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EMERGING TECHNOLOGY SERIES

Food Processing Technologies for Africa

Prepared for UNIDO
by
Hamid A. Dirar (ed.)

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UNITED NATIONS
INDUSTRIAL DEVELOPMENT
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Vienna, 1997

EMERGING TECHNOLOGY SERIES

Technology is now at the core of competitive strategies of successful industrial firms. The new and rapidly evolving generic technologies, such as biotechnology, new materials and information technologies, offer many opportunities and challenges for broad competitive strategies. They engender entirely new products, services, markets and businesses. Their impact is trans-sectoral, radically improving the competitiveness of products, processes and services of firms in a large number of traditional industrial sub-sectors. New materials improve product specifications and lower production costs in engineering and chemical industries; biotechnologies save energy and raw materials in chemicals, pharmaceuticals and food processing, while the pervasive applications of information technologies allow companies in all industrial sectors to re-engineer critical processes, improve overall efficiency and raise productivity across functional areas. Monitoring and access to information is now a key to competitiveness.

Experience in newly industrialized countries shows that access to reliable technical information can be instrumental in allowing manufacturers to leap whole periods of technological development and adopt state-of-the-art systems directly – without needing to undertake a painful and costly development phase. Up-to-date economic information and analysis of global economic trends and the prevailing industrial situation in other countries is likewise indispensable – and the gateway to identifying industrial needs, opportunities, constraints and priorities of the country and region concerned. Monitoring technological advances and economic analysis provide the basis for the formulation and effective implementation of appropriate industrial programmes and projects by both public and private entities. For developing countries, with their limited resources and often greater susceptibility to the negative aspects of technology-led change, such activities are doubly important. Yet many developing countries still lack the critical elements for technology monitoring of emerging technologies and their implications for national development strategies. If they are to maximise the benefits and minimize the negative effects of technology on social and economic development, developing countries must manage technology in an appropriate manner – and monitoring is an essential element of that management process.

One of the objectives of UNIDO is to carry out a set of coherent activities at the national, regional and international levels, to help developing countries at different stages of development to acquire, apply, develop and manage technologies against a global background of technological change. Investment and technology play a vital role in the industrial growth of developing countries, as well as their gradual integration into the international economy. Although most developing countries now have liberal regimes for investment and technology transfer, this is not a sufficient condition for industrial growth. There is a need for a wide-ranging investment and technology approach that will not only attract and retain the inflows of investment and technology, but also make the optimum use of them for the domestic economy. UNIDO's wealth of experience in industrialization, combined with its worldwide network of contacts makes the Organization an ideal partner to assist developing countries in building up their investment and technology partnerships. The Organization is a focal point of industrial technology; it is a global source of industrial information; and it is an honest broker for industrial cooperation.

Through this new series of publications on emerging technologies in developing countries, which supercedes the ***Industrial Technology Monitors*** and the ***Technology Trends Series***, UNIDO plans to sensitize industry and governments to the need for and requirements of technology monitoring and assessment in the areas of new and emerging technologies. These technologies play a catalytic role in the development process of the new global pattern of rapid and accelerating technological change, sweeping trade liberalization, far-reaching deregulation of markets – including the privatization of state-owned enterprises and commercialization of R&D – and the globalization of international business.

Investment and Technology Promotion Division
March 1997

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PREFACE

In November 1996, the World Food Summit ended with governments pledging to reduce the number of undernourished people worldwide from the current level of 840 million to 400 million by the year 2015. In an attempt to address the injustice of such widespread and persistent hunger and starvation, nearly all governments at the Summit agreed that the international community should identify freedom from hunger as a fundamental human right.

The goal of any country is development. Development is the development of people: improving their standard of living and quality of life. This can be achieved only through optimal generation, mobilization and utilization of natural and human resources.

Industrialization is the engine of development: processing raw materials into usable goods and services; giving products added value; increasing economic returns, employment and purchasing power; and strengthening linkages with other sectors of the economy. This will lead to self-reliant development.

The exaggerated urbanization and concentration of investments in urban-oriented, capital-intensive activities have produced a severe imbalance in developing countries between the elite groups who monopolize power and wealth and the majority of rural people who remain poor. It is because of urbanization that both industry and agriculture have suffered. There should be a net transfer of surpluses and income to rural areas, correcting the historical inequities and imbalances. This calls for rural industrialization. Such rural industrialization should really be based on generating, mobilizing, utilizing and maximising natural and human resources that are available in rural areas.

Africa posseses a significant proportion of the world's mineral, agricultural and human resources. Yet inefficient utilization of these resources impedes global economic welfare – not just the welfare of Africa alone, and a new approach is needed based on the fact that the continent's development is beneficial to the global economy and *vice versa*, and ought therefore to be a global concern.

In view of the high dependence of most African countries on the agricultural sector as a source of employment, sustenance and export earnings, and bearing in mind the relatively low level of development of this sector, any effective strategy to accelerate the pace of industrialization must focus on the promotion of agro-based industries. The continent's high population growth rates and rapid urbanization require an expansion of high-quality food processing activities and rural industrial growth to help guarantee food security and facilitate the expansion of non-farm employment. The politically explosive connection between rural poverty, rural-urban migration, urban unemployment, squatter camps, environmental degradation, erosion of indigenous knowledge (particularly of rural populations), and attendant social ills underlines the need for the effective integration of policies for rural and industrial development in Africa.

This publication, which follows up on some of the subjects reviewed in papers on the applications of biotechnology to food processing in Africa broached at an expert group

meeting on the applications of biotechnology to food processing in Africa, discusses some of the technology options for food processing technologies appropriate for the African continent. A number of programmes and projects could be undertaken commercially, while others would require further research, development, and pilot and proving plants. A technical symposium on food fermentation technology held in Dakar (Senegal) discussed at length the need for setting up a professional organization of African biotechnologists whose mission would incorporate developing new and enhancing existing African biotechnology networks, scientific databases, culture collections, electronic exchange networks and help desks, bioinformatics nodes and technical training of biotechnologists in these and related fields.

A first step to ensure that effective policy measures for creating an enhancing environment for the development of appropriate biotechnology, is to create an awareness in policy makers as well as the public in Africa of the potential benefits of biotechnology. It should, however, be ensured that programmes and their potential products meet the real needs of the countries they serve. This requires a demand-oriented approach to technology development and implies that the objectives of governments should not be to build a biotechnology capacity at all costs, but to target socio-economic development first, and then to consider any possible role for biotechnology.

African governments, need to identify priority projects where biotechnology R&D can best provide solutions to problems and yield socio-economic benefits in tangible time.

Overview of UNIDO Activities

Biotechnology is broadly defined as the application of life sciences and engineering to transform living organisms and their components in order to produce goods and services. Conventional biotechnology has been practiced for millennia all over the world including Africa. Fermented foods made through traditional biotechnology, such as *kenkey* of Ghana, *uji* of Kenya, *iru* and *gari* of Nigeria, *gariss* of Sudan; *idli* of India, *tempeh* of Indonesia, *koji* of Japan, *kimchi* of Korea, and *pozol* of Mexico, are well known. These fermented foods can be upgraded and expanded using improved microbial starter cultures, technologies and processes.

The discovery of the structure of genetic material heralded the advent of modern biotechnology. The rapid advances in biotechnology and genetic engineering have already resulted in products of commercial success in the fields of agriculture, health and environment. In 1991, the biotechnology product sales of G7 countries alone amounted to nearly US$ 10 billion. It is estimated that this figure could go up to US$ 100 billion world-wide by the year 2000.

Traditional and modern technologies provide increasing opportunities and should be availed to a greater extent to meet the expanding food requirements of Africa where the population is predicted to double in the next two decades. The symposium in Dakar aimed at reviewing the state-of-the-art developments in selected food technologies with special reference to lactic acid and cassava fermentations and mushroom cultivation, and promoting these in Africa.

Before dealing with the subject proper of the symposium, a brief review of the initiatives of the United Nations Industrial Development Organization (UNIDO) in promoting biotechnology in developing countries in general, and Africa in particular, are presented. Such a review seems appropriate in view of the interest to evaluate the potentials of biotechnology and apply them for the industrial and economic development of Senegal.

Realizing the vast potential for the application of advances in biotechnology and genetic engineering to industrial development, UNIDO initiated a number of programmes directed at the national, regional and international levels from as early as 1981.

National programmes

UNIDO monitors biotechnology developments in developing countries. In a technical publication following such a mission the research and development priorities of a given country are reviewed at the government, academic and industry levels, including patenting aspects. In the past UNIDO has carried out expert missions on request to

advise African countries on policy and programme formulation in relevant areas of biotechnology and genetic engineering within the context of national priorities. African countries so far covered under such missions were Algeria, Ethiopia, Kenya, Nigeria, Senegal, Sudan and Zaire. Other activities cover a training workshop on genetic engineering techniques in Zimbabwe, a conference in Thailand on marine biotechnology and a study to promote biotechnology product commercialization among small-scale entrepreneurs in Ghana.

One of the more recent UNIDO projects that has met with quite considerable success concerns the efforts to offer the campesinos in the Chapare region of Bolivia an alternative to the cultivation of coca. The projects comprised a group of eleven activities ranging from a fruit juice processing plant, tea production, essential oil plants, a vinegar production plant, a milk and cheese plant and five small solar dryers for the production of cassava flour and cassava chips. These plants are all operating in good working conditions, their production and management problems have been identified and business and investment plants have been drawn up for each of them to yield profits. All the plants now have a positive cash flow and sales.

In Ghana, meanwhile, a project is being finalized for the use of sorghum in malt and beer processing. The idea is to develop and improve malting and brewing technologies to replace imported barley malt with locally produced sorghum/sorghum malt. The pilot malting plant being set up under the project is presently being installed and will be commissioned shortly.

Another very successful UNIDO sponsored project that has been going on for some time, has been assistance to Viet Nam's Food Industries Research Institute (FIRI). This project's original objective was to strengthen the capability of the institute in carrying out applied research in bio- and fermentation technology as applied to the food industry in the production of such items as beer, yeasts, fish sauce, food additives, bacertial anylases, glucose from cassava, etc. This was achieved through the provision of direct support services to industry in the design, selection and application of process technologies, as well as in quality control of raw materials and finished products. Naturally, training of technical personnel from industrial plants and units in specific processing technologies, bacteriological, physical and chemical (including instrumental) testing and quality control, product development, product management, etc., was arranged and undertaken in Australia, the United Kingdom, France, Indonesia, Germany and Thailand (in the latter country a study tour had been undertaken to investigate similar relationships as with FIRI, between local industry with research institutes,). As a result of the efforts and dedication of the local counterparts, as well as that of the experts and consultants involved, some projects have advanced from the laboratory to the pilot plant or commercialization stage. In fact, one of these (a small brewery) is now so successful that it is marketing its product and even financing some of the research being undertaken at the institute that initially set it up as a spin-off, to the extent that it is being demonstrated as a successful example of joint efforts.

Within the framework of the **Alliance for Africa's Industrialization**, UNIDO's Studies and Research Branch is organizing informal consultations on agro-related industrial

development in Africa with a small group of selected experts from Africa and developed countries. These consultations are undertaken in cooperation with the relevant units within UNIDO, in particular the Agro-based Industries Branch, the Engineering and Metallurgical Industries Branch and the Chemical Industries Branch.

These consultations take place within the context of preliminary research being undertaken by UNIDO on agro-related indusrial development in Africa. The issue of agro-related industrial development has assumed increased importance in the light of globalization, and of Africa's potential comparative advantages in these industries, which cover both forward and backward linkages. This was fully recognized at the launching of the **Alliance for Africa's Industrialization**, which encompasses – as one of its main pillars – strengthening of agro-industrial development in line with UNIDO's thematic priority on Africa and the least developed countries, specifically linking industry and agriculture.

UNIDO has an extensive programme of assistance to agro-related industrial development in Africa. There is strong justification for further strengthening and supporting these programmes through additional resources, with a focus on both food security and industrial competitiveness.

Agro-related industries account for over half of Africa's manufacturing value added, manufactured exports and employment. Stimulating their growth can make a key contribution towards poverty alleviation, the achievement and maintenence of food security, enhancement of agricultural productivity and the expansion of foreign resource inflows to Africa.

Agro-related industrial development has stalled during the past decade. Investment has fallen. The privatization programme has failed to take off in several countries, and export and foreign financing opportunities remain limited. UNIDO is concerned to make a contribution towards relieving these constraints on socio-economic development in Africa.

The consultations being undertaken will seek to examine the practicability of ideas presented in the research papers and to identify a viable strategy for accelerating the development of agro-related industries in Africa. It will review the existing evidence on performance and constraints on the development of specific branches, focusing on strategic issues of relevance to their development, with a view to identifying viable approaches for structuring UNIDO's contribution towards the further development of key branches of agro-related industry. UNIDO's programme is concerned with developing the type of strategies that can restructure agro-related industries in such a way that it boosts their contribution towards achieving better food security, poverty alleviation and increased agricultural productivity while at the same time increasing foreign resource inflows to the African continent. UNIDO's programme is also expected to facilitate on-going private, public and multilateral programmes in these areas and serve as a catalyst for their expansion.

Following these consultations, field missions will be undertaken to selected countries to elaborate the approach and scope for assistance, and on the basis of these missions, a

major UNIDO-wide initiative covering technical assistance, financing, negotiations, investment and economic research will be developed within the framework of integrated teamwork with all relevant units of the Organization, in view of the overarching nature of agro-industrial development that permeates many of UNIDO's activities – for example, policy issues, small and medium enterprise development, investment and technology, industrial information, the role of women in industrialization and human resource development.

In addition, the Industrial Sectors and Environment Division implements several biotechnology technical cooperation projects in several countries, including the manufacture of pharmaceuticals and vaccines. One activity of particular relevance to the present paper is an international convention on *Food Ingredients: New Technologies, Fruits and Vegetables* that will take place in Cuneo (Italy) from 15-17 September 1997. The event will consist of the presentation of research papers from industry and from public and private R&D centres in developing and industrialized countries selected by a committee composed of representatives from six Italian and foreign universities, and UNIDO. The convention is being organized and co-sponsored with Allione S.p.A. (Italy), a private company which has also agreed to set up small pilot plants to demonstrate the new technologies being proposed. More than 500 participants from all over the world are expected to attend the convention, coming from industry including multinationals, industrial associations and R&D centres, and from the academic community. The areas of cooperation with the Allione group of companies encompass training and study tours for experts; technical visits for representatives of R&D centres from temperate climate countries with a view to eventual transfer of technology and investment projects – for instance in the production of baby-food in Africa and Asia and the production of non-conventional fruits and vegetables from temperate climates, and tropical products for export; projects for the development of new processed products, particularly from tropical areas; and for the utilization of databanks. A compendium of the innovative technologies selected from developing country R&D centres will also be published.

Regional programmes

UNIDO promoted the organization of regional expert meetings in biotechnoly of the first and second Arab Conferences on Perspectives of Modern Biotechnologies in the Arab Countries of 1989 and 1993. On the Latin American scene, UNIDO initiated a regional network for biotechnology in Latin America in 1981 and implemented several technical assistance programmes in Argentina, Chile, Costa Rica, Cuba, Mexico, Peru and Venezuela.

The Organization also participated in the Pan African Ministerial Symposium on Biotechnology held at Algiers in February 1992 when a decision was taken to establish an African Agency for Biotechnology.

UNIDO supported a project for the development of small scale, low cost and energy efficient technology suitable for rural areas for the processing of cassava into *gari*.

Under a subcontract agreement with UNIDO, the Federal Institute of Industrial Research Organization (FIIRO) in Lagos, Nigeria, in collaboration with the African Regional Centre for Engineering Design and Manufacturing established a *gari* processing plant. The plant is versatile enough to produce other African foods derived from processed cassava, such as flour, *Lafun* and starch. UNIDO, along with FIIRO, conducted training workshops on the development and transfer of *gari* processing technology for rural areas in several African countries to promote cooperation in the region.

International programmes

Among the earliest initiatives of UNIDO to promote biotechnology in developing countries was the proposal at a Plenipotentiary Meeting in Madrid in 1982 to establish an international centre of excellence in this field. The International Centre for Genetic Engineering and Biotechnology (ICGEB) thus established has two components, one at Trieste (Italy) and the other at New Delhi (India).

Functioning as a fully-fledged institution since in 1987, ICGEB ran for a number of years as a project of UNIDO with the assistance of a Preparatory Committee before becoming autonomous. ICGEB has turned out to be an unique inter-governmental centre engaged in research in biotechnology and genetic engineering, reaching a high level of scientific excellence within a relatively short time. Research at the New Delhi component is focused in three areas: mammalian biology; plant biology and structural biology, whereas the Trieste component undertakes studies on molecular biology of viruses; microbial degradation of lignin; protein structure and function, including molecular modeling, pathology and immunological aspects. Besides R&D, the Centre conducts training programmes by way of workshops, colloquia, conferences and symposia. In addition, research grants are awarded to affiliated centres under a collaborative programme to conduct research in their relevant areas. An advanced computer service laboratory is situated at the Trieste component of the Centre which serves to provide remote access to a wide range of major domain databases.

Currently 40 countries are full members of the ICGEB including 14 from the African region. The Centre's scientific programme is guided by a committee of scientific advisors and has affiliations with national institutions in 29 countries.

The Centre has already imparted short- and long-term training to over 2000 scientists and researchers from member countries. A number of industrial enterprises are approaching ICGEB for funding on collaborative ventures. ICGEB is keen to see that the results of its research will lead to products of benefit to developing countries. Agreement has been entered into with two companies to commercialize products developed at the Centre, including a diagnostic kit for AIDS infection. In view of the gravity of AIDS infections, the diagnostic technology was initially offered to African ICGEB member countries. Four of these countries have expressed an interest to acquire the technology and measures are underway to arrange licenses. Steps have been taken to patent a peptide with the potential to develop into a vaccine against Hepatitis B infection. The Centre is also becoming increasingly involved in harmonizing perspectives relating to biotechnology safety, intellectual property rights and patenting policies in its member countries.

Specific projects

Awareness building

Since 1982, UNIDO has been disseminating the state-of-the-art developments in biotechnology and genetic engineering to developing countries through a quarterly bulletin, formerly called the **Genetic Engineering and Biotechnology Monitor** and now integrated into the **Emerging Technology Series**. The bulletin, while including abstracts on scientific and commercial developments, carries invited articles on special topics and aims to increase current awareness in the field.

At the invitation of the United Nations ACC Task Force on Science and Technology for Development, UNIDO commissioned an article on the contribution of biotechnology to sustainable development within the framework of the UN System. Among the recommendations in this paper is a proposal to organize a Consultative Group on Biotechnology to accelerate R&D and commercialization, and stimulate the support and spread of environmentally friendly biotechnologies worldwide.

The AGENDA 21 – Task Manager in biotechnology

The Agenda 21 Programme, which comprises the recommendations arising from the United Nations Conference for Environment and Development (UNCED, Rio de Janeiro, 1992) includes provisions for specific activities in biotechnology. An Inter-Agency Committee for Sustainable Development (IACSTD), which implements the Programme, assigned UNIDO to be Task Manager in biotechnology. UNIDO will play a key role in coordinating the activities of all other UN agencies involved in work contributing to the Agenda 21 Programme in biotechnology. UNIDO's responsibilities and functions as Task Manager include the provision of consolidated technical inputs on UN System-wide implementations of Agenda 21 in specific areas such as information exchange, inter-agency contact, joint activities and programmes and common strategies. The Task Manager will also contribute to the report of the Secretary General of the United Nations to the General Assembly of the Organization with focus on common strategies for the implementation of Agenda 21.

Biosafety Guidelines and Code of Conduct

The advent of recombinant DNA (rDNA) technology provides unprecedented opportunities to genetically modify organisms (GMOs) at will and induce them to make useful products with precision and speed. The realization of the potential of biotechnology often entails the release of GMOs and/or products arising from such organisms from laboratories and production centres into the environment. There is considerable global concern that the release of GMOs to the environment may have deleterious effects on the environment. It is therefore evident that a basis must be laid for an orderly application of biotechnology that ensures the protection of human and animal health. There is a general need for a better awareness and perception on the part of countries of issues related to health, safety and environment and consumer protection that require consistently safe biotechnological practices.

With the above considerations in view, UNIDO, as part of the UNIDO/UNEP/WHO/FAO Working Group on Biosafety, and in association with some 50 experts including from OECD, developed an International Voluntary Code of Conduct for release of GMOs into the environment. Basically, the Code attempted to harmonize the existing guidelines, capturing the minimum commonly accepted principles into an international framework in the form of a Code of Conduct for the release of GMOs. The guidelines expressed in the Code are meant to be user friendly and serve to promote R&D and environmental applications of GMOs with an overall aim to promote the process of biotechnology progress. They provide guidance to national authorities to take quick decisions on proposals for the introduction of GMOs; help industry to commercialize GMO-based products; bring transparency and avoid trade barriers and facilitate consumer confidence and acceptance. The salient paragraphs of the Code have been annotated.

A volume entitled **Genetic Modified Organisms: A Guide to Biosafety** has been prepared by UNIDO and is available as a commercial publication. The project work had a catalytic effect in UNIDO's related activities on the subject and the ICGEB has included biosafety in its programme yearly training courses.

UNIDO has established a biotechnology information network and advisory service (BINAS) which offers remote access to databases of national biotechnology legislations, regulatory authorities and experts. BINAS simplifies the process of making informed decisions on the choice of available regulatory options and provides a decision support tool to the governments. It assists the biotechnology industry to identify competent authorities responsible for permits of field trials and commercialization of transgenic products, as well as the information requirements that are needed for the purpose.

Commercialization of biotechnology

Industrialized nations have already established firm linkages between research and industry and have therefore been successful in commercializing products of biotechnology. In contrast, developing countries lag behind in this respect. An in-depth review of the factors for success, constraints and modalities of their circumvention formed the subject of an Expert Group Meeting (EGM) on Commercialization of Biotechnology organized by UNIDO at the end of 1991 from which a number of important recommendations emanated, including the need for accelerating human resources development in developing countries, particularly in bioengineering and downstream processing, and in management skills; a need to sensitize the public and governments on biotechnology developments through science parks, and rewarding scientists for their innovations with product focus.

Marine biotechnology

Marine biotechnology provides industrial opportunities particularly to countries with vast ocean resources and marine ecosystems. Besides aquaculture, marine organisms provide industrial and medicinal products of economic value. UNIDO, in collaboration with the World Bank, organized an International Workshop in November 1993 at Bangkok (Thailand) to identify priorities for research and development in marine

biotechnology with high commercial application in developing countries.

Bioremediation

Under an SIS (Special Industrial Services) project, Trinidad and Tobago was given consultancy services in oil recovery. Expert missions were mounted to Algeria, Indonesia, Mexico and Venezuela and more recently Kuwait, at the request of the respective governments in order to acquaint them as to the potentials of hydrocarbon technology. Requests were received from different countries to organize international conferences on the subject.

Biodiversity conservation and sustained utilization

UNIDO is promoting genetic resources prospecting which offers developing countries a valuable opportunity to identify, conserve and effect their sustainable utilization. In an Expert Group Consultation convened at UNIDO in October 1993, issues such as current developments and trends in biodiversity prospecting, including policy and technology management and intellectual property rights were discussed in depth.

Fermented foods

An expert group meeting on food processing was conducted in Nigeria at the end of 1991 to popularize and accelerate commercialization of African foods. In addition, UNIDO implemented a project on lactic acid fermentation in the Republic of Korea to enable the development of a nutritious traditional beverage derived from the fermentation of soya bean and rice. The beverage, called *risogurt*, is rich in protein enhanced with an added attractive flavour. An international training programme was conducted in food fermentation technology in Seoul to participants from Asia and Africa. UNIDO intends to promote such projects in Asia, Africa and Latin America. As an extension of the Korea project, UNIDO is planning for a feasibility study to set up an international biotechnology food research network.

Conclusion

African nations are largely based on agriculture economy. Both the Lagos Plan of Action and the programme for the IDDA (Industrial Develoment Decade for Africa) accord highest priority to the attainment of self sufficiency in food production, and to the building of an industrial sector in Africa. Agro-scientists and modern biotechnologists feel that the application of a blend of old and new technologies holds the key in this respect. The African Agency for Biotechnology highlights the importance of using biotechnology in providing foods with a high nutritive value. Through the application of biotechnology, new ways can be found to augment the quantity and quality of existing foods and other edible materials.

In the light of the UNIDO programmes in biotechnology enumerated above, and as a follow-up of the UNIDO's Expert Group Meeting (EGM) on Food Processing held in Ibadan in 1991, the Dakar symposium on food fermentation technologies was convened under the auspices of the African Agency for Biotechnology and the IDDA Programme. We are grateful to both the Institute for Food Technology and the African Regional

Centre for Technology for generously acceding to be co-sponsors of this symposium.

Among the recommendations at the Ibadan meeting were the formation of a network on lactic acid fermentation technology for African scientists including linking it to a global network being established by UNIDO; extending support of a network on food fermentation and processing technology to stimulate research activities and information exchange within Africa and with the other regions of the World; and transferring mushroom growing technology to Africa. Accordingly, the topics chosen for this symposium are lactic acid and cassava fermentations and mushroom cultivation technology which have great potential for application in Africa.

INTRODUCTION

Africa and the twenty-first century

Only a trickle of years remains of the waning twentieth century. The cosmos is in the throes of giving birth to the twenty-first century. The often heard "turn of the century" is here once more. The year 2000 might not have any unique essence in the measure of absolute time, yet the year 2000 is a milestone of special flavour, bestowed on it by the minds of men and women who gave it, too, the awe and reverence it is surely to claim. In this context it is a year unique for that sector of mankind with any bearing on modern civilization. Thus ushering in a new era, the twenty-first century, suffice it to say that the year 2000 is the harbinger of scientific finesse and fineness. In that year, just three years from now, the world will pause and each continent will look back in time and make an inventory of its past achievements, then each will proceed to take the "turn". Africa will do the same. But how is the strapping continent going to take the "turn"? Hopefully, resplendent in the iridescent raiment of science and technology. The "Dark" continent of yowling beasts of the nineteenth century, has long gone past that darkness into the dappled shades of patchy science during the twentieth century, and her scientific children will surely take her into the bright lights of science and technology in the twenty-first century. But all this could be wishful thinking if enlightened Africans do not become aware of the colossal work ahead. The problems besetting the continent are multi-pronged and they have to be faced daringly. The tripple-scourge of hunger, disease and poverty takes a front seat among these problems and it is best attacked at the hunger link. Many turns of the century have passed without the Africans being aware of them, but this time millions of them expect the new century to bring with it a realization of their long pent-up dreams and hopes for a better life. But better life begins with abundant wholesome food. Africa is now better equipped as it braces itself to pick up the gauntlet thrown down at her by the new century. Her weapons are science and technology, in particular biotechnology.

Biotechnology and Africa

Biotechnology, the science of the future, holds unlimited opportunities for food production and combating hunger as it does for other facets of life, such as the development of therapeutics and diagnostics in combating human and animal disease. This applied science can be defined as the use of cells and tissues, as well as parts thereof, in providing commodities or services. In the area of primary food production great strides have been made in the production of high-yielding, pest and pathogen-free plants and animals via tissue culturing and other techniques. Wide opportunities have been opened in the fields of biological nitrogen fixation employing such bacteria as *Rhizobium*, while other bacteria such as *Bacillus thurengiensis* have been used in the biological control of agricultural pests. In addition, the management of agricultural wastes through harness-

ing the enzymatic capabilities of various microorganisms to convert waste into useful products such as fuels, chemical solvents, antibiotics, soil conditioners and microbial biomass is leaving an impact on our daily life.

Biotechnology can be divided into two major divisions: modern biotechnology and traditional biotechnology. In the first category enters recombinant DNA technology and gene manipulation, where genetically modified molecules, cells and organisms in pure clones are used for the production of substances or for diagnostic purposes. Some scientists would like to describe this kind of biotechnology as sophisticated or refined biotechnology. Traditional biotechnology, on the other hand, is not so sophisticated and usually does not involve tampering with DNA. It is best exemplified by such fermentations as biogas generation, composting, silage production and even the old industrial microbiology.

Part of this latter category is what can be called rural people's biotechnology, which includes indigenous food fermentations, retting of fibrous plants, some kinds of leather tanning, farm manure preparations, ink fermentation and a number of other examples, including rudimentary mushroom cultivation and some kinds of folk medicine preparations.

In fact, biotechnology, in its broadest sense, has been with us since time immemorial, but in its modern sense it is proving to be a powerful tool to bring about profound changes in our lives and to help us solve many of our problems today and in the future. Now, where does Africa stand with respect to this science and technology of the twenty-first century? At this very moment there are young African scientists busy using restriction enzymes and ligases, cutting and splicing genes from one being into the genome of another. This they are mostly doing, not on African soils, but in Western laboratories where they get their training. A number of these young and hopeful scientists have returned to Africa with the knowledge and expertise they acquired, only to discover that they do not have the necessary equipment to do what they used to do back in Europe or America. For the African common ruck, to learn how to chop up DNA and rearrange the chips without translating the action into useful products would be nothing but another extravagant luxury. The young scientists having come back home find themselves fitting in no planned programme to make use of their vital knowledge. The policy makers have not yet brought themselves to the level of sophistication for these young scientists to give them the opportunity to contribute to the development efforts of their people. The private sector is still in its infancy; there are no companies like Biogen, Genentech, Amgen, Genzyme, etc., which are so hung up on biotechnology they derive their names from the Gene!

Still, young African scientists have to train themselves in these new sciences in order that the critical mass of trained scientists in modern biotechnology can be attained in the continent. The time will come when they and their disciples will be badly needed. In the meantime, Africa could easily develop traditional biotechnology and establish a firm fermentation industry. Among these traditional fermentations is food fermentation.

3. Fermented foods and Africa

By definition, food fermentation, even that practiced in the huts of Africa, is in fact biotechnology: the process uses microbial cells and enzymes to produce a commodity, the fermented food itself. It is true that food fermentation in its traditional state forms the bottom rung of the ladder of biotechnology, nevertheless it is biotechnology all the same. But this is not the issue. The important thing is how to develop these food processes. The tradition of food fermentation in Africa is so deep-rooted it is recoded for posterity in poetry:

* To weed sorghum it takes a lad with rolled up sleeves
* One who always recalls the sweet words of young ladies
* Congratulations to him who weeds it the second time and watches it grow
* But the ultimate reward is in the jar of fermented dough

Food preparation in Africa is the realm of women, and what goes on in their simple cottages, shacks and hovels in this respect is very impressive. The African woman's catalogue of technology and technique of fermented food preparation must be a garden of wonder for the poet and a treasure for the technologist. This is a culture developed and preserved over thousands of years, as told by historical and archaeological finds in parts of the continent. Yet very little of this is actually known in the circles of modern science and technology. Needless to say, the indigenous knowledge concerning these foods has to be documented as it is disappearing rapidly under the pummelling impacts of introduced food cultures, especially those coming from Western cultures. It is logical to look at this native food industry as the basis of a viable modern food technology in the continent. These foods must be preserved, researched and brought up to the modern ways of production, considering that food provision is one of the most urgent matters that local governments are to address in Africa.

A fermented food is one in which microorganisms have grown on or in its raw material, and by doing so have brought about in it fundamental changes in texture, colour, chemical composition and flavour. In Africa, most food fermentations are spontaneous in the sense that pure cultures of microorganisms are not purposely added as starter cultures. Back-slopping, which is the addition to the fermenting milieu of a portion of the previously prepared product, adds a mixture of microorganisms of unknown identity.

In order to grow and multiply, these tiny beings must get biological energy and body building blocks. They do this by breaking down the various organic molecules constituting the raw foodstuff. This they achieve through the help of a group of proteinaceous compounds – enzymes – which they elaborate. Enzymes are the tools with which the microbial cell makes and breaks things, thus bringing about their effect on the food. Thousands of enzymes, each specialized in a specific chemical reaction, are made by the microbial cell. They are either kept inside the cell or released free in the foodstuff. The information for synthesizing each of these enzymes resides in the DNA of the cell and is more or less faithfully preserved. As the genetic make up of each microbial species differs from that of the other, each kind of microbe brings about a change in the food that is dictated by its particular compliment of enzymes. Some of these chemical compounds

produced by enzymes in the food would have a more pronounced presence and so impart their characteristic taste or smell on that food. Thus, we find some microorganisms produce acids such as lactic acid, and make the product characteristically sour, whereas other microorganisms attack proteins and produce strong-smelling compounds and ammonia.

The activities of microorganisms in foods are not restricted to these two important outward manifestations. A microorganism can produce a myriad of new chemicals, including aldehydes, amines, alcohols, ketones, dyes, sugars, vitamins, amino acids, etc. When, in the case of spontaneous fermentations, a number of microbial species contribute to the process, as in most African fermented foods, one would expect a welter of chemicals to be produced in the particular food. In fact, the process is further complicated by the influence of the prevailing temperature and humidity, and by such factors as the chemical composition of the starting material and its buffering capacity, the provision of aid, exposure to radiation and the compatibility and interaction of the microbial strains present. Chemicals produced by one microbe can be scavenged and transformed by another. Some strains cross-feed others, while other strains antagonize yet others, and the final chemical composition of a fermented food is beyond the guess of anyone. But we can have an idea of that when it is remembered that the aroma developed in some fermented food products could be contributed to by hundreds of chemicals! No wonder then that such organisms with such abilities are considered part of the national resources of any country. They are isolated, characterized and whenever possible, used in the production of enzymes, acids, antibiotic-like compounds, vitamins and amino acids. They are preserved as a repository of genes in microbial gene banks so useful in biotechnology.

The importance of fermented foods and the nutritional and health benefits that accrue from their simple methods of preparation have been enumerated on many occasions. These foods are all made from local raw materials, a matter of vital importance to poor rural people. The foods themselves are liked by the local people, even though foreigners might find some of them extremely repugnant. The simple methods of preparation require only the simplest of equipment and tools, the pestle-and-mortar, the quern and the earthenware pot being central to their preparation. As most of them are both fermented and sun-dried, they keep well and are easy to carry (especially favouring nomadic tribes). By the simple methods of fermentation, the spectrum of edible foodstuffs can be broadened as some poisonous raw materials can be reclaimed by the process, the poison being dissipated by fermentation. Fermented foods are widely held to be light on the stomach and easy to digest. Research has shown that in many cases the nutritional value of fermented food is improved over that of the original starting raw material, e.g., by having more vitamins or amino acids. Fermentation also reduces cooking time, as, for instance, fermented meat takes much less time on the fire. It improves flavour and often produces enjoyable foods that impart variety on a normally monotonous starchy basic diet. Anti-nutritional factors such as enzyme inhibitors and flatulence factors, are often destroyed by fermentation. Even the texture and colour of the food are changed by this process. No specially prepared starters are required and this makes the fermentation process cheaper. Furthermore, in Africa, most fermentations of

foods are of the lactic kind and here lies one of the greatest benefits of the process. Lactic and other organic acids and the lowered pH value which results from their presence, guard against the spread and development of disease-causing microorganisms in the food, a blessing in the high temperatures of the tropics.

Nonetheless, we still have to learn more of the full role played by fermented foods in the life of the African: socially, religiously, economically and nutritionally. This role, as well as the roots of the practice of food fermentations in Africa, are all matters still shrouded in the haze of mystery and tenebrious obscurity. But we know for sure that the fermented foods of Africa of today are the outcome of realistic, down-to-earth practice which took into consideration both the material substances and the environmental conditions of the continent. These practices have taken into account the hard and harsh realities of an implacable and capricious climate, as well as a precarious food supply. These foods serve to forestall famine and make up for the nutritional shortfalls that riddle the daily life of many of the rural Africans, resulting in disease, child predicament and misery.

As mentioned before, the artist, scientist and technologist behind all this indigenous knowledge is the African woman. She has been brewing these foods since ancient times. The key to her success is that she considered the environment around her: she is indigenous, the problems she wanted to solve are indigenous, her raw food materials are indigenous and the tools and clay pots she uses are indigenous. When the ancient African kingdoms of the central Nile grew prosperous, their elite imported Mediterranean wines in large, burnished, sophisticated wheel-made clay jars. When archaeologists uncovered these jars in our time, they found the old unburnished small clay pots made by the local African women, side by side with these beautiful, imported amphorae, and they wondered why the African woman did not adapt the new jars for her food purposes. The imported jars disappeared in time, while the African woman's pot continues with us to this day. The African pot outlived the imported jar because it is more suited to the African life, both then and now. The imported jar could not survive because the African woman did not find it fit for Africa, just as today she does not find plastic containers particularly suitable for preparing fermented foods. This should give us something to think about when we discuss the modernization of the processes of food fermentations in Africa – we should watch our steps.

Modernization and industrialization of African fermented foods

For all their glory, traditional African food fermentations are not without shortcomings. The methods of production of the various foods are primitive, taking into consideration modern ways of food preparation. Some of the procedures followed are obviously time-consuming and have probably evolved during times when time itself was not as precious as it is today. The safety aspects or measures, which admittedly do alleviate danger, are not impeccable, and even when lactic acid bacteria dominate the microflora involved in the fermentation and produce their compliment of anti-microbial substances such as acids, alcohols, hydrogen peroxide and antibiotic-like secondary metabolites, the food is not completely safe. The methods of preparation of these foods were originally developed within the confines of a hut and meant for small-scale, family-size

production, a situation not quite congruent with modern, urbanized life. Efficiency is low and storage losses high. The packaging material and the aesthetic look of the food are not always what urban communities would like to have. The product, of course, is not standardized, its composition varying from batch to batch. These drawbacks and the fact that no attempt has been made to upgrade the methods of production of these local foods have, no doubt, helped pave the way for the introduction of foreign foods which invaded the African urban market and already began pervading the rural areas. One consequence of this is that the traditional knowledge behind the production of local foods began to dwindle and in time it will be lost for ever. Modernization and industrialization would be one way to salvage these foods.

To produce an indigenous food through modern ways has numerous benefits. The product would be taken out of its isolation in its original locality to reach consumers throughout the country. In fact, modernization of the production techniques of a food opens channels of exportation for it. For instance, the Asian soy sauce, or shoyu, can now be found in European and American restaurants. Modernization, of course, results in a standardized food product packaged in an attractive casing, which makes it appealing to urban dwellers who have the real purchasing power. However, even more important is the fact that modernization of such a food product would subject it to official quality control measures and so the consumer would be protected against pathogenic microorganisms and fraudulence in chemical composition, wholesomeness and expiration date. The hygienic production of a food is much more easily monitored when modern ways of production are followed, e.g., by application of the principles of the Hazard Analysis Critical Control Point (HACCP). Whether the small-scale intermediate technology or the traditional large-scale production system is employed, these advantages of modernization hold true.

The issue of industrialization, however, is not as simple as it may seem at first. There are a number of sensitive aspects to be dealt with: economic, social, scientific and technological. Scientific laboratory research, field and socio-economic research, choosing the level of technology to suit the particular country, choice of the sector of the population to be targeted by industrialization – all are vital issues in this respect and all need time, funds and effort. Obviously then, the development of an indigenous fermented food into a modern product needs the concerted effort of a number of groups and agencies: scientists, technologists, social workers, economists, local entrepreneurs, womens groups and policy makers. Among these, scientists are probably the one group that would have to bear the brunt of responsibility for such development, and once a project is conceived, they have to suffer the labour pains of its birth.

But scientists and other research workers cannot achieve much without the help of politicians and the policy makers. These two groups have to work in unison if industrialization of fermented foods is to be realized. There is often a rift between scientists and policy makers and that has often resulted in a faltering progress which, more often than not, comes to a standstill. It should perhaps be demanded of the scientists that they spend more time and effort to enlighten politicians as to the importance of the issue of modernizing the processing of indigenous foods, in order to secure their vital support. After all, if these foods, simple as they are, were not essential for the well-being of those

who make and consume them, they could not have survived in this era of modern food technology. Scientists and research workers have much to build on in order to solicit the sympathy of a heeding ear among policy makers.

Scientists and researchers themselves are also beset with considerable problems. In a number of debilitated economies in Africa they form a niche of negligence and their living conditions are appaling. It is hardly logical, therefore, to expect the excellence in research needed for a viable attempt at the modernization of the food industry under the circumstances. Even if such scientists could feed their children, they still need the tools to work with: a descent laboratory and the chance for on-the-job training to acquire new skills and access to recent literature. This point concerning the living standards of African scientists is often ignored or overlooked when research problems are discussed with respect to Africa, but it is in fact a crucial point.

In spite of the many difficulties that could block the road, the modernization of indigenous fermented foods is not such an impossibility; living examples can be cited. Japan is perhaps the best example to quote where originally home-made products such as shoyu are now produced by the most sophisticated methods of food and fermentations technologies known to date. Even in Africa itself, there is ample experience in this respect, where in South Africa sorghum beer and *mahewu* are produced via modern methods. In Nigeria, *gari* and *ogi* would be two more examples of a success story of modernization of the processes of production of a local food. In all cases, it is worth recalling that scientific research formed the linch-pin of success and policy makers blessed the endeavour.

UNIDO and the Dakar Symposium

UNIDO has taken stronger steps towards helping African nations develop their biotechnology capabilities, particularly in the area of foods, since the beginning of the International Decade for the Development of Africa, when it held a symposium in Ibadan, Nigeria, in 1991. In that meeting experts, scientists and entrepreneurs discussed the applications of biotechnology to food processing in Africa. The symposium was such a success that UNIDO decided to follow it up with a sequel at Dakar, Senegal, in December 1993, the proceedings of which are presented in this volume. UNIDO is planning a third meeting to be held in southern Africa, perhaps in 1997, and her efforts to improve the biotechnology situation in Africa will extend to the end of the Decade and beyond. But this should not be taken to mean that the international organization wants to see the matter of biotechnology and food mired in an imbroglio of semantics and academia. On the contrary, UNIDO's mandate is to help nations in the field of industrial development; its aim with respect to all application of biotechnology, especially in the area of food fermentations, is to see at least some of these processes go through the pipeline of modernization and industrialization. The meetings should be looked at as a mobilizing step to rally the scientific forces of the continent and to pave the way to the final goal.

Great benefits resulted from these two meetings mentioned above. UNIDO succeeded in bringing under one roof scientists, technologists and entrepreneurs from all corners of the continent where they exchanged ideas and knowledge and set up plans for future progress in the wide field of biotechnology and particularly in the special area of food processing and food fermentation, as well as tackling some organizational issues.

At the Dakar meeting, three themes were selected on which to base the discussions: lactic acid fermentations, cassava fermentation and mushroom cultivation.

The importance of lactic fermentation of foods has caught the attention of a wide range of agencies and persons concerned with the issue of food safety in Africa. Food fermentation scientists have long observed that most food fermentations in Africa were of the lactic acid type and soon the possible protective effect of this became clear. Lactic acid, in its undissociated molecular form, and the low pH resulting from it both have an antimicrobial effect. The food is preserved by destruction of spoilage organisms or by their inhibition and the consumer is protected against disease by the destruction of, or the inhibition of pathogenic microorganisms. Lactic acid bacteria, which is the primary microbial group involved in the production of the acid, exert their antimicrobial or antagonistic effect not only by their production of the acid, but also through the production of other antimicrobials, such as hydrogen peroxide and substances of secondary metabolism that simulate the effect of antibiotics.

The role of lactic acid bacteria in retarding the growth of, or destroying disease-causing microorganisms, becomes all the more important in the warm climate of Africa where pathogens can develop in a food very fast if not checked, especially since the temperature degree of 37^0C, blood temperature, is in fact the ambient temperature for long hours in many parts of the continent. The role of lactic fermentations in combating disease is even more important if we take into consideration infants and babies and their weaning foods under the prevailing unhygienic environments in many parts of Africa. For these reasons lactic acid bacteria became a target for research by many laboratories and agencies the world over. Isolation, purification, identification, classification and genetic and biochemical characterization of these organisms are now the subject of many researches. In the field of organization, research scientists dealing with lactic acid bacteria (LAB) are being inter-connected through networking (LABNET) in an attempt to boost efforts for better research. In the Dakar meeting, examples were given of acid-fermented African foods and the results of researches carried out on them, enriching our knowledge of the extent of application of this technique of food preservation in the continent. Serious attempts to develop starter cultures for lactic-fermented foods were also presented. In the development or modernization of such lactic fermentation products one has to deal with the development of starter cultures. This is not an easy job as the selection of the right starter strains from the natural microflora of the food, their dissemination and preservation, all need considerable and precise research.

Cassava is very likely now the most important staple crop in Africa. Introduced by the Portuguese in the 15th century from the New World, it has spread very rapidly, particularly in the populous West Africa, where it quickly dominated the dietary scene. The tuber, which is the major edible part and starch source, is normally fermented in

various ways before processing into food products. Cassava tubers are well known for their content of a poisonous substance, linamarin. This cyanogenic glucoside breaks down to release the toxic component hydrocyanic acid (HCN). It was originally thought that the enzymes of the fermentation organisms carried out this breakdown, releasing the poison, which was subsequently lost to the atmosphere and the environment, thus resulting in the detoxification of the tuber. However, more recent research suggests that the detoxification process is carried out by enzymes endogenous to the tuber itself, in spite of the fact that many microbial strains isolated from the fermented microflora possess the enzyme linamarase necessary for the breakdown of linamarin. It is therefore more likely that microorganisms contribute little to this process but are important in the development of the required flavour in the finished product. There is still some controversy over which organisms are important in the fermentation, but there seems to be some agreement that lactic acid bacteria could be the major group involved. The enzyme, linamarase, of the tuber and whatever assistance it may get from the organisms of the spontaneous fermentation appear not to be sufficient to dissipate all the linamarin present, as unfortunately, residual toxicity has been detected in ready-to-eat cassava foods. This means that people in cassava-eating regions of the continent are subjected regularly to toxicity by HCN. This is a situation which calls for remedial measures. One way of improving the overall cassava processing methods is the use of starter cultures, particularly of lactic acid bacteria, which possess, among other desirable characteristics, the ability to produce effective enzymes to do away with all the toxicity under controlled conditions. The presentation given on cassava fermentation at the symposium discussed these matters and raised important questions.

Mushroom cultivation is admittedly a little distant from the two preceding themes of the symposium, but it is considered part of biotechnology, being a form of microbial biomass production. Moreover, mushroom culture is like any microbial culture involving the growth of microorganisms on a substrate, and in fact it is a form of fermentation in which the spent substrate is a form of compost used for soil conditioning. Besides, mushrooms are a form of food like cassava and the lactic-fermented food products. But the propagation of mushrooms is of a special importance here, because, in spite of its great value as a food source, it has been largely neglected in Africa, although many communities in the continent have the tradition of collecting mushrooms from the wild for local consumption. It is therefore one of UNIDO's aims to encourage mushroom production in Africa as a cheap source of precious food. Many kinds of agricultural wastes can be used for growing these valuable macrofungi to produce tons of protein-rich fruiting bodies, which could enrich the African table and fetch extra income to farmers. The methods of production suitable for Africa are expected to be simple and not capital intensive. Both imported and local fungi are to be tried and the most suitable adopted. The papers presented at the symposium give good examples of encouraging trials in countries from different parts of Africa.

At the end of the symposium, which was part of the broader meeting of Afristech 1993, it was demanded of the participants to come up with recommendations on practical steps to promote the advancement of cassava, lactic fermentation, mushroom technologies and other aspects of biotechnology in Africa. An excellent and comprehensive report of this is available on request, but for reasons of space only a summary is presented in the appendix.

CUMULATIVE SUMMARY

The scientific papers presented at the Dakar meeting can be grouped into three categories: lactic acid fermentations, cassava fermentation and mushroom culture. In the following paragraphs an attempt is made to give compiled summaries of these papers.

1. Lactic acid fermentations

In the paper presented by **Moussa Souane** the topic of lactic fermentation of foods is considered from a broad point of view. Lactic acid fermentation is considered to be one of the first biological processes to draw attention to the benefits of food fermentation. Both acid and ethanolic fermentations have contributed to mankind's survival over the millenia because of their preservative effect and their lethal influence on pathogenic microorganisms. Lactic acid fermentation in fact has a number of advantages: it is a low-cost process, the acid produced improves product stability, the resulting foods are culturally accepted in many regions, it results in improved digestibility of cereals, improves the nutritional value, has positive health effects and results in the detoxification of otherwise poisonous raw foodstuffs. However, in spite of the fact that some lactic-fermented foods have been well-studied, many traditional foods are not so lucky with respect to scientific research and technological development. The author divides the techniques used in the production of these traditional acid-fermented foods into two major groups:

A. Non- salt-controlled systems: cereals, starchy products and milk.

B. Salt-controlled systems: vegetables, fish, meat, mixed ingredients.

The primary lactic acid bacteria (LAB) involved in the fermentation of these foods belong to the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus (Lactococcus)*. Some of these are homofermentative and convert sugars, such as glucose, primarily into lactic acid via the glycolytic pathway, whereas others are heterofermentative, producing from glucose lactic acid as well as acetic acid, ethanol and carbon dioxide via the phosphoketolase pathway of metabolism.

In conclusion, the author states that numerous lactic acid fermented foods exist that are based on single or mixed substrates and the activities of a limited number of microbial associations involving few species. The author finally recommends that inter-disciplinary research on acid-fermented African foods be carried out, particularly in the area of developing starter cultures.

Cherl-Ho Lee's paper deals with the importance of lactic acid bacteria in the non-dairy food fermentations. The paper gives a review of the classification of LAB and discusses their metabolic pathways and then turns to discuss examples of non-dairy fermented foods in which LAB are the dominant microbial agents. Important examples of fermented meat, fish, cereal, tuber, legume and vegetable products from different parts of the world are discussed and the involvement of LAB in their production examined. The

most important microorganisms in acid fermentation of foods belong to the bacterial family Lactobacillaceae, which consist of four genera: *Streptococcus, Pediococcus, Lactobacillus and Leuconostoc.* These organisms appear morphologically as diplocci, tetracocci, streptococci or in the form of rods, which may be found single or in chains.

Metabolically, these bacteria follow different metabolic pathways in their breakdown of sugars, which they convert to acids as a rule. In general, however, a species could follow either a homolactic fermentation in which glucose is converted to lactic acid mainly, or a heterolactic fermentation, which leads to the production of lactic acid plus other products such as acetic acid and carbon dioxide in more or less equimolar quantities.

A general consideration of the patterns of microbial population growth in the various acid-fermented foods reveals a succession of dominance of these organisms. *Leuconostoc mesenteroides* appears to initiate the process of fermentation in most cases of cereal and vegetable foods. The organism is often replaced by *Pediococcus cerevisiae, Lactobacillus brevis* and/or *L. plantarum*, depending on the nature of the substrate. Sometimes the growth of *Lactobacillus* is suppressed by yeast growth, which could be desirable in some cases, such as in sourdough or alcoholic fermentations, but detrimental in cases like fish fermentations.

In their presentation, **Alan Reilly** and **Andrew Westby** state that lesser developed countries require food processing technologies that are technologically appropriate, suitable for tropical regions, and that are affordable in rural and urban economies. Fermentation is one such technology, which in fact has a number of advantages: it is a food preservation method, it improves food safety, it enhances flavour and acceptability, increases variety in the diet, improves the nutritional value of the food and it reduces the anti-nutritional factors in foodstuffs. There is a wide range of perishable foods which rely on lactic acid fermentation as a means of preservation.

The dominance of lactic acid bacteria in food fermentations is a result of their ability to proliferate in a carbohydrate-rich anaerobic environment and to their rapid suppression of food spoilage and pathogenic bacteria. This ability of LAB to suppress the growth of other microorganisms can result from the production of organic acid accompanied by a reduction in pH, the production of hydrogen peroxide, production of antibiotic-like substances and/or nutrient depletion. In addition to its role as a food preservation technique, lactic fermentation can render foods safer to consume. Lactic acid bacteria have been reported to inhibit the growth of foodborne pathogenic bacteria. This is of particular importance in the case of foods for weaning infants. This issue of safety is of great concern in the tropics where the high temperatures and low standards of hygiene and sanitation allow pathogenic bacteria, fungi and toxin-producing species to proliferate. Therefore fermentation of foods as a low-cost, low-technology processing operation should be fully appreciated as a safety measure.

Anti-nutritional factors such as lactose in milk, cyanogenic glucosides in cassava, phytate in cereals and legumes, and oligosaccharides in legumes are aspects of concern with respect to health, and although fermentation organisms have been associated with their removal from foodstuffs, some, such as the cyanogeneric glucosides of cassava, have been shown to be removed by enzymes endogenous to the tuber itself.

Finally, the authors discuss the future of fermented foods and state that it is difficult to make generalizations on the subject as most communities are in a dynamic state of socio-economic change. For example, rural-urban migration is one major problem facing many countries and it is expected that by the end of the century, 40 per cent of the world's population will live in urban areas and the traditional food production systems have to adapt to such a situation. One important aspect in the future of fermented foods is the development of suitable starter cultures which can speed up the fermentation, keep the original characteristics of the food, improve safety and perhaps improve the nutritional value of the food. Use can even be made of the modern recombinant DNA technology to plan a suitable starter culture. The authors observe that previous research on fermented foods has concentrated on the characterization of the microbiological, chemical and biochemical changes that take place during the fermentation process, and suggest that future research should adddress the needs of developing countries for development.

The development, maintenance and use of starter cultures for an indigenous fermented food make the subject matter of the paper by **Claude Champagne**. Here the practical experience of modernizing the production of a traditional cheese type in Canada is given with the intention of showing African scientists the problems expected in such an endeavour. The questions raised and answered in this presentation are similar to those often facing African scientists concerned with the industrialization and commercialization of African traditional fermented foods. Helpful guidelines are given for food scientists to make full use of this Canadian experience and these can be presented as follows:

1. Determine what additional processing steps are needed to complement the lactic fermentation in order to ensure safety.

   In the Canadian experience, although acid fermentation is considered helpful in destroying pathogens, this is not taken to mean that the foods thus produced are completely safe. They have to be given an additional treatment, such as salting, smoking, drying, ripening period, spices, etc., in order to assure safety. For example, cheese made from unpasteurized milk has to be stored for 60 days to give enough time for the anti-microbial factors in it to completely destroy the pathogens, otherwise milk for cheese making must be pasteurized.

2. Determine the LAB species involved in the fermentation of the product in question.

   For the development of starters, LAB isolated from the highest quality product or from a closely related one can be used.

3. Expect regional differences in the starter compositions to be developed to meet consumer preference.

4. Examine the possibility of using thermophilic cultures for starter.

Using thermophylic starter cultures has the advantage of bringing about fermentation in a short time and at the same time preventing the growth of mesophilic contaminants. However, one should avoid fermentation temperatures around 37^0 C, which are suitable for the growth of pathogens.

5. Determine if phage attack might pose a problem in a given food fermentation process.

   The use and development of many different mixed cultures for the starter might be a necessity in order to combat the bacteriophages. But the development of such mixed cultures in great numbers is not easy because strain compatibilities and phage sensitivity patterns must be known.

6. Determine the appropriate form in which the starter cultures are to be supplied: frozen or freeze-dried.

   The author suggests that freeze-dried cultures are more convenient, especially for small-scale production enterprises. Such cultures store well at refrigerator temperatures and can even be used after a week at room temperature.

7. In the preparation of the bulk starter at the processing site, determine the critical points in the process and provide the technicians with the necessary analytical tools to immediately find out if a mistake has been made.

8. It should be determined if the creation of culture suppliers is warranted.

   The necessity to maintain many mixed culture collections could be quite a burden for small-scale food processors. It would be more convenient to secure the cultures from specialized culture suppliers.

Another success story involving the modernization of traditional production comes from an African country, Morocco, as told by **Abed Hamama**. In the traditional process of *jben*, the Moroccan cheese, milk is allowed to undergo spontaneous souring at room temperature for two or three days. The coagulated milk is then drained on a cloth or mat. The curd is squeezed in the cloth to further remove the whey. The curd in the bag is hung up for two or more days until it attains the desired texture. Next, the cheese is surface-salted or immersed in brine. In recent years, however, farm and urban dairy shops flourished and the traditional, family-level rural process has been modified by the use of rennet to bring about the coagulation of curd, which is then pressed in aluminium or plastic moulds. Finally, weighed blocks of the cheese are packages in wax paper for sale. The product is consumed within a week's time.

The microbiology of the fermentation in this process is dominated by lactic acid bacteria, but fungi, general coliforms and even faecal coliforms are also present in substantial counts. Worse, microbial pathogens and food poisoning organisms such as *Salmonella sp., Yersinia enterocolitica* and *Listeria monocytogenes* have been isolated from *jben* samples.

Research efforts to produce a standardized cheese of improved microbial quality and safety have shown that it is possible to produce *jben* of high quality from pasteurized milk in combination with the use of rennet and selected LAB starter cultures. A good starter culture used here consisted of a mixture of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* var. *diacetylactis* and *Lactobacillus casei* subsp. *casei*. The new cheese had a consistant composition, was free of faecal coliforms and pathogens and had a low yeast and enterococcal count.

The seasonality of fresh milk used for *jben* making prompted the researchers in Morocco to investigate the possible use of recombined milk (powdered skimmed milk and butter oil) in making the cheese to ensure a continuity of production throughout the year. Preliminary research gave promising results and further research should lead to success. Researchers are also probing the possibility of using nisin-producing LAB strains as components of starter cultures in order to further boost safety aspects of the cheese. Studies have shown, here too, that acid fermentation alone was not sufficient to ensure safety from pathogens.

From Ethiopia, **Melaku Umeta** discusses two fermented foods in which lactic acid bacteria dominate the microflora involved in their processing. One of these products is *tef injera*, which is a pancake-like sour bread and constitutes the staple of the country. The fermentation of the dough begins with the kneading of a fine flour of the cereal called *tef (Eragrostis tef)* with a thin paste from a previous batch and water, and allowing the mixture to undergo fermentation for 48 to 72 hours. At the end of this period the liquid portion separates from the solid portion and is decanted and discarded, reportedly to reduce the acidity. Now a part of the solid dough is mixed with ample water and boiled for a short time. This is then mixed with the mother dough in the fermentation container and another stretch of fermentation (30 minutes to two hours) is allowed after adding more water and mixing. During this period the dough rises and then settles, indicating the end of the second stage of fermentation. The dough is next thinned with water and baked into *injera*.

Gram-negative bacteria, including certain members of the Enterobacteriaceae, initiate the fermentation and bring about a slight drop in pH. By the time the pH drops to about 4.0 members of *Lactobacillus* become the dominant microorganisms and the population of yeasts begins to rise. When the pH reaches 3.8, lactic acid bacteria will form the major group and there is a dense growth of film yeast on the surface of the separated liquid phase. Research has shown that lactic acid and acetic acid are the major organic acids responsible for both the drop in pH values and the sour taste of *injera*.

The second acid-fermented product is *kocho*, made from *ensete (Ensete ventricosum)*, a plant of the banana family. In the preparation of *kocho* the whole plant is uprooted and the pigmented leaf sheath scraped away. The non-pigmented pseudostem and corm are then pulverised using a pestle with a multi-pronged end, and after the juice is removed, the material is placed in a large pit lined with fresh *ensete* leaves. The pit is then covered and the stuff allowed to undergo fermentation for about a week. In the meantime the root is covered with leaves and given time to decompose. When the proper time has elapsed,

the root is crushed and mixed with the other material in the pit. The whole substance is then well-mixed and pressed before covering with fresh *ensete* leaves and heavy weights placed on it. Fermentation takes weeks, months or even years in colder regions.

The most dominant microorganism during the first week of fermentation is *Leuconostoc mesenteriodes* with *Streptococcus faecalis* coming second in importance. At this point the lactobacilli take over and *Lactobacillus coryneformis* and *L. plantarum* dominate, reducing the pH value to about 4.2.

A discussion of African indigenous knowledge concerning lactic acid and lactic acid bacteria is given by **Hamid Dirar**. Lactic acid-bearing materials are used for a number of purposes in Africa. For instance, the best thirst-quenching foods and beverages seem to be those containing lactic acid. The author gives information about a food product which is prepared for the sole purpose of quenching thirst. Its technology is based mainly on the provision of relatively large quantities of the acid and on facilitating the administeration of it into the stomach without tasting the sourness in the mouth.

Examples are also given of cosmetic and medicinal uses of lactic acid-bearing materials. One important example is the use of soured camel milk in the treatment of the fatal disease called *kala-azar* (Leishmaniasis) when the patient is kept on this product as the sole diet until good health is attained.

Examples are also given of the use of lactic acid produced by fermentation at the cottage level for more or less technological purposes. In one instance, the acid is needed to lower the pH of the substrate to allow yeast growth in the brewing of sorghum beer. Here, lactic acid bacteria are first encouraged to grow and produce the acid and then the microflora are completely eliminated by a rigorous heat treatment. Now the acid-bearing substance that remains is used to grow the yeast. In yet another example, the bacteria are used to produce the acid which is needed to free starch from other grain components in the cottage-level starch production.

The African woman, who carries out lactic fermentation almost daily, knows how to deal with LAB in the course of her daily life. Of course she does not see the cells themselves, but she seems to be aware of their presence. She puts into practice the enrichment technique to select for and encourage their growth whenever she desires that. On the other hand, she has developed techniques of her own to suppress the growth of these bacteria when that is needed under certain conditions of food production.

2. Cassava fermentation

Eric Giraud enumerates the merits of cassava as a staple crop that feeds half a billion souls the world over. He says that the crop is highly productive and can grow in a wide range of climatic conditions and soil types, as well as under various conditions of cultural constraints characteristic of large parts of Africa, Asia and America. Cassava is an efficient converter of solar-energy and its production requires little care. Furthermore, it can be kept in the soil for years after maturation and so can serve as a famine reserve. However, cassava contains a toxic substance, linamarin, and is seriously deficient in

protein. A variety of food products are made from cassava, their differences stemming from differences in the fermentation process, which is an essential step in food preparation.

Various investigators have tried to find out which microorganisms are involved in cassava fermentation and what role they play. However, no unanimous decision has been taken in these respects, but there seems to be some agreement that lactic acid bacteria make the primary group of microflora in the process. Their major role seems to be in the development of flavour. At first, researchers thought that microorganisms were responsible for the breakdown of linamarin, resulting in the detoxification of cassava, but later this theory was abandoned, in spite of the fact that microorganisms that produce linamarase, the enzyme responsible for the breakdown of linamarin, have been isolated from fermenting cassava. It is now generally accepted that linamarin is broken down by an enzyme endogenous to the tuber itself. Any time the integrity of the tuber tissue is violated, such as by pulping or crushing, the enzyme comes into contact with the cyanoglucoside and destroys it within a few hours. First, the cyanogenic glucoside, linamarin, is hydrolized by linamarase to give glucose and acetone cyanohydrin, which in turn, undergoes spontaneous dissociation to cyanhydric acid and acetone. Cyanhydric acid is a volatile compound and is rapidly eliminated in the atmosphere.

Nevertheless, foods made from cassava have been shown to contain traces of cyanogenic glucosides, which possess a hazard to human health. Apparently, the endogenous linamarin of the tuber is not sufficient to bring about complete detoxification. It is therefore thought that the use of starter cultures with linamarase activity could help wipe out the last traces of toxicity and improve the whole process of food preparation. An "ideal" starter strain would have the following capabilities: fast growth, tolerance of high acidity, production of ample amounts of lactic acid, hydrolysis of linamarin, ability to hydrolyze starch for growth and genetic stability. The author and associates were able to isolate a strain of *Lactobacillus plantarum* which appeared to meet most of these criteria, but no significant improvement was noticed in detoxification when the organism was used in cassava fermentation.

There are also attempts to raise the protein content of cassava foods. In one such case protein content is claimed to have increased from about 3.5 per cent to 12 to 14 per cent by growing certain moulds on the pulp of the tuber.

Olusola Oyewole reports on research results on the submerged fermentation process of cassava. According to the author, the fermentation of cassava can be achieved by one or the other of two methods: solid-state fermentation or submerged fermentation processes. In the first procedure the cassava tuber is not soaked in water. Here the tubers are either grated, packed into a bag and fermented under pressure, or they are sliced and spread out in the sun. In the submerged process the tubers are soaked in water in the form of whole peeled or unpeeled roots or sliced roots.

The reported research has concentrated on three aspects of the submerged fermentation: optimization of the traditional process, a study of the lactic microflora involved and the development of a starter culture.

A wide spectrum of microorganisms has been found to be involved in the traditional process of fermentation. Bacteria of the genera *Bacillus, Lactobacillus, Klebsiella, Leuconostoc* and *Corynebacterium*, as well as moulds and yeasts belonging to the genera *Aspergillus, Geotrichum* and *Candida* were characterized. A pattern of succession has been observed in the microbial growth. *Bacillus, Corynebacterium* and *Klebsiella* which produce amylase enzymes were found to grow first. They were then inhibited by the lactic acid produced by LAB, while the final stages of fermentation were invariably dominated by *Lactobacillus plantarum*. A number of the LAB involved in the fermentation were found to be capable of hydrolyzing linamarin and most of these belonged to *L. plantarum*. In addition, over 80 per cent of the lactobacilli were found to be able to hydrolyze startch. Accordingly, a strain of *L. plantarum*, with linamarase and amylase activity, as well as ability to produce ample acid, was investigated as a single-strain starter culture. More research is still needed in this respect, especially in the light of the fact that research has indicated that the different organisms implicated in the natural fermentation of cassava play specific complementary roles in the process. For example, *L. plantarum* was found to produce the highest amount of acid, *Bacillus subtilis* the highest rate of retting and *Candida krusei* the characteristic flavour. Even when a single bacterial strain is employed as a starter organism there is always the possibility of improving the performance of this strain through recombinant DNA technology.

With gentle irony, **Ira Robertson** drives his message home. He discusses cassava fermentation from a wider angle of view, an overall biotechnological approach with the material as an industrial resource with wide possible uses, not only as food. Cassava could be fermented at the village level to produce protein-rich palatable food for children; it could be protein-enriched as cattle feed. Cassava could furthermore be used as a carbohydrate source for the production of industrial alcohol by fermentation; it could be used to produce butanol by fermentation, too. Both alcohol and butanol can replace, at least partially, petroleum products especially for driving engines.

The author reports on the results of the Zimbabwean research on cassava. Use of virus-free material and improvement through breeding programmes have resulted in great increases in crop yields. Yields of 20 to 30 tonnes per hectare have been realized, a dramatic improvement over the African average of only six tonnes per hectare. On the processing side, the protein content of cassava has been raised to 17 per cent through a process of dry fungal fermentation. The interest here is on using cassava as cattle feed since the crop is not really popular in Zimbabwe as food for humans.

The author believes that the scientific knowledge concerning the industrialization of cassava is ample and the technology is available, and so he wonders why Africa does not make use of this situation. He goes on to list the possible problems and constraints to be encountered and gives the experiences of a number of non-African countries in this respect and shows how they tackled the problems they faced. He concludes that only certain favourable circumstances have to be around to warrant the use of any cassava-based industry and make it economically viable. In this respect the role of the politicians must not be ignored; an example is given of how politics and politicians can influence

social attitudes. Attention is drawn to the economic, political and international realities which will always influence any implementation of an industrial project involving cassava utilization.

3. Mushroom biotechnology

Mushrooms can make an excellent food and income resource. Their cultivation and consumption in the developed countries and in quite a few countries of the Third World has been a success for decades. Africa is lagging far behind in this aspect of food production. But **John Okhuoya** tells us that mushrooms have been traditionally known to Africans as a source of food and medicine. Local people in Nigeria not only collect mushrooms from the wild, but they even practice some kind of rudimentary care and cultivation of these macrofungi. Of these techniques the author mentions:

i. Site preservation: where the farmer takes notice of the site of mushroom natural growth in the wild and takes some measures to protect the stand, e.g., by putting up a fence around it. The site is visited each growing season and the product harvested.

ii. Log preservation: where logs known to support mushroom growth are cut, placed in the shade and watered regularly. The harvest is collected each season.

iii. Soil burial of sclerotia: which involves the burying of sclerotia in the soil under shade or in a cool place to grow and give fruiting bodies, when both sclerotia and fruiting bodies are harvested and consumed.

The native Nigerians also have methods for the preservation of excess mushroom harvest. Mushroom material could be preserved either by drying in the sun or by smoking.

Research carried out by the author and his co-workers has resulted in the characterization and identification of many of the popular edible mushrooms found in Nigeria. Each of the traditional mushroom growing techniques mentioned above was found to fit certain kinds of mushrooms. Site preservation, for instance, is associated with *Termitomyces* spp., log presevation with the growth of *Auricularia* sp., *Schizophyllum* sp. and some *Pleurotus* spp., while the soil burial technique favours *Pleurotus tuberregium*. A number of other mushrooms collected from the wild have also been identified.

Research then focussed on the cultivation of the mushroom *P. tuberregium*, a very widely used species in the country. Both the fruiting body and the sclerotium of this fungus are consumed in Nigeria. Various substrates for the production of fruiting bodies, sclerotia and spawn were tested. It was found that substrates could carry a number of fungal contaminants and other pests, such as nematodes. Of the substrates tested, oil palm fruit fibre was found to support extensive mycelial growth of the fungus and so was used to develop spawn. Loam soil was found to give the highest yield of mushroom among the different substrates tried for the production of fruiting bodies. Higher yields were obtained with substrates inoculated with sclerotia than those inoculated with spawn. Sclerotia are better produced on substrates that did not support much mycelial growth of the fungus. The yield of sclerotia was highest on sawdust.

The author suggests that research scientists should carry out studies intended to build on the indigenous know-how of the native African, as this would be a cheaper and easier technology to sustain.

The Ugandan experience in mushroom biotechnology is related by **Frederica Nkakyekorera**. The introduction of mushroom technology in 1991 targeted specifically women farmers to help them earn more income. From the start it was decided that the technology be simple and feasible for this sector of small-scale farmers. The original mushroom strains introduced were *Pleurotus* spp. and came from Egypt. In Egypt they were grown on rice straw as substrate, but this material is not widely available in Uganda. It was therefore decided to carry out research on the local waste substances and find out which could replace rice straw as substrate. Cotton seed hulls were found to be the best among many substances tried as substrate. Wheat, barley and rice straws also gave high yields and were recommended for use by farmers who had no access to cotton seed hulls.

As the substrates on which mushrooms are propagated are mostly ligno-cellulosic materials, they must be pretreated in order to facilitate their breakdown by fungal enzymes produced by the mushroom. At the same time the substrate must be at least partially sterilized to reduce competition and contamination. The procedure copied from Egypt was to boil the substrate for two hours and then drain it to a specific moisture content by hanging it out in the air. In Uganda, this has posed a problem as there is more rain and some farmers are not patient enough to wait for the substance to dry up. High moisture could lead to suffocation of the mushroom mycelium. In such circumstances it was discovered that simple steaming of the substrate was more acceptable.

Spawn production in Uganda depends on inoculating pieces of the mother culture into bottles of sterilized wheat or sorghum grains to give mother spawn, which is then used to inoculate other similar bottles to make the final spawn to be distributed to the farmers. Inoculation is done by placing the substrate and spawn in a plastic bag, which is sealed and incubated at ambient temperature. Within three to four weeks the mycelium would be ready to be induced to produce its fruiting bodies. This is done by changing the environmental conditions of temperature, relative humidity, light intensity and aeration. Within four to seven days after these changes have been made, pinheads appear on the mycelium and later develop into edible fruiting bodies. In Uganda at least six flushes of harvest can be obtained, but the quality of the mushrooms deteriorates after the third flush. The spent compost is used as manure for agricultural land. In 1993 new mushroom strains were introduced into Uganda from Poland, Italy, Thailand and Mauritius.

Ralph Kirby and **Maria de Serra** report on an interesting situation in South Africa. In the 19th century nitrogen-fixing acacia shrubs were introduced into the country for a number of reasons including the provision of bark for the tanning industry and to help stabilize the sand dunes along the coast. But the acacia went rampant, invading areas where they were unwanted and created a big problem. As a combating measure, the trees were cut down and the stems reduced to chips which remained as waste on the soils.

As a remedial measure, to make use of the chipped wood, the possibility of growing mushrooms on them was studied. Three *Pleurotus* strains were tried and they proved promising. As yet, however, productivity is very low and further studies, testing more mushroom strains, are being undertaken at present. The idea is to find a way to help the farmers whose lands have been invaded and make use of this waste in an income-generating small-scale mushroom production scheme.

Sanchai Tontyaporn tells the story of mushroom technology in Thailand. Recounted here is a true success story taking place in a developing country which African countries can make use of. In Thailand, mushroom cultivation was successfully developed as far back as 1937 and by 1938 the technology was extended to rice farmers. Today, at least seven mushroom species are produced commercially in Thailand. Annual production (1992) is 80,000 tons, fetching US$ 80 million. The bulk of the harvest is consumed locally, while 10 per cent of it is exported. Thousands of farmers are engaged in mushroom production, some permanent, some seasonal.

Mushroom cultivation systems in Thailand may be divided into two kinds: production in polypropylene bags and production in beds. In the bag method, pararubber sawdust is the major substrate. It is packed in the heat-resistant bags and sterilized. Then the spawn is inoculated into the substrate and after the required incubation period the mushrooms are ready for fructification, maturation and harvesting. Wood-colonizing mushrooms are usually produced by this technique.

The bed-type production method can be further divided into open-field culture and protected culture. In the open-field culture, the low-bed variant is followed, where pretreated rice straw is packed in a wooden frame and spawn is placed on top of it, along the rim of the bed. A second and a third layer of substrate and spawn are likewise laid on top of the first. Now these are covered with a loose layer of rice straw and the whole thing is finally covered with a plastic sheet over which is placed a layer of straw to prevent light from entering. Within nine days mushroom primordia begin to appear and harvesting begins on the 12th day.

In the protected culture method, cotton waste imported specially for mushroom growing, is used as substrate. The method is rather more exacting and requires a larger capital input.

Current and future research in Thailand aims at improving the mushroom strains now employed and at improving substrate mixes and spawn production, as well as controlling diseases and pests. Collection and exploitation of local mushroom strains also make part of these research efforts.

Agricultural wastes are not only suitable for use in mushroom production; they constitute important growth media for a number of other microorganisms, which produce vital commodities. The paper presented by **Nabil Magdoub** gives us an example of this as it reports on the production of vitamin B_{12} (Cyanocobalamin) and on ethanol production, both from the agricultural waste material, whey, a by-product of the cheese industry and

a potential environmental pollutant if not properly managed. It is, however, unfortunate that the paper is too long (80 pp.) to be included in this publication, and therefore only the present summary can be given of it here.

In this research, strains of *Propionibacterium* were employed for the production of B_{12}. The standard fermentation medium consisted of ultra-filtered cheese whey, to which cobalt chloride was added. Preliminary research resulted in the selection of two bacterial strains, out of an original five, as most promising. The rest of the experiments used these two only. A number of additives to the basic medium were found to improve B_{12} production. These include: choline chloride, sodium lactate, yeast extract, kefir whey, rape oil meal extract and glutamic acid.

Whey or milk permeate can similarly be used to produce ethyl alcohol via the mediation of suitable, lactose-fermenting yeast strains. In the research at hand, five strains of *Kluyveromyces marxianus* have been tried for the production of ethanol from cow milk permeates. The standard fermentation medium consisted of permeate (4.5 per cent lactose) obtained by ultra-filteration of cow's milk. The pH was adjusted to 4.5 and the medium sterilized by autoclaving at 121^0C for five minutes. The various factors which were thought to affect production of ethanol were tested. One strain of the yeast was found to give its highest yield of the alcohol after incubation at 30^0C for five days in milk permeate containing initial lactose concentration of 15 per cent, initial pH of 4.5 and inoculum level of 12 per cent. The remaining four strains performed best at 20 per cent lactose under the same conditions of incubation as above. Yeast extract and ammonium sulphate were found to stimulate ethanol production at specified concentrations. Similarly, gamma irradiation of the yeast strains improved alcohol production noticeably when the organisms were cultivated under the conditions mentioned above.

I. LACTIC ACID FERMENTATION

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Lactic Acid Fermentantion as a Low-Cost Food Process and Preservation Technology

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Abstract

Lactic acid fermentation is probably one of the first biological processes from which human beings have discovered the benefits of fermentation.

It is believed that processes required for food material fermentation were present on Earth when mankind appeared on the scene, millions of years before microorganisms started converting and recycling organic material. Cabohydrates from damaged wet seeds and fruits may be converted by microorganisms to glucose, then to organic acids, alcohol and CO_2 yielding alcohol and/or acid fermented products. Acid and alcohol fermentation have been responsible for mankind's survival over millenia due to the preservative effects of both alcohol and organic acid and their lethality for some disease-producing microorganisms and parasites.

Acid fermentation is still widely used throughout the world as the basis of most traditional processing technologies of milk, cereals, fruit, vegetables, legumes, meat and fish, mainly involving various species of lactic acid bacteria.

Numerous advantages are attributed in the literature to lactic acid fermentation of food materials (stability, digestibility, flavour and nutritional improvement).

However, a major feature is that over millenia, whenever the required fermentation conditions were respected, the desired acidic fermentation pattern was constantly reproduced in spite of the non-sterility of the substrate.

In this paper some traditional fermented foods chosen as model systems are described, emphasizing the factors leading to the dominance of some species of lactic acid bacteria during fermentation.

It is suggested that model studies could lead to the discovery of specific biotypes for the selection of useful strains for food processing and preservation. The studies could also permit the identification of parameters for low-cost food processing technologies based, for instance, on controlled solid state and non-sterilized substrate fermentation, using specific starter cultures.

Believing that any improvement of traditional fermentation processes should be guided by preliminary studies of ecological parameters which govern them, it was concluded that there was a necessity for a deeper research on some representative fermented foods in order to develop and disseminate low-cost fermentation technologies for small-scale industries in Africa through joint cooperative research networks.

INTRODUCTION

Lactic acid fermentation is probably one of the first biological processes from which human beings discovered the benefits of fermentation.(1)

It is believed that the processes required for food material fermentation were present on Earth when mankind appeared on the scene.(2) According to recent scientific thinking microorganisms were the first forms of life to evolve on Earth, (fossil microorganisms have been discovered in rocks 3.3 – 3.5 billion years old); plants were the next forms, followd by various species of animals which evolved over a hundred million years, followed later by Man.(2,3) Accordingly, a million years before mankind appeared, microorganisms started converting and recycling organic material. Carbohydrate from damaged wet seeds and fruits may be converted to glucose, then to organic acids, alcohol and carbon dioxide ($C0_2$) yielding alcohol and/or acid fermentated products. Archaeological evidence of intensive use of ground grains in the Nile and Sahara regions between 12,000 and 10,000 B.C.(4) suggests that Man was already accustomed to acid fermented cereal foods 13,000 years ago.

Acid and alcohol fermentation have been responsible for Man's survival over millenia due to the preservative effect of both alcohol and organic acids and their lethality for some disease producing microorganisms and parasites. Acid fermentation is widely used throughout the world as the basis for most traditional processing technologies of cereal, fruits, vegetables, legumes, meat and fish, involving mainly various species of lactic acid bacteria.(1,5)

Numerous advantages have been attributed to lactic acid fermentation :

- Low-cost food preservation process, particularly if associated with drying;(6,7)
- Organic acid (lactic acid and acetic acid, etc.) produced contribute to the stability of the product;
- Fermented foods have been used for centuries and are therefore culturally accepted;(7)
- Improvement of the digestibility of cereals, e.g.:

— reduction of the level of phytic acid and polyphenol (tannin) which interfere negatively with starch and protein digestibility and mineral absorption; [8,15]
— improvement of nutritional value: bio-enrichment with microbial protein, amino acid, lipids and vitamins;[6,16,17]
— positive health effects of lactic acid fermented foods: hypocholesteraemic (still not clearly defined) and anticarcinogenic effects, antibiotic synthesized by some strains;[16, 18]
— detoxification of food material, e.g., cyanide removal from cassava.[19, 21]

Although some lactic acid fermentation processes have been extensively studied and industrialized (yoghurt, commercial production of lactic acid), many traditional lactic acid fermented foods are still regarded as low quality, with the status of household or artisanal foods.

However, the renewed interest in natural and health foods in developed countries and the need for alternative low-cost food preservation and processing technologies in developing countries justify further studies on lactic acid fermented foods.

In this paper factors leading to the dominance of lactic acid bacteria in some traditional fermented foods chosen as model systems will be studied in order to define conditions for the development of low-cost lactic acid fermentation processes.

TRADITIONAL FOOD FERMENTATION TECHNOLOGIES

Worldwide distribution of fermented foods

As shown in table 1, lactic acid fermented foods are widely distributed throughout the world.

Cereal-based foods represented mainly by beverages, porridges and some breads are numerically dominant, particulary in developing countries. These cereal-based foods, which are often supplemented with protein-rich products (legume and milk products), are generally consumed as the main dish. Other products are fermented vegetables, fish and meat.

Various local taboos and food habits, instead of scientific knowledge, may have allowed the success and survival of fermentation practices (or arts) over millenia. On them are also based the optimal fermentation conditions that yield products with the traditionally required properties.

Microorganisms and biochemical processes involved

Microorganisms

As indicated in table 1, the main microrganisms involved in the acid fermented foods described belong to the genera *Lactobacillus, Leuconostoc Pediococcus* and *Streptococcus (Lactococcus)*. These four genera constitute the "central citadel" of the group

"lactic acid bacteria", now recognized as consisting of Gram-positive, non-sporing, carbohydrate fermenting lactic acid producers, acid-tolerant, of non-aerobic habit, catalase-negative, typically non-motile and unable to reduce nitrate.[22]

However, other genera, which could be found in fermented products and are lactic acid producers, are excluded by this definition ; these are *Bifidobacterium, Sporolactobacillus, Micrococcus* and some strains related to *Bacillus coagulans (Bacillus laevolacticus).*[1,22,23,24]

Metabolic pathways during the fermentation

Lactic acid bacteria ferment sugars according to two main pathways:

- The glycolytic pathway in which glucose or fructose is fermented into two molecules of lactic acid by the homofermentative strains;[25,26] example: *Lactococcus, Pediococcus,* some *Lactobacilli*;
- The phosphketolase pathway in which glucose, fructose or some pentoses (ribose, arabinose, xylose) are fermented into lactic acid, acetic acid, ethanol and carbon dioxide by the heterofermentative strains;[25,26] example: *Leuconostoc* and some *Lactobacillus.*

However, homofermentative bacteria have the enzymic capability to be heterofermentative and thus the pathway and enzymes are under precise control, resulting in the formation of specific end products under certain conditions of growth; for example, when carbohydrates are limiting, homofermentative lactic *Streptococci (Lactococci)* can produce more heterofermentative end products.[27]

Lactobacillus plantarum has been classified as a facultative homofermenter because it is capable of producing some enzymes of the heterofermentative pathway, including phosphoketolase, which allow it to ferment pentoses.[25,28]

A variant of the phosphoketolase pathway is the bifidus pathway, in which *Bifidobacterium* converts glucose or fructose into lactate and acetate in the ratio 2 : 3.[5]

Several factors influence the ability of lactic acid bacteria to completely ferment sugar, including initial sugar concentration, pH, salt concentration, temperature and buffer capacity of the substate.[25]

In addition to sugar, organic acids such as malic, tartaric and citric acids, which are present in many vegetables, may be metabolized by lactic acid bacteria:

- *Lactobacillus, Leuconostoc* and *Pediococcus* convert malic acid to L-lactic acid by malolactic fermentation;[29]
- Some strains of *Lactobacillus plantarum* isolate from silage degrade citrate into acetate and oxaloacetate.[25]

Lactic acid bacteria can produce either L(+) or D(-) lactate stereoisomers; the unequal production of isomers is caused by relative activities of specific D and L lactate dehydrogenase that reduce pyruvate to lactate.[25] *Streptococcus* and *Bifidobacterium* produce the L(+), *Pediococcus* produces the DL (racemic) and the L(+), *Lactobacillus*

the D(-) L(+) and DL and *Leuconostoc* the D(-) isomers from glucose.[5] However, the four genera, *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc* produce the L(+) isomer from malic acid.[29]

Both L(+) and D(-) lactic acids are metabolized by mammals;[25] L(+) lactic acid is completely transformed, while D(-) is slowly and partially metabolized (up to 30 per cent is normally excreted in urine.[30] Based primarily on concerns about the ability of infants to metabolize the D(-) isomer, a World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) expert commitee recommended that the D(-) lactic acid be avoided in infant food and to limit consumption by adults to 100 mg/kg body weight per day. This recommandation was revised in 1974 to retain the recommandation that D(-) or DL lactic acid not be used in infant foods, but no limit was set for an acceptable adult intake.[23]

Amino acids are occasionally decarboxylated by lactic acid bacteria to give biogenic amines, which have been implicated in food poisoning incidents, usually from fish consumption; the decarboxilation of histidine to histamine by *Pediococcus Cerevisiae* was demonstrated.[23,27]

Typical fermentation systems

Lactic acid fermentation processes may be classified according to the nature of the substrate concerned (cereal, vegetable, meat or fish, etc.), or to the factors used to control the microbial growth (salt, temperature, etc.), or both.

Accordingly, we may distinguish the following systems and sub-systems based on the use or not of salt as the selective agent of the microflora:

* Non salt-controlled systems (cereal and other starchy products, legumes, milk).
* Salt-controlled systems (vegetable, fish and meat or mixed ingredients).

Non salt-controlled fermentation systems

Fermented milk

Archeological evidence depicted cow-worship and milking activities in the Libyan Desert dating from 9,000 B.C., and records indicate that the Sumerians used fermented milk 5,000 years ago.[31] However, the consumption of fermented milk may be as old as that of fresh milk.

Nearly every civilization has consumed fermented milk of one type or another. The type of fermentation that occurs varies from area to area and is governed to a considerable degree by the environment; acidophilus milk, Bulgarian buttermilk, yoghurt, kefir, kumiss and others are avaible.[31] Milk contains most of the nutrients required for the growth of fastidious lactic acid bacteria and the lactose cannot be utilized by all species of microorganisms. As bacteria are ubiquitous, they are destined to inoculate milk and ferment it. For centuries, housewives have relied upon natural fermentation at ambient temperatures; then inoculation from sour milk containers or previously fermented milk

was utilized, which allowed for the selection and dominance of specific lactic acid bacteria giving a type of fermented product.

Various microbial associations are discribed: *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for the yoghurt, Lactobacilli and yeast for the *kefir*, *Streptococcus lactis, Lactobacillus casei* and yeast for *kumiss*,[31] and *Lactobacillus, Streptococcus* and yeast for the *Mbanick* of Senegal.[32]

Lactic acid fermented cereal products

The first evidence of bread baking may be dated to 7,000 to 3,000 B.C. (Neolithic).[33] However, the consumption of cereal fermented food may have started earlier. Cereal lactic acid fermentation may be in solid-state (wet flour or grits), semi-solid-state (sourdough) and liquid-states. Cereals and the flour prepared from cereals are always heavily seeded with microorganisms, which may have been introduced from the soil, air, water, or post-harvest and processing equipment. The activity of each contaminant will be influenced by the water content of the product as well as other environmental factors.

Solid state (flour, grits) fermentation

In Senegal, cereal grains (millet, maize, sorghum) are wetted, husked in a mortar, washed, drained and slightly dried before milling (figure 1). During these operations acid producing bacteria, which are rare on the original grains, increase quickly in number, while the product becomes sour. The final flour is granulated into fine grits or small balls, which are allowed to ferment for a further six to twelve hours. The grits are steam-cooked to give a fermented "couscous", called *thiarae*, while the balls are steam-cooked to give *thiakry*, or boiled to give a porridge called *lakh*.

The fermented grits and balls may be preserved by sun-drying prior or after steaming. The entire process may take 12 to 24 hours, depending on the degree of sourness desired. The fermented flour may also be wetted, moulded into different shapes, and steamed or baked on a fire to give a bread-like product called *nakh* or *munko*. A bread drink called *kudy* may be prepared by dispersing the baked bread in water, dextrinizing the starch with a water extract from the leaves of *Leptadenia hastata*. Dominant microorganisms belong to the genera *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* yeast and undetermined acid-producing rod-shaped bacteria.[34]

Since husking and washing may lead to the elimination of most of the external microflora of the seeds, some of these dominant microorganisms may be brought by water, the processing containers, or maybe just the microflora originally adhering to the immature milky grains. The growth of undesirable microorganisms may be limited by quick acidification, drying and or heat treatment (boiling or steaming).

The solid state fermentation may have been developed in regions where water is rare (the Sahel zone of Africa) and where cultural and religious considerations do not allow the consumption of alcoholic beverages.

Another solid state fermented product is the Thai *khanom Jeen*: raw rice is soaked, drained and fermented for three day before grinding; *Lactobacillus* sp. and *Streptococcus* sp. are involevd in the acid fermention.[5]

Semi- solid state (dough) fermentation)

In dough-state fermentation two different products may be desired according to the functional properties needed:

- a sourdough for the processing of acid-leavened bread or pancake; (sourdough, bread, *injera, puto, kisra*);[5]
- a sourdough used as a multipurpose base for steam-cooked bread, porridge, gel-like product, gruel and paste; example: *mawe* of Benin, *kenkey* of Ghana.[35, 36]

A great number of lactic acid producing bacteria of the genera *Lactobacillus, Leuconostoc, Lactococcus* and *Pediococcus* have been found in sourdough. Proper leavening is obtained by the action of yeast and/or heterofermentative lactic acid bacteria which produce carbon dioxide, ethanol and acetic acid. Their growth will establish in the dough a partial anaerobic and acidic environment unfavourable for the growth of undesired aerobic and/or acid-intolerant spore forming bacteria and moulds.[31,35]

In the sourdough prepared for steamed bread, *puto* and *injera*, the heterofermentative *Leuconostoc mesenteroides* initiates the fermentation.[5]

The supplementation of the cereal dough with milk products or legumes (*idli*) will make the substrate more suitable for the fastidious high acid producing lactic acid bacteria, which will allow a stronger acidification.

Liquid state fermentation

Acid porridges or drinks are prepared from cereals and eaten in various amounts in different parts of the world, particulary in Africa.

The product may be fermented according to four main types of processes:

- spontaneous fermentation of the raw flour suspended in water, example: *uji* of Kenya,[5, 38] *ogi* of Nigeria;[5,36]
- fermentation of cooked suspension using raw flour (wheat) as starter, example: *mahewu* of southern Africa;[5,36,39]
- fermentation of the cooked gruel in special fermentation gourds,[38] which may contain specific microflora, example: *uji* of Kenya;
- fermentation of a mixture made by suspending hot cooked rice in water, the mixture being partly renewed every three to four days by adding a fresh hot cooked rice water suspension, (figure 2); example: *jangsu* of Korea.[40-41] In this case, water may be considered as the source of fermentation inoculum.

Lactic acid bacteria are the main organimsm in these fermentation systems:

- in *ogi*: *Lactobacillus plantarum;*[5]
- in *uji*: *Lactobacillus plantarum* and *Leuconostoc mesenteroides*[5] or *Lactobacillus plantarum* and additionally *L. cellobiosis, L. fermenti, Pediococcus diacetilactis* and *P. pentosaceus;*[38]
- in *mahervu*: *Lactobacillus* spp. and *Streptococcus (Lactococcus) lactis;*[5]
- in *jangsu*: *Leuconostoc mesenteroides*, two amylolytic bacteria, *Lactobacillus* spp. and *Streptococcus (Lactococcus)* spp., and *Pediococcus* spp.[40-41]

In the case of *jangsu* the periodic renewal of the medium allows the progressive selection of lactic acid bacteria as shown in figure 3.

These observations may suggest that even if salt is not used to selectively control the microbial growth, the conjunction of several process parameters allow the dominance of lactic acid bacteria:

- Presence of lactic acid bacteria in the subtrate and its environnement at pre-harvest, harvest and post-harvest stages (cereal grains, milk), but generally in a very low proportion.
- Substrates containing specific fermentable sugar, particularly di- or trisaccharides (lactose, maltose, saccharose, raffinose) more quickly metabolized by the substrate specific lactic bacteria than by the contaminants.
- The inhibitory effect of the carbon dioxide, the acetic acid and alcohol produced in these low-sugar fermentation conditions which favours the hetero-fermentative metabolic pathway.[25, 27, 28, 42, 43, 44]
- The gas(CO_2) trapping effect of the dough and the submerged culture (liquid state fermentation) which creates a partial anaerobiosis.

However, the non-salt controlled fermented products are not stable; they need to be cooked, dried or refrigerated to avoid over-souring or a drop in acidity.

In the controlled fermentation, in addition to the above factors other parameters are the preservative agent (salt) or stimulatory agents (carbohydrate).

Salt controlled fermentation system

The discovery of salt probably brought about one of the greatest contributions to mankind's survival.

The preservative and organoleptic properties of salt were recognized long before history was recorded. The use of salt for preservation of vegetables and meat is an ancient

practice. However, since salt was formerly expensive and impure, probably relatively low salt concentrations were used.

Certainly when low quantities of salt were added to vegetables (or meat and fish), the product acquired an acid flavour, which partially balanced the excessive salty flavour and undoubtedly appealed to the consumer.

Due to the availability of salt and the diversity of food materials and food customs, varieties of salt-controlled fermentation practices exist throughout the world.

Fermented vegetable products

Preservation of vegetable material by fermentation depends upon the reduction of the activities of native enzymes, inhibition of oxidative chemical changes and the inhibition of spoilage microorganisms. It depends upon the combined effect of acid, salt, carbon dioxide, the low oxidation reduction potential and other minor factors.[31]

The important parameters are cleanliness and quality of raw materials, temperature and proper salt concentration to withdraw sufficient liquid from the vegetables to deter softening, but to also allow the rapid initiation of the fermentation in order to acidify the product and create anaerobic conditions.[31]

Leuconostoc mesenteroides initiates the fermentation and imparts desirable flavour derived from the acids (acetic and lactic acids), alcohol and other fermentation products, and carbon dioxide.

The bacterial sequence includes *Leuconostoc mesentoroides, Lactobacillus brevis, Pediococcus cerevisiae* and *Lactobacillus plantarum,* and occasionally *Streptococcus faecalis.*

In general, shredded or chopped cabbage is dry-salted in making *sauerkraut*, while cucumbers, olives and other vegetable material in large pieces are brined.

The Korean *kimchi* is a mixture of vegetable material with cabbage, radish or cucumber as main ingredients. To make *bachu kimchi* cabbages are first soaked in 15 per cent salt brine for three to seven hours, drained and mixed with powdered red pepper, garlic, green onion and ginger. A small amount of acid may also be added. The optimum salt concentration is 3 per cent.[5, 45]

An acidity of 1.0 to 1.2 per cent acid may be obtained in a dry salted fermenting vegetable by the activity of *Leuconostoc mesenteroides* and up to 2 to 2.5 per cent acid may be produced if sufficient sugar is present. In fermentation in brine, an acidity beyond 1.0 per cent is rarely attained.[31] Under optimal conditions an acidity of 0.6 per cent is attained in *kimchi* after three days.[5, 45]

Salt concentration and the distribution and fermentation temperature are very important in these systems. For example, excess salt, i.e., 3.5 per cent or more, is detrimental to the growth of LAB, particularly to *Leuconostoc mesenteroides.*

Excessive salt may result in proportionaly excessive growth of homofermentative organisms which produce little carbon dioxide so important in the establishment of anaerobiosis.

Between 7.5 and 18^{0}C, *sauerkraut* fermentaion is brought about by *Leuconostoc mesenteroides* and an acidity of 0.8 to 1.2 per cent of lactic acid is attained in less than one month, with a salt content of 2.25 per cent. At a higher temperature homofermentative bacteria dominate progressively with a progressively declining product quality.[31]

For *kimchi*, the optimum pH and acidity for the best taste is 4.2 and 0.6 per cent (as lactic acid) respectively, and this is attained after three days of fermentation at 20^{0}C and 3 per cent salt content.[5, 45]

In these low-salt fermented vegetable systems *Leuconostoc mesenteroides* initiates the fermentation under optimum fermentation conditions. This heterofermentative species produces lactic and acetic acid, alcohol and carbon dioxide, yielding an environment condition favourable for other LAB and for the stabilization of ascorbic acid and the colour and firmness of the vegetable.[5, 31]

The factors that allow the early dominance of *Leuconostoc* may be their ability to grow in the vegetable exudate right after harvesting and at low temperature, increasing acidity and anaerobiosis in the product.[31] In the case of *kimchi*, the relatively higher salt content, the presence of spices such as ginger, which has a stimulatory effect on some, and LAB,[46] or garlic, which has antimicrobial properties,[1,47] may contribute to the selectivity of the product.

Fermented animal products (milk, meat, fish, etc.)

Food products of animal origin are highly perishable due to the richness in nutrients suitable for microbial growth. The storage life of perishable fish and meat has been extended with the addition of carbohydrate and salt.

The amount of added salt and carbohydrate primarily control the extent of acid fermentation,[5] however, other parameters allow for the selective growth and dominance of lactic acid bacteria.

The Gajami Sikhae system

Fermented products made with fish and vegetable ingredients (cereal, spices) are common side dishes in South East Asia.[5] The Korean *gajami sikkae* is a good representative.

Small pieces of salted (6 per cent) *gajami*, a flat fish belonging to the genus *Solea* are mixed with hot cooked millet, powdered red pepper, garlic and ginger, and left to ferment at 5 to 10^{0}C for three days.[1, 47]

Lactic acid bacteria, mainly *Leuconostoc mesenteroides, Lactobacillus plantarum* and *Lactobacillus sake*, constitued the dominant microflora, in spite of their rareness in the raw material. As garlic was proved to have an inhibitory effect on the microorganisms isolated from raw material but not on the lactic acid bacteria, it was suggested that this

spice played an important selective role in this system and probably in the other fish or meat-salt-carbohydrate systems were it is also included.[1,47] Ginger, another spice included in these systems, has an antioxidant property[48] and is said to contain Mn^{2+} ions, which are stimulatory to lactic bacterial growth.[49]

The fermented sausage of the West (salami) and those of Thailand and Viet Nam are fermented on a similar basis.

From the studies of these fermentation systems we may better understand the important role of salting in non-sterile food fermentation systems. The salt concentrations used, 2 to 6 per cent, are low enough to allow the growth of lactic acid bacteria while inhibiting some spoliage microorganisms. However, the effect of salt is always reinforced by additional parameters.

- fermentation in an air-tight container so that an anaerobic environment more inhibitory to spoilage microorganisms could be realized by the depletion of oxygen and the production of CO^2 during the fermentation; example: *sauerkraut*, brined cucumber.
- addition of growth stimulators for lactic acid bacteria, such as fermentable carbohydrates (sugar, cereal); example: sausages, *kimchi*, fish-carbohydrate blend (*gajami sikhae*).
- addition of a growth factor such as Mn^{2+} ions from spices; example: *kimchi*, sausages, *gajami sikhae* and the like.
- fermentation at relatively low temperatures, (7 to 20^0 C) to slow down the the pathogen, but mainly to favour the growth of *Leuconostoc mesenteroides*, which produces acetic acid, alcohol and carbon dioxide, inhibitory factors which are also important for the flavour of the product.

The studies of these traditional fermentation systems show that they are dominated by microbial associations composed quite invariably by *Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae* and some streptococci, with their ratios depending on the environmental conditions. Accordingly, these fermentation systems may be regarded as special biotypes where highly adapted strains could be selected and used for the development of improved fermentation processes.

FUTURE DEVELOPMENT AND RESEARCH

This review shows the great variety of lactic and fermented foods throughout the world.

Some of them have been extensively studied and industrialized in the developed coutries; these are vegetables (*sauerkraut*, olives, cucumbers) milk products (yoghurt, cheeses) meat products (sausages) and cereals (sourdough).

In Africa, mainly starchy products (cereals and cassava) are studied; elements of microflora are isolated and identified, but studies on microbial ecology of these foods are rare.

Research on lactic acid fermented vegetables, meat and fish products in Africa are also rare. It is in this continent that more research is needed in lactic acid fermentation technology, not only because of the multiple advantages of fermented foods (improved nutritional value and digestibility, etc.), but for the fact that fermentation is the basis of most traditional household and artisanal commercial food processing activities.

In other words, up-grading food processing and preservation capacities to industrial levels would depend necessarily upon the improvement of the fermentation process.

For that purpose the following research areas may be identified:

- Solid state fermentation and extrusion cooking of cereals and cassava

Case of cereals:

* Microbial ecology of the existing fermentation processes
* Selection of specific LAB
  - — Thermophilic strains
  - — Osmophilic strains for growth in low-moisture substrate
  - — Animo acid- or vitamin-producing strains for nutritional improvement

Figure 4 shows an optional process flow chart.

Case of cassava:

* Microbial and biochemical studies on the fermentation process
* Selection of specific LAB
  - — Glucosidase-producing LAB for hydrolysis of cyanogenic glucosides involved in cassava fermentation[21]
  - — Thermophilic strain

The optional process outlined in figure 4 includes two stages of fermentation: the first for bacterial growth and linamarinase production in mashed cassava, the second step after addition of soy flour which would increase and stabilize the pH (by the buffering effect of the protein). At the second step, enzyme digestion should take place if the optimum pH is reached.

The extrusion could be done in conditions similar to those described in the literature.[1, 48]

During the extrusion cooking, the free cyanide would be eliminated by the puffing of the product.

The extrusion cooking would also partially sterilize the product as shown in table 3.

Study on the development of low-cost exturders adapted to these operations could be realized.

Fish and meat preservation by lactic acid fermentation and drying:

Further studies on the use of vegetables or cereals and spices in mixture with fish or meat to hasten the acidification should be considered.

Vegetable preservation by lactic fermentation:

Study on the process, storage and packaging problems.

CONCLUSIONS AND RECOMMENDATIONS

Numerous lactic acid fermented foods exist around the world and concern most foods of animal and vegetable origin.

The process involves a single substrate (cereal, vegetable, legume, meat or fish) or a composite substrate (a blend of two or more substrates), liquid, semi-solid or solid state fermentation.

In respect to fermentation parameters used and desirable product properties to be attained, generally a limited number of microbial associations, including few species are involved.

Accordingly, regional or international network studies on model fermentation systems may help increase the efficiency of research activities and improve the use of available resources.

In Africa, the following research areas could have priority:

- Solid state lactic fermentation, extrusion cooking and drying for the production of instant cereal food and beverages, and precooked detoxified cassava products.
- Development of starter cultures for use at the household or artisanal level in the production of traditional food and beverages;
- Lactic acid fermentation and drying for the preservation of fish and meat;
- Low salt preservation of vegetables;
- Development of cassava detoxification process by using lactic acid fermentation and extrusion cooking.

All this research should be done in an interdisciplinary way in order to take into account not only the microbiological aspects but the social, economic, engineering and packaging problems.

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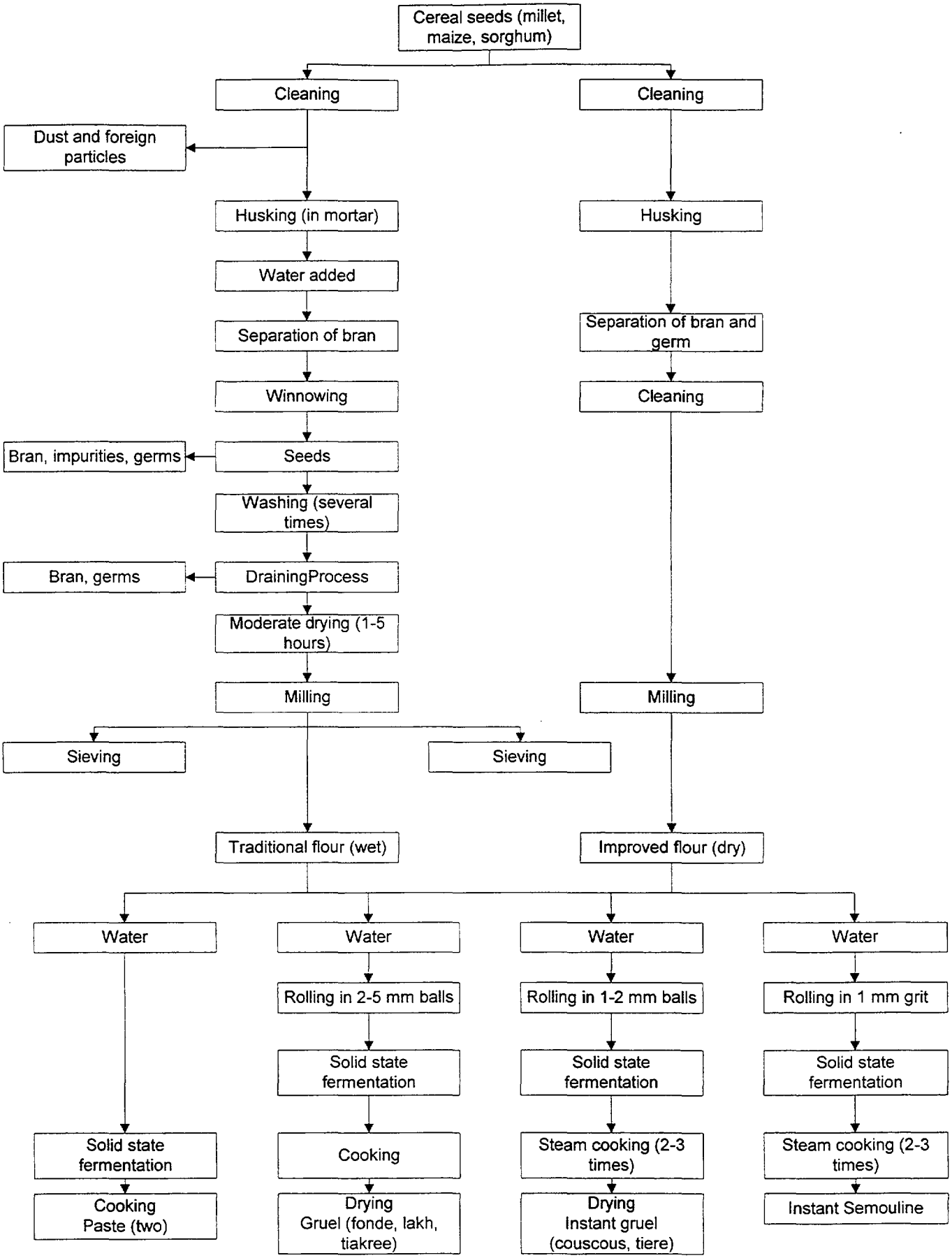

Figure 1: Traditional Senegalese foods from cereals (main process flow charts)

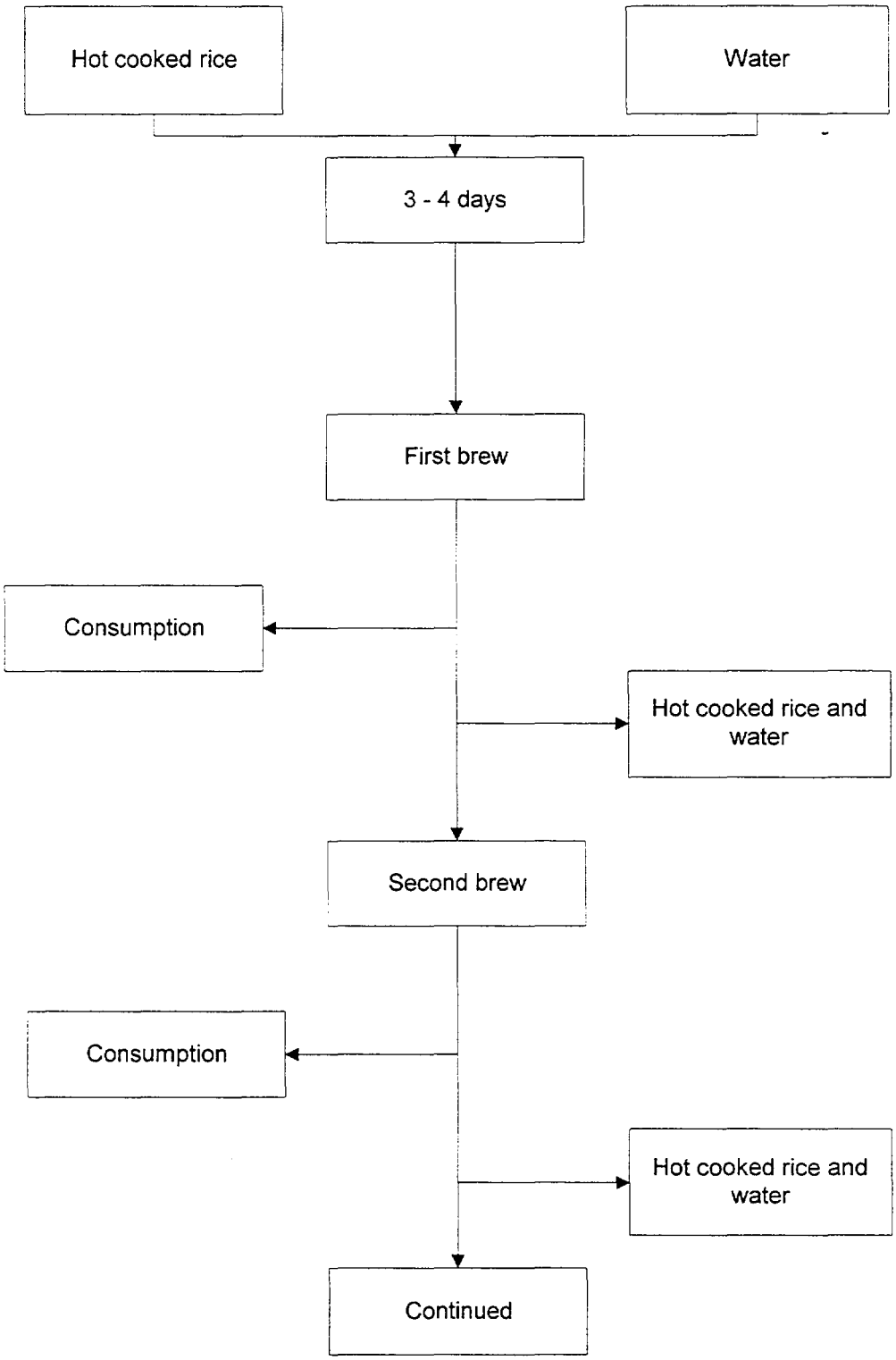

Figure 2: Traditional processing of Jangsu

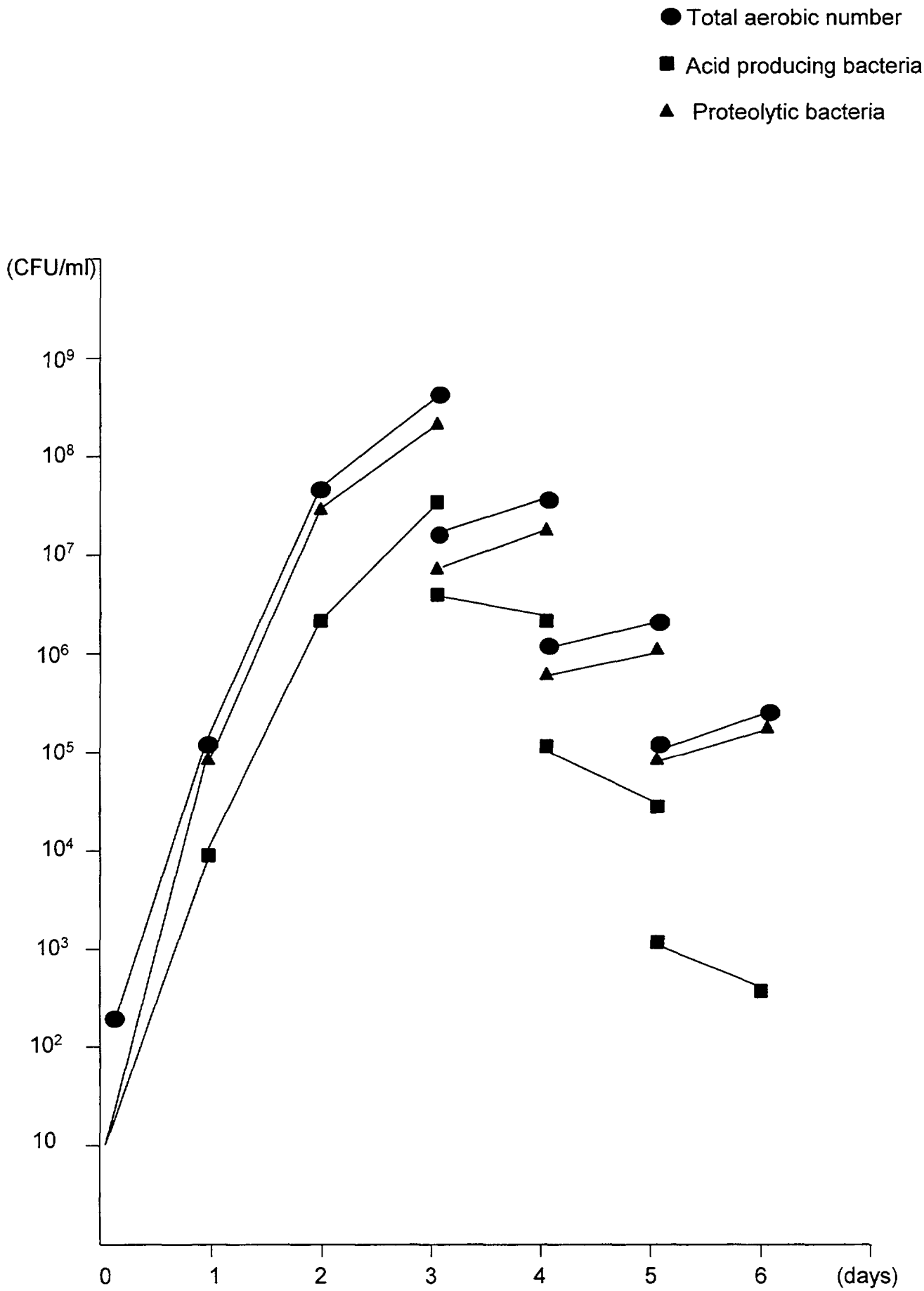

Figure 3: Changes in microflora during Jangsu fermentation (with periodical renewal of media)

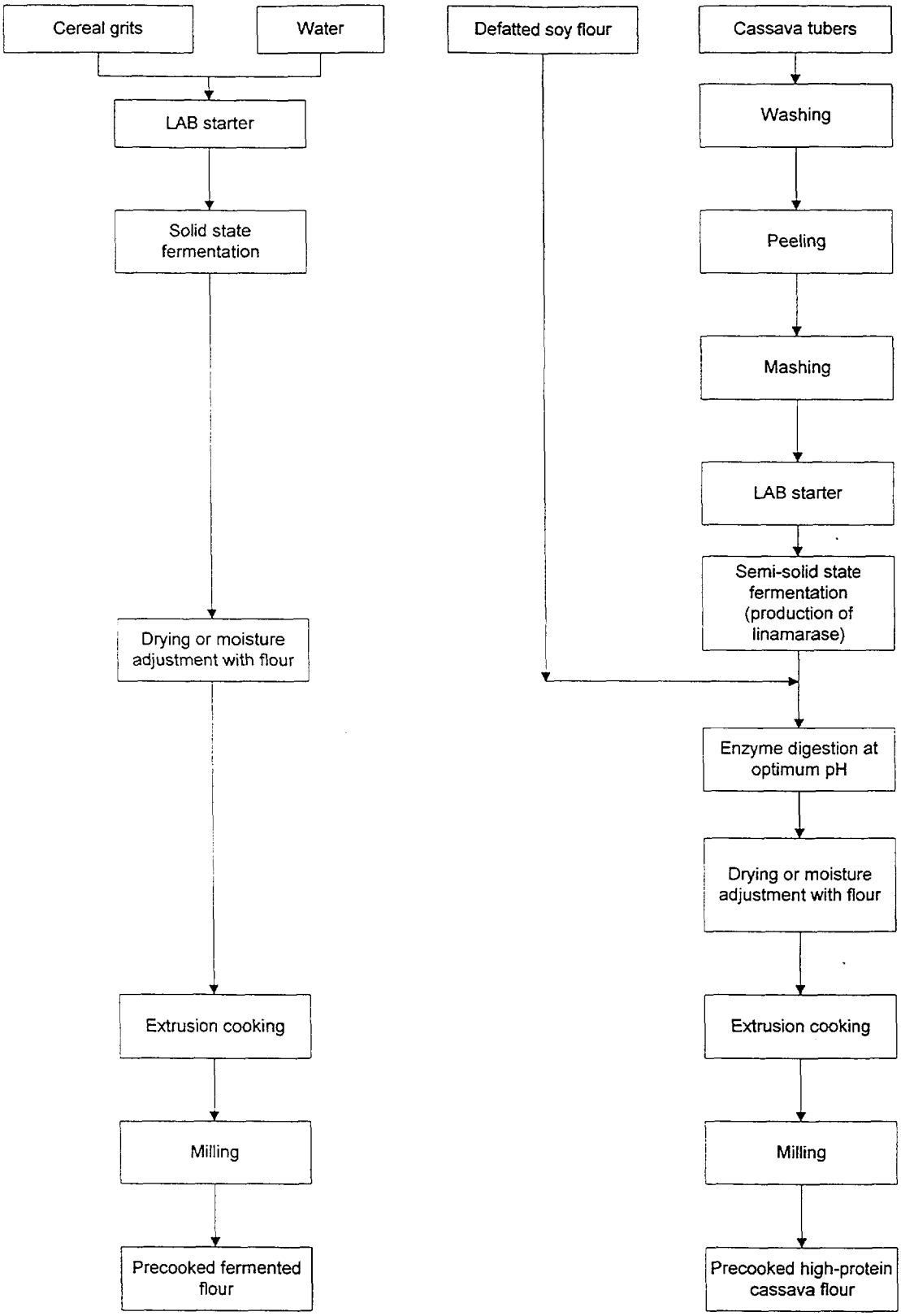

Figure 4: Optional process for production of precooked fermented cereal or cassava flour using a low-cost extruder

Table 1: Lactic fermented foods and beverages

Name	Area or country	Substrate	Microorganisms involved	Food use	References
Cereal alone					
Busa	Tartars of Crimea Turkestan	Rice or millet and sugar	*Lactobacillus* and *Saccharomyces*	Fermented drink	1
Bousa	Egypt	Wheat	Unknown	Thick acidic liquid with an alcoholic odour	1
Kenkey	Ghana	Maize	Unknown	Fermented steamed mush (sourdough)	1
Sorghum beer (Bantu beer, kaffir beer, leting, joala, utshival amqomboti, igwele)	South Africa	Maize, sorghum	Lactic acid bacteria,yeast	Thick acidic drink	1
Mahewu (Magou)	South Africa	Maize	*Lactobacillus delbrueckii* and other lactics	Sour, non-alcoholic beverage	1
Leting (low alcohol)	Basutos	(see sorghum beer)	Unknown	Acid beverage	1
Joala (high alcohol)	Basutos	(see sorghum beer)	Unknown	Acid beverage	1
Utshwala (high alcohol)	Zulu, Swaziland	(see sorghum beer)	Unknown	Acid beverage	1
Ting	Pedi (Basuto tribe)	Meal	Unknown	Porridge	1
Metogo	Pedi	Kaffir corn, maize and kaffir millet	Unknown	Non-intoxicating beverage	1
Mabjalwa	Pedi	Kaffir corn, maize and kaffir millet	Unknown	Beverage	1
Kaang-kopuwai (Kaanga-pirau, Koangawai)	Maori (New Zealand)	Maize	Other areas unknown	Gruel	1

Table 1: Lactic fermented foods and beverages (continued)

Name	Area or country	Substrate	Microorganisms involved	Food use	References
Braga	Romania	Millet	Unknown	Acidic alcoholic drink	1
Pozol	South eastern Mexico	Maize	Complex of moulds, yeast and bacteria	Fermented dough diluted with water is drunk as a basic food	1
Enjera	Ethiopia	Tef or other cereals	*Leuc. mesenteroides, P. cerevisiae, L. plantarum*	Pancake	5
Kisra	Sudan	Sorghum, millet	*Sacc. cerevisiae, Lactobacillus* sp.	Pancake	5
Uji	Kenya, Uganda, Tanzania	Maize, sorghum, millet	*Acetobacter* sp., *Leuc. mesenteroides, L. plantarum*	Sour porridge main meal	5, 38
Flour, cere (Senegalese couscous)	Senegal, Gambia	Corn, millet, rice	*Lactobacillus* spp., *Lactococcus* spp.	Steam cooked sour semole	1
Lakh, Fonde	Senegal, Gambia	Corn, millet, rice	*Leuc. mesenteroides*	Sour porridge and gruel	1
Jang-Su	Korea	Rice	*Lactobacillus, yeast* Sp., *Leuconostoc mesenteroides, Pediococcus* Sp., *Streptococcus thermophilus*	Sour solid particles in liquid menstrum	1

Table 1: Lactic fermented foods and beverages (continued)

Name	Area or country	Substrate	Microorganism involved	Food use	References
Burukytu	Savannah regions of Nigeria	Sorghum vulgare and cassava	Lactics and *Candida* sp.	Creamy liquid with suspended solids	
Tarbana	Turkey	Parboiled wheat meal and yoghurt (2 :1)	*Sacc. cerevisiae*, Lactics	Dried, used in soup	1
Kishk (Iranian name is Kushuk)	Egypt, Syria and Arab world	Wheat, milk	Lactic acid bacteria	Dried, balls; disolves rapidly in water	1
Khaman	India	Bengal gram	Unknown	Batter, steamed and seasoned	1
Rabdi	India	Maize and buttermilk	Unknown	Sour corn mush	1
Idli	India, Sri Lanka	Black gram and rice	*Leuconostoc mesenteroides, Lactobacillus delbrueckii, Lactobacillus lactis, Streprococcus lactis, Pediococcus cerevisiae, Streprococcus faecalis*, yeasts	Breakfast food	1
Cereal and starch or proteinaeeous supplement:					
Ambali	India	Ragi flour, rice and buttermilk	Unknown	Sour food	1

Table 1: Lactic fermented foods and beverages (continued)

Name	Area or country	Substrate	Microorganisms involved	Food use	References
Dosa	India	Black gram and rice	*Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lactobacillus fermenti*, *Streptococcus faecalis*, *Bacillus* spp., yeasts	Breakfast or snack food	1
Systems with lactic fermentation, secondary but important					
Sourdough bread	Western world	Wheat, rye meal and yoghurt (2 : 1)	*Lactobacillus* sp., yeast, lactics	Sourdough bread	1
Soy sauce (Sheyu)	Japan, China, Taiwan, USA	Soybeans and wheat	*Aspergillus oryzae*, *Saccharomyces rouxii*, *Pediococcus halophilus*, *Lactobacillus delbrueckii*	Seasoning	1
Kecap	Indonesia and vicinity	Soybeans and wheat	*Aspergillus oryzae*, *Lactobacillus* spp., *Hansenula* spp., *Saccharomyces* spp.	Flavouring agent	1

Table 2: Growth of *Bacillus laevolacticus* and *Saccharomyces* spp. on rice-soybean blend in solid state culture at 45^0C (viable cell count: c.f.u./g product) (1)

		Fermentation time				
Substrate	**Microorganisms**	**0 hours**	**6 hours**	**12 hours**	**18 hours**	**24 hours**
Rice flour	Total aerobic	$1.9x10^4$	$1.4x10^6$	$2.8x10^8$	$1.5x10^{10}$	$1.3x10^{10}$
	Bacillus laevolacticus	$3.0x10^3$	$3.0x10^5$	$2.9x10^8$	$1.5x10^8$	$1.3x10^8$
	Saccharomyces spp.	$1.0x10^3$	$3.0x10^5$	$7.0x10^6$	$1.0x10^6$	$1.0x10^6$
Rice-DSM	Total aerobic	$2.2x10^5$	$1.2x10^7$	$1.6x10^9$	$3.0x10^9$	$8.8x10^8$
	Bacillus laevolacticus	$1.6x10^5$	$2.4x10^6$	$5.6x10^8$	$3.0x10^9$	$8.8x10^8$
	Saccharomyces spp.	$1.0x10^3$	$3.0x10^5$	$6.0x10^7$	$1.0x10^7$	$3.0x10^7$

Table 3: Effects of extrusion temperature on microbial survival in pre-fermented rice-soybean blend (1)

(Colony forming units per gram of substrate)

Aerobic microflora	Non-extruded sample	Extrusion temperature			
		100^{0}C	**105^{0}C**	**110^{0}C**	**113^{0}C**
Total count	4x108	1.1x104	1.5x103	$6x10^2$	5x102
Lactic bacteria (*Streptococcus thermophilus*)	5.1x108	1.1x104	2.1x103	$4x10^2$	$<10^2$
Spore forming bacteria (*Bacillus*)	1x103	$3x10^2$	$2x10^2$	$<10^2$	$<10^2$
Yeast	3x106	$<10^2$	$<10^2$	$< 10^2$	$<10^2$

Extrusion conditions"

Substrate:	rice-soybean blend – 5/1 moisture content – 35 per cent pH – 4.2 particle size – < 1mm
Autogenous single screw extruder:	feeding rate – 150g/min screw speed – 300 rpm L/D ratio – 15 compression ratio – 3 : 1

Importance of Lactic Acid Bacteria in Non-Dairy Food Fermentation

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Abstract

Lactic acid fermentation is the oldest and most widespread food processing technology. Besides the well-known milk products, many indigenous food processing technologies of cereals, legumes, tubers, fruit, vegetables and fish in Asia and in Africa are based on acid fermentation utilizing various types of lactic acid bacteria. This paper reviews the classification of these organisms and their lactic acid producing characteristics. The importance of lactic acid bacteria is emphasized by highlighting the various indigenous food products made by the acid fermentation of cereals, tubers, legumes, vegetables, fish and meat in different regions of the world. The microorganisins involved in different products are examined, and the general trends of microbial pattern established in different types of foods are illustrated.

INTRODUCTION

Lactic acid fementation is probably one of the first biological processes from which human beings discovered the benefits of fermentation. The wide use of lactic acid fermentation technology throughout all regions of the world and the variety of raw materials used for the fermentation support this view. Lactic acid fermentation is important to Africans for their staple cereal food preparation, for Europeans, Arabians and Indians for the preservation of meat and dairy products, and to Asians for the preservation of vegetables and fish.

Lactic acid fermentation of milk products such as yoghurt and cheese has been extensively studied in Western countries, and is highly industrialized. Commercial production of lactic acid by fermentation at a high technological level has also been established.

However, few studies on the lactic acid fermentation of cereal and vegetable foods have been performed. Consequently, the level of industrialization of these products is very low, and they have mostly remained as low quality household level products.

Much of the indigenous acid fermented food processing techniques of Africa and Asia has been found to convert under-utilized food materials, including cereals, legumes, tubers, fruit and vegetables, into palatable food and beverages. This paper presents the status of non-dairy lactic acid fermentation in different regions of the world and the future prospects for food and beverage processing by using these indigeneous techniques.

Table 1: The Genera of Lactic Acid Bacteria, Type of Fermentation and Main Products Obtained[2]

Genus/Subgenus	Type of Fermentation	Main Products (Molar Ratio)	Configuration of Latic Acid
Streptococcus	homolactic	Lactate	L(+)
Pediococcus	homolactic	Lactate	DL and L(+)
Lactobacillus	homolatic	Lactate	
Thermobacterium	homolatic	Lactate	D(–), L(+), DL
Streptobacterium	heterolactic (facultative)	Lactate : Acetate (1 : 1)	
Betabacterium	heterolactic	Lactate : Acetate: CO_2 (1 : 1 : 1)	DL
Leuconostoc	heterolactic	Lactate : Acetate : CO_2 (1 : 1 : 1)	D(–)
Bifidobacterium	heterolactic	Lactate : Acetate	L(+)

The Microorganisms

The most important microorganisms for acid fermented foods belong to the family of Lactobacillaciae and this is differentiated into four genera; *Streptococcus, Pediococcus, Lactobacillus* and *Leuconostoc*. In addition, *Bifidobacterium*, which formerly also belonged to this family, has been proposed to be classified under the genus Actinomycetals. Bifidobacteria are practically anaerobic, however the other lactic acid bacteria can take up oxygen in limited amounts and are therfore called microaerophilic.[1]

Latic acid bacteria appear morphologically as diplococci, tetracocci, streptococci and as a rod, which may be presented singly or in chains. The type of fermentation and the configuration of lactate produced depends on the genera of the lactic acid bacteria, as shown in table 1.

Although the type of fermentation occuring depends on the presence of certain genera of lactic acid bacteria, a homolactic fermentation in some cases can be converted into a heterolactic one by changing the fermentation conditions. The metabolic pathways of glucose in lactic acid bacteria are proposed to be different, as shown in figure 1; glycolysis, bifidus-pathway and 6-P-gluconate-pathway.[2] The homolactic fermentation through glycolysis is represented by the equation: $C_6H_{12}O_6 \rightarrow 2CH_3\text{-}CHOH\text{-}COOH$.

However, a 100 per cent conversion is never attained. Various amounts of by-products, such as ethanol, acetic acid, formic acid, carbon dioxide and others may be formed. Andersson and Hedlund.[3] found a great variation in the acid profiles from 24 different cultivars of latic acid bacteria cultivated in MRS broth, as shown in table 2.

Fermentations with a yield of more than 80 per cent of the theoretical value of lactic acid are considered homolactic. In heterolactic fermentation the by-products and lactic acid are produced in about equal molar amounts.[2]

Table 2: Production of Organic Acid from 24 Isolates Cultivated in MRS Broth at 20 to 30 Days[3]

Acid	Lowest conc. (%)	Highest conc. (%)	Mean value (%)
Oxalic	0.23	0.93	0.52
Citric	0.06	0.41	0.12
Tartaric	–	–	–
Malic	0.02	0.06	0.04
Succinic	0	0	
Lactic	0.57	1.25	0.84
Formic	0	0	
Acetic	0.17	0.29	0.23
Propionic	0	0	
Butyric	0	0	

Lactic acid having an asymmetric carbon atom, occurs naturally in the dextrorotatory L(+)-form, the levorotatory D(–)-form or as the racemate. The formation of optically active and racemic lactic acids in nature can follow two different pathways, as shown in figure 2.

In animal and human cells L(+)-lactic acid is present and hence only the corresponding L-lactate dehydrogenase is found. For this reason the configuration of lactic acid is very important from the nutritional point of view. The intake of larger amounts of D(–)-lactic or DL-lactic acid can result in an enrichment of D(–)-lactic acid in the blood, and hyperacidity of urine may occur. These findings caused the WHO to limit human consumption of D–)-lactic acid to 100 mg/kg/d.[2]

Lactic acid bacteria are fastidious microorganisms. For normal growth apart from the carbon source, they need nitrogen, partly in the form of amino acids, several vitamins, growