.

#### **1.2.4 Defined Starter Cultures Developed Using the Diagnostic Tools of Advanced Biotechnologies**

The use of DNA-based diagnostic techniques for strain differentiation can allow for the tailoring of starter cultures to yield products with specific flavours and/or textures. Random amplified polymorphic DNA (RAPD) techniques have been applied in, for example, Thailand, in the molecular typing of bacterial strains and correlating the findings of these studies to flavour development during the production of the fermented pork sausage, *nam*. The results of these analyses led to the development of three different defined starter cultures which are currently used for the commercial production of products having different flavour characteristics (Valyasevi and Rolle, 2002).

#### **1.2.5 GM Starter Cultures**

To date, no commercial GM micro-organisms that would be consumed as living organisms exist. Products of industrial GM producer organisms are, however, widely used in food processing and no major safety concerns have

been raised against them. Rennet which is widely used as a starter in cheese production across the globe is produced using GM bacteria.

## **1.3 General Analysis**

Socio-economic factors have played a major role in the adoption and application of microbial inoculants in food fermentations. In situations where the cost of food is a major issue, uptake and adoption of improved biotechnologies has been generally slow. Demand for improved inoculants and starter culture development has been triggered by increasing consumer income, education, and new market opportunities.

### **1.3.1 Socio-Economics of the Consumer Base**

The consumer base of traditionally fermented staple foods in most developing countries is largely poor and disadvantaged. Price, rather than food safety and quality, is therefore a major preoccupation of this group when purchasing food. Fermented foods provide that target group with an affordable source of food, and make a substantial contribution to their food and nutritional security. These foods are generally produced under relatively poor hygienic conditions at the household and village level. Fermentation processing is practised largely as an art in such contexts. Interventions designed to upgrade processes used in the production of these traditionally fermented staples have been largely carried out through donor-funded projects and have focused primarily on reducing the drudgery associated with the fermentation processes. Improvements have also targeted the up gradation of hygienic conditions of fermentation processes and the introduction of simple and “appropriate” methodologies for the application of inoculants, such as the use of back slopping. While the uptake of simple back slopping technologies at the

household level has, in general, been very good by that target group, the uptake of defined starter cultures has been less successful, owing to cost considerations.

With growing incomes and improved levels of education in urban centres across a number of developing countries, dietary habits are changing and a wider variety of foods is being consumed. Fermented foods are no longer the main staples, but are still consumed as side dishes or condiments by that target group. The demand of that target group for safe food of high quality has begun to re-orient the traditional fermented food sector, and led to improvements in the control of fermentation processes through the development and adoption of defined starter cultures, the implementation of GHPs and HACCP in food fermentation processing, and the development of bioreactor technologies, coupled with appropriate downstream processing to terminate fermentation processes and thus extend the shelf-life of fermented foods. The packaging of fermented products has also improved.

### **1.3.2 Changing Consumer Demand Trends**

Apart from their changing dietary patterns and their demand for safety and quality, higher-income consumers demand convenience and are increasingly concerned about deriving health benefit from the foods they consume. Many of these consumers also show a preference for shopping in supermarkets. Consumer demand for deriving wellness through food consumption has stimulated the development of industrial fermentation processes for the production of functional ingredients such as polyunsaturated fatty acids and probiotic cultures for use as food ingredients in developing countries. These functional ingredients are currently applied in the fortification of fermented foods as well as in the production of dietary supplements in countries such as India. The growth of supermarkets in developing countries has promulgated the

need for standardized products of a reasonable shelf-life that meet safety and quality criteria. Packaged fermented products such as kimchi, miso, and tempeh, for example, are widely available in supermarkets across Asia. The production of traditional beer in a powdered format and in ready-to-drink containers in Zambia is a very good example of product development that has taken place in response to consumer demand for convenience, both in local and export markets. Shifting consumer preferences in South Africa, away from basic commodity wine to top-quality wine, is yet another example of how market demand has led to research and biotechnological innovation in the wine industry. Biotechnological innovations in that country are currently focused on the improvement of *Saccharomyces cerevisiae* strains to improve wholesomeness and sensory quality of wines.

### **1.3.3 The Enabling Environment for Starter Culture Development**

A considerable amount of research in developing countries has focused on the identification of starter micro-organisms associated with the fermentation of these staple foods. The greatest strides in starter culture development have, however, been realized in countries that have prioritized the development of technical skills, the infrastructural support base and funding support for research into the up gradation of fermentation processes. Linkages between research institutions and the manufacturing sector have also been critical to the successful introduction of starter cultures. Collaborative initiatives among research institutions have also had a major positive impact on biotechnological developments in developing countries. Collaboration among African institutions and their counterparts in the North has greatly facilitated improvements in biotechnological research and capacity development in the area of food biotechnology on the continent. One major success story in this regard has been collaborative projects involving Burkina Faso, Ghana and institutions in the



Netherlands. This programme facilitated the typing and screening of microbial cultures associated with fermented African foods as a basis for starter culture development. Results of this work (Mengu, 2009) led to improvements in the production of gari, a fermented cassava product and dawadawa, a fermented legume product. Issues related to the protection of intellectual property rights (IPR) are of growing concern with respect to starter culture development.

### **1.3.4 Proactive Industrial Strategies**

Biotechnology developments have been most successful in areas where proactive approaches are taken by industry. The Thai food industry successfully creates perceived quality by launching new product logos and associating these new products with biotechnology or with the fact that they were developed using traditional biotechnology, such as starter cultures. The goal of the industry is to project an image of itself as producing products of superior quality and safety that represent progressiveness based on a higher level of technology.

### **1.3.5 Export Opportunities for Fermented Products**

Increasing travel due to globalization has changed the eating habits of consumers across the globe. Export markets for fermented foods have grown out of the need to meet the requirements of developing country diaspora in these markets as well as to satisfy growing international demand for niche and ethnic products. Indonesian *tempe* and Oriental soy sauce are well known examples of indigenous fermented foods that have been industrialized and marketed globally. The need to assure the safety and quality of these products in compliance with requirements of importing markets has been a driving force for the upgrading of starter cultures as well as for diagnostic methodologies for verification of their quality and safety.

Growing interest and trade in fermented food products is also likely to lead to the greater use of the DNA barcode for identifying the origins of specific fermented food products produced in developing countries.

## **1.4 Actualisation: Case Study of Flavour Production from Alkaline-Fermented Beans (West Africa)**

This Case Study on the indigenous fermentation of the locust bean, dawadawa (fermented locust bean), is a classic example of how traditional fermentations can be exploited for the production of high-value products such as flavour compounds. The work, however, was undertaken by a large cooperation with little involvement of local researchers. Returns on commercial successes derived from this study did not go back to the people who invented the traditional method of producing this indigenous fermented food. This Case Study, therefore, serves to highlight the critical issue of IPR of traditional production systems. Dawadawa is produced by alkaline fermentation of the African fermented locust bean (Steinkraus, 1995). It is an important condiment in the West/Central African Savannah region (Odunfa and Oyewole, 1986). Similar fermented food products can be found throughout Africa, with regional differences in the raw materials used as processing inputs or in post processing operations. Similarly fermented products are referred to as “kinda” in Sierra Leone, “iru” in coastal Nigeria, “soumbara” in Gambia and Burkina Faso, and “kpalugu” in parts of Ghana (Odunfa and Oyewole, 1986). Foods produced by alkaline fermentation in other parts of the world include “natto” in Japan, “*thua noa*” in Thailand and “kinema” in India (Tamang, 1998). These are mainly used as culinary products to enhance or intensify meatiness in soups, sauces and other prepared dishes. The production of dawadawa involves extensive boiling and dehulling of the beans, followed by further boiling to facilitate softening. Spontaneous fermentation of the softened

beans is subsequently allowed to take place over 2–4 days. Micro-organisms associated with the fermentation include *Bacillus subtilis* (Ogbadu and Okagbue, 1988), *B. pumilus* (Ogbadu and Okagbue, 1988), *B. licheniformis* (Ogbadu, Okagbue and Ahmad, 1990), and *Staphylococcus saprophyticus* (Odunfa, 1981). During the fermentation process, the pH increases from near neutral to approximately 8.0, temperature increases from 25 °C to 45 °C and moisture increases from 43% to 56% (Odunfa and Oyewole, 1986). At the same time, a five-fold increase in free amino acids takes place, and glutamate, a flavour enhancer, increases five-fold during the process. Mechanisms of flavour production during the fermentation process, as well as flavour principles generated during dawadawa fermentation processing, have been studied by international food manufacturers and been used as a basis for the development of flavours for incorporation in bouillon-type products (Beaumont, 2002).

## 1.5 Discussion

Fermented food products play a significant socio-economic role in African countries and the developing world. The importance of traditional fermented foods has been reviewed. These products also contribute to the protein requirements of the indigenous consumers [Achi OK., 2005]. Lowering the pH of food products through fermentation is a form of food preservation [Ananou S, Maqueda M, Martínez-Bueno M and Valdivia E; 2007]. This is a self-limiting process in that further reduction of pH may be inimical to the producing organisms. As a result the pH normally stays just below 5. Other benefits of fermentation include improvement of food quality through food digestibility to increase essential amino acids, vitamins and protein in the era of diminishing food quality, fermentation can play a role in complementing the food fortification programme instituted by the WHO. Cereal grains are also susceptible to

contamination by naturally occurring mycotoxins both on the fields and during storage. The fermentation of maize meal has been demonstrated to detoxify these toxins making maize meal safer for human consumption. Children are the hardest hit segment of the population when a nation faces food crisis. Donor countries would normally supply mainly maize to fight off hunger. Fermentation of maize meal makes the final product suitable to serve as weaning food since the bacteria responsible for fermentation also produce vitamins and amino acids during their growth and serve as single cell proteins after cooking. Moreover, maize is more likely to be readily available in a poor setting where a balanced diet is not available. Therefore, fermentation technology is of great importance in ensuring food safety, preservation and food flavouring [Nout MJR and Motarjemi Y.; 1997]. Fermentation is also known to soften food texture and alter its composition in such a way that it will require minimal energy both in cooking and preservation process. Thus, less fuel will be used for cooking and eliminates the need of preservation as fermentation increases the shelf life of food. These advantages make fermentation a highly desirable technique in the rural communities of the third world where resources for cooking and preservation are scarce. Fermentation technology also has the potential of meeting the world's food supply demand if adequately developed into the industrial scale [Nout MJR and Motarjemi Y.; 1997]. In a Joint FAO/WHO Workshop held in Pretoria, South Africa, the importance of antibiotic activity and nutritional benefits of LAB was revisited [Motarjemi Y and Nout MJ.; 1996]. In this workshop, a gap of knowledge was identified and the participants were unanimous that further research was required to further expand the usefulness of food fermentation especially its antibiotic activities against parasites, viruses, and bacteria. Additionally, assessment of physicochemical effects of fermented foods on consumers and the establishment of starter cultures for commercial market were identified as priorities [Motarjemi Y and Nout MJ.; 1996]. The handlers of

traditional foods also need to be educated on food hygiene, as there are many instances where food is contaminated by bad handling after cooking. LAB fermentation fits into primary care initiatives and can reduce child mortality by supplying the minimum required nutrients [Motarjemi Y.; 2002]. In addition to its potential use to tackle malnutrition, the technology is a low cost means of food preservation.

There is need to educate the African citizens on the need of consuming fermented foods and food safety. Although fermented foods are generally safe, and in the view that certain antimicrobial factors are present, lack of standardization in the methods used, the environment and the hygiene of the people that prepare them, will determine the quality of the product. Safety is of paramount importance. Personal hygiene should be practiced to complement the overall benefits of fermented foods. The greatest drawback in the development of fermented food products in Africa is that many products are produced under primitive conditions, resulting in low yield and poor quality, including short shelf-life [Achi OK; 2005]. Other problems include the lack of appeal in the presentation and marketing of the food products, as well as the fact that the processes are often laborious and time-consuming [Nout MJR, Kok B, Vela E, Nche PF and Rombouts FM; 1995]. The technology needs to be improved through research to advance its potential for food safety and nutritional value. Imported products should not stifle the development of traditional food products at a national and international level. With the current technologies, it should be possible to be innovative about many of the foods produced using fermentation and indigenous knowledge systems. The challenge is to ensure that technology is used to add value to such products, such as increased shelf-life, flavour and appealing packaging and labelling. Old ferments are not an efficient way of preserving the LAB probiotic organisms as poor survival has been reported in these products. Microencapsulation technology is a new technique which can be

used to preserve and propagate LAB cultures for mass production of fermented foods [Kailasapathy K; 2002]. Current research conducted by our group include the isolation and identification of the microorganisms associated with *amahewu* and *incwancwa* production. This is hoped to preserve the cultures for future use as starter cultures and as a base to extend the product-range of fermented foods. In addition, antimicrobial peptides produced by some LAB can be used as lead compounds in drug discovery. It is also important to document these traditional indigenous technologies in order to preserve them for future generations, as the old days practices keep changing from time to time. This will also create a reference database for future generations of food research scientists, nutritionists and food regulatory bodies and policy makers in different ladders of government.

## **1.6 General Recommendations**

It is important that countries recognize the potential of fermented foods and prioritize actions to assure their safety, quality, and availability. A number of specific options can be identified for developing countries to help them make informed decisions regarding adoption of biotechnologies in food processing and in food safety for the future.

### **1.6.1 Regulatory and Policy Issues**

Governments must be committed to protecting consumer health and interests, and to ensuring fair practices in the food sector. There has to be consensus at the highest levels of government on the importance of food safety, and the provision of adequate resources for this purpose. Government policy that is based on an integrated food-chain approach is science-based, transparent and includes the participation of all the stakeholders from farm to table must be put in place. The importance of the regional and international dimensions of the use

of biotechnologies in food processing and safety must be recognized. Priority must be accorded to promoting fermented foods in the food-security agendas of countries. Governments must also provide an enabling environment that is supportive of the growth and development of upstream fermentation processes such as the production of high-value fermented products, such as enzymes, functional-food ingredients, and food additives.

### **1.6.2 International Cooperation and Harmonization**

The organization and implementation of regional and international fora are critical requirements for the enhancement of national organizational capability and performance and for the facilitation of international co-operation. Further, the setting up of administrative structures with clearly defined roles, responsibilities, and accountabilities could efficiently govern processed foods and safety issues.

Biotechnology-based Standard Operating Procedures (SOPs) for food safety should also be documented for use in authorized laboratories. National food control databases for the systematic collection, reporting, and analysis of food-related data (food inspection, analysis, etc.) with set regulations and standards based on sound science and in accordance with international recommendations (Codex) are key requirements.

### **1.6.3 Education Policy**

While the consumption of fermented foods is growing in popularity among higher-income consumers thanks to increasing interest in wellness through diet, the consumption of fermented foods by lower-income consumers in many developing countries is perceived to be a backward practice.

Strategies should therefore be developed for the dissemination of knowledge about food biotechnology and, particularly, fermented foods. Targeted consumer education on the benefits of consuming fermented food products and on applying good practice in their production is required.

Food biotechnology should be included in educational curricula in order to improve the knowledge base in countries on the contribution of fermented foods to food and nutritional security and to generate awareness of the growing market opportunities for fermented foods and high-value products derived from fermentation processes.

#### **1.6.4 Information-Sharing**

Access to specialized technical information on biotechnology and biotechnological developments in the food processing sector are critical and necessary inputs and support systems for guiding and orienting the research agendas of countries. The necessary information systems should therefore be developed to facilitate rapid access to information on biotechnological developments across both the developed and the developing world.

#### **1.6.5 Legislation and Policy on Technologies**

Expertise in legislation and technology licensing, as well as knowledge about how to nurture innovation and turn it into business ventures, are critical requirements for developing countries. Successful technology transfer requires all of these elements and an environment that is conducive to innovation. Government policy in developing countries should therefore prioritize technology transfer that helps create new business ventures, an approach that requires government support such as tax incentives and infrastructure investment.



### **1.6.6 Intellectual Property Rights (IPR)**

Many of the traditional fermentation processes applied in developing countries are based on traditional knowledge. Enhanced technical and scientific information is required in order to claim ownership of the traditional knowledge of the craft of indigenous fermented foods. Lack of technical knowledge has resulted in the failure to realize the benefits of the industrialization of indigenous fermented foods by individuals who are the rightful owners of the technology. Greater focus is required on issues of relevance to IPR and on the characterization of microbial strains involved in traditional fermentation processes. Emphasis must be placed on IPR education for scientists. National governments should put in place the requisite infrastructure for IPR to facilitate the process. At the institutional level, this infrastructure would include technology management offices for assisting scientists in procedures relating to intellectual property matters. The processes used in the more advanced areas of agricultural biotechnology are generally covered by IPR, and the rights are generally owned by parties in developed countries.

### **1.6.7 Communication and Consumer Perceptions**

Communication between various stakeholders is critical in proactively engaging with consumers. Communication must be established with the public at large on processed food and associated hazards. Communication gradually builds confidence and will be critical to advancing the application of biotechnologies in food processing and safety. The primary role stakeholders are incorporated in the discussion and decision-making process. The need for specific standards or related texts and the procedures followed to determine them should also be clearly outlined. The process, therefore, should be transparent.

Public awareness and education are critical to the success of food bioprocessing and food safety in developing countries.

Greater attention must be directed toward understanding consumer and producer (processor) perceptions on food safety and quality in developing countries.

If foods are to be promoted as being safe and healthy, their nutritional and safety attributes must be transparently demonstrated by presenting scientific data to substantiate the nutritional and health benefits and by applying good manufacturing/hygiene practice and HACCP as safety measures to ensure that issues of consumer concern are addressed.

### **1.6.8 Technical Capacities and Technology Transfer**

Traditional fermented foods should be viewed as valuable assets. Governments should capitalize on these assets and add value to them by supporting research, education, and development, while building on and developing the indigenous knowledge base on food fermentations.

Government agencies in developing countries should focus on the development of technical capacities to deal with emerging technical issues.

The technical capacities of academic and research institutes should be strengthened in the fields of food biotechnology, food processing, bioprocess engineering, and food safety through training and exchange programmes for researchers. Such programmes should emphasize collaboration with both developed and developing country institutions engaged in work on food biotechnology, starter culture development, bioprocess engineering, and food safety.

Training capabilities in food biotechnology and food safety should be developed within developing country institutions through the introduction of degree courses in order to broaden the in-country technical support base for food bioprocess development. Given the similarities among fermentation processes across regions, an inventory of institutions engaged in food biotechnology in developing countries would be an asset in facilitating networking among institutions. Food processors, policy-makers, and equipment manufacturers should also be integrated into the networking activities.

The development of appropriate levels of bioreactor technology with control bioprocess parameters will be necessary to improve the hygienic conditions of the fermentation processes.

Research and infrastructural development to enable the cost-effective production of defined starter cultures in a stable format (i.e. cultures which do not require refrigeration and have prolonged shelf-life under ambient conditions) should be prioritized.

Infrastructure development to facilitate the transfer and adaptation of fermentation technologies developed at the laboratory level to the household and village and, where necessary, the enterprise level should be prioritized.

Appropriate levels of equipment will also be required to facilitate the downstream processing of these products.

Traceability systems that facilitate the differentiation and identification of food products should be prioritized in order to broaden market opportunities for these products.

A food-chain approach to assuring food safety should be prioritized by governments.

Food safety management systems should be strengthened by implementing systematic food safety measures such as GHP, GMP, and HACCP in food fermentation operations. Diagnostic kits are important tools for monitoring and verifying the level of sanitation in processing plants.

Highly sensitive and rapid diagnostic kits are invaluable for monitoring and rapidly detecting chemical and microbiological hazards that pose a threat to human health, with high precision and sensitivity. The development of low-cost diagnostic kits suitable for use by small processors would greatly facilitate food-safety monitoring. Development should target the realization of multiplex diagnostic systems with the capacity to detect several pathogens or many chemical contaminants using a single diagnostic kit. The development of diagnostic kits at a national level could further reduce their cost of production. Given the regional specificity of bacterial pathogens at the species and subspecies levels, such diagnostic kits should be developed with specificity and sensitivity to the species or subspecies that are prevalent in a specific region. Investment is therefore needed for the development of expertise, facilities, and the infrastructure for the mass production of antibodies, cell culture technology and for the formation of technical know-how on assembling the requisite components of diagnostic kits.

The development of national hazard -profile databases that document the prevalent pathogens in different regions will be critical. Such information would be useful for further research into the development of diagnostic kits with high precision and sensitivity and in implementing HACCP as well as risk assessment research. The culture collection of identified infectious agents in the hazard profiles could play an important role for specific antibody production for use in the development of immunoassay diagnostic kits.

For developing countries to make full use of the available biotechnologies in their traditional food fermentations, an understanding and acquisition of expertise in the following areas are essential:

### **1.6.9 Art of Fermentation**

A clear understanding by the master brewer of every step used in the fermentation is needed. This is the art of fermentation. Although the master brewers might not have scientific backgrounds, they could normally ensure a proper fermentation as a result of years of experience. Without knowledge of the art of traditional food fermentation, a scientist cannot provide a scientific explanation for the process and proceed to provide assistance in improvement of the process.

### **1.6.10 Microbiology**

It is essential to know which microorganisms involved in food fermentations are useful and how the physiology and metabolism of these microbes are affected by the physical and chemical environments of fermentations, as well as how their microbial activities in turn affect the fermentation processes. Microorganisms normally break down carbohydrates, proteins, and lipids present in the raw materials to be fermented by releasing enzymes into the medium. As the raw materials are hydrolysed, the environment is changed, as sometimes reflected by a drop in pH value. Moreover, the breakdown products such as peptides and amino acids can be further converted into smaller volatile molecules that are odoriferous and hence improve the flavour characteristics of the fermented foods.

### **1.6.11 Upstream and Downstream Processing**

Normally raw materials are pre-treated before fermentation. It is important to comprehend how such pre-treatment could affect the fermentation process. In soy sauce fermentation, whole soybeans are steamed to make the soy protein more easily hydrolysable by the proteases of *Aspergillus oryzae*. In so doing, too much moisture is introduced and wheat flour must be added to lower the moisture content to a level that does not favour early bacterial growth and hence prevents spoilage of the fermentation. Downstream processing does not affect the bioprocess involved. However, it could alter the normal organoleptic properties of the product, especially when downstream processing involves heating, such as in the pasteurization of soy sauce. Heating causes a change in the flavour of soy sauce due to nonenzymic browning reactions, which could result in the production of pyrazine compounds.

### **1.6.12 Biochemistry**

An understanding of the biochemical activities of the microbes actively participating in the fermentation could help to explain the change in the texture of the raw material as well as the origin of flavouring substances often present in fermented foods. Flavour and texture are important properties of fermented foods. Elucidation of flavour production in such fermentations could result in the development of processes for producing of flavouring materials by fermentation, as in the production of cheese flavours by *Penicillium roquefortii*.

### **1.6.13 Fermentation Equipment and Techniques**

Practical experience in the use of both solid-state and submerged culture fermentation equipment is very useful. Normal training includes submerged

culture bioreactors but not solid-state fermenters. It is useful to know both types of fermentations because traditional food fermentations often involve solid state fermentation. In soy sauce fermentation an initial solid-state fermentation is followed by a submerged fermentation step. Systems that measure and control pH, dissolved oxygen, temperature, and moisture help to make these bioprocesses more efficient and reduce the time required for production of a quality product.

## 1.7 Conclusion

Fermentation of traditional foods, as a hurdle technology, is profitable in terms of food quality, preservation, and decontamination of toxins, often found in food. Together with food safety, the nutritional and flavour profile of the products need to meet the expectations of modern consumers. Education of communities about benefits of consuming fermented foods needs to be part of health education. This technology needs to be further developed to enhance safety and ease of application in a rural poor-resource setting. Development of convenient starter cultures and processing methods will ensure that many people in Africa will reap the benefits of indulging in fermented foods and beverages both during cultural ceremonies and during their normal daily activities.

With the rapid progress in the biological sciences, both basic and applied aspects, it has been possible to gain a better understanding of the mystery that has surrounded fermentation processes. The types of microorganisms involved has been isolated and identified, and the physiology and metabolism of these organisms have been studied. Hence, traditional fermented foods can now be made better, faster, and more economically. The application of available knowledge to improve traditional food fermentations in developed countries has far outpaced that in developing countries. The terms “old biotechnology” and “new biotechnology” have been used- “old” to mean the undirected manipulation

of microorganisms and plants, such as by mutagenesis and selection of the better strains. In this old biotechnology it is prudent to include directed control of the physical and chemical environments of the fermentation process, which could result in better performance of the useful microbes. Though mutation increases the ability to select better strains, there can, of course, be little directed alteration of genetic material. The new biotechnology, such as recombinant DNA techniques, overcomes this problem. The new biotechnology can, of course, be of tremendous help in producing super strains of microbes that could enable acceleration of fermentation processes, provide more efficient utilization of raw materials, and produce better-quality products. How best can African nations apply these biotechnologies to traditional fermented foods? Should it be application of the “old” before the “new”, “new” without the “old”, or “old” and “new” simultaneously? In their enthusiasm to promote the new biotechnology for traditional fermented food applications, scientists from developed countries should not forget the different environments that exist in developed and developing countries. In developed countries the old biotechnology is already well understood and practiced efficiently in fermented food industries. African countries may need to acquire a better understanding of the old biotechnology before efficiently absorbing and implementing the new biotechnology to its fullest.

## References

- [1] Achi OK. The potential for upgrading traditional fermented foods through biotechnology. *Africa Journal of Biotechnology*. 2005; 4(5): 375-380.
- [2] Aderiye BI and Laleye SA. Relevance of fermented food products in southwest Nigeria. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*. 2003; 3: 1-16.
- [3] Anukam KC and Reid G. African Traditional Fermented Foods and Probiotics. *Journal of Medicinal Food*. 2009; 12(6): 177-1184.



- [4] Barrett, T., Fang, P. & Swaminathan, B. 1997. Amplification Methods for Detection of food-borne Pathogens. *In: H. Lee, S. Morse, & O. Slovak, eds. Nucleic Acid Amplification Techniques: Application to Disease Diagnosis*, pp. 171–181. Boston, USA, Eaton Publishing.
- [5] Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D and Webb C. Cereal-based fermented foods and beverages. *Food Research International*. 2003; 36(6): 527-543.
- [6] Bruinsma, D. H., and M. J. R. Nout. 1990. Choice of technology in food processing for rural development. Paper presented at the symposium “Technology and Rural Change in Sub-Saharan Africa”, Sussex University, Brighton, U. K., Sept. 27-30, 1989. *In: Rural Households in Emerging Societies: Technology and Change in Sub-Saharan Africa*. M. Haswell and D. Hunt (Eds.). New York: Berg Publishers.
- [7] Codex Alimentarius Commission. 2001. *Ad-hoc inter-governmental task force on food derived from biotechnology*. Report of the Codex Alimentarius Commission, Geneva, July 2001. Alinorm 01/34. Rome, FAO.
- [8] Guandalini S. Probiotics for children: Use in diarrhoea. *Journal of Clinical Gastroenterology*. 2006; 40(3): 244-248. *Food Microbiology*. 2002; 75: 213-229.
- [9] Hesseltine, C. W. 1981. Future of fermented foods. *Process Biochemistry* 16: 2-13. Publishers.
- [10] Hesseltine, C. W., R. Rogers, and F. G. Winarno. 1988. Microbiological studies on amylolytic Oriental fermentation starters.
- [11] Holzapfel, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.* 75: 197–212.
- [12] Hutkins, R. W. 2006. *Microbiology and biotechnology of fermented foods*. Blackwell Publishing.
- [13] Ikediobi. C. O and E. Onyike. 1982. The use of linamarase in gari production. *Process Biochemistry* 17: 2-5.
- [14] Kailasapathy K. Microencapsulation of Probiotic Bacteria: Technology and Potential Applications. *Current Issues in Intestinal Microbiology*. 2002: 3: 39-48.

- [15] Leuchtenberger, W., Huthmacher, K. & Drauz, K. 2005 Biotechnological production of amino acids and derivatives – current status and prospects. *Applied Microbiology and Biotechnology* 69: 1–8.
- [16] Mengu, M. 2009. Overview of FP and Africa FP lessons learnt and success stories: Research on traditional African fermented foods. *Proceedings of the Science and Technology Europe-Africa Project Workshop*, Nairobi, Kenya. Available at: [www.st-eap.org/pdfs/report\\_st-eap\\_ws\\_mar2009.pdf](http://www.st-eap.org/pdfs/report_st-eap_ws_mar2009.pdf).
- [17] Mensah P. Fermentation -- the key to food safety assurance in Africa? *Food Control*. 1997; 8(5-6): 271-278.
- [18] Mensah, P., A. M. Tomkins, B. S. Drasar, and T. J. Harrison. 1991. Antimicrobial effect of fermented Ghanaian maize dough. *Journal of Applied Bacteriology* 70(3): 203-210.
- [19] Merican, Z. & Quee-Lan, Y. 2004. Taiwan Processing in Malaysia: A Technology in transition. In K. H. Steinkraus, ed. *Industrialization of Indigenous Fermented Foods*. New York, Marcel Dekker.
- [20] Motarjemi Y, Käferstein F, Moy G and Quevedo F. Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization*. 1993; 71(1): 79-92.
- [21] Motarjemi Y. Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology*. 2002; 75: 213-229.
- [22] Mokoena MP, Chelule PK and Gqaleni N. Reduction of Fumonisin B1 and Zearalenone by Lactic Acid Bacteria in Fermented Maize Meal. *Journal of Food Protection*. 2005; 68: 2095-2099.
- [23] Mosha TCE and Vicent MM. Nutritional value and acceptability of homemade maize/sorghum-based weaning mixtures supplemented with *rojo* bean flour, ground sardines and peanut paste. *International Journal of Food Sciences and Nutrition*. 2004; 55(4): 301 - 315.
- [24] Nout, M. J. R., and B. J. Davies. 1982. Malting characteristics of finger millet, sorghum, and barley. *Journal of the Institute of Brewing* 88: 157-163.

- [25] Nout, M. J. R., and F. M. Rombouts. 1990. Recent developments in *Tempe* research. *Journal of Applied Bacteriology* 69(5): 609-633.
- [26] Nout, M. J. R. 1991. Ecology of accelerated natural lactic fermentation of sorghum-based infant food formulas. *International Journal of Food Microbiology* 12(2/3): 217-224.
- [27] Nout M. J. R., Kok B, Vela E, Nche PF, and Rombouts FM. Acceleration of the fermentation of kenkey, an indigenous fermented maize food of Ghana. *Food Research International*. 1995; 28(6): 599-604.
- [28] Nout, M. J. R. 1990. *Food Laboratory News* 6(2)20: 10--12. Fermentation of infant food.
- [29] Nout M. J. R and Sarkar PK. Lactic acid food fermentation in tropical climates. *Antonie van Leeuwenhoek*. 1999; 76(1): 395-401.
- [30] Odunfa, S. A. 1981. Micro-organisms associated with fermentation of African Locust bean during iru preparation. *J. Plant Foods*, 3: 245-250.
- [31] Odunfa, S. A., & Oyewole, O. B. 1986. Identification of *Bacillus* species from iru, a fermented African Locust bean product. *J. Basic Microbiology*, 26: 101-108.
- [32] Odunfa, S. A., & Oyewole, O. B. 1997. African fermented Foods. In B. J. B. Wood, ed. *Microbiology of fermented foods*, Second Edition, Vol. II, pp. 713-715. London, Blackie Academic, and Professional.
- [33] Ogbadu, L. J., & Okagbue, R. N. 1988. Bacterial fermentation of Soy bean for daddawa production. *J. Appl. Bacteriol.* 65: 353-356.
- [34] Ogbadu, L. J., Okagbue, R. N., & Ahmad, A. A. 1990. Glutamic acid production by *Bacillus* isolates from Nigerian fermented vegetable proteins. *World J. Microbiology. Biotechnol.* 6: 377-382.
- [35] Ray, R. C., & Sivakumar, P. S. 2009. Traditional and novel fermented foods and beverages from tropical roots and tuber crops: Review. *Int. J. Food Sci. Tech.* 44, 1073-1087.
- [36] Rolle, R. & Satin, M. 2002. Basic requirements for the transfer of fermentation technologies to developing countries. *Int. J. Food Microbiology*. 75: 181-187.

- [37] Sauer M, Porro D, Mattanovich D and Branduardi P. Microbial production of organic acids: expanding the markets. *Trends in Biotechnology*. 2008; 26(2): 100-108.
- [38] Steinkraus, K. H. 1995. In: K. H. Steinkraus, ed. *Indigenous fermented foods involving an alkaline fermentation*, 2nd ed., pp. 349-362. NY, Marcel Dekker.
- [39] Steinkraus KH. Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control*. 1997; 8(5/6): 311-317.
- [40] Spicher, G. 1986. Sour dough fermentation. *Chemie Mikrobiologie Technologie der Lebensmittel* 10(3/4): 65-77.
- [41] Tamang, J. P. 1998. Role of micro-organisms in traditional fermented foods. *Indian Food Ind.* 17, 162-167.
- [42] Valyasevi, R. & Rolle, R. S. 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope of their improvement. *Int. J of Food Microbiology*. 75: 231-239.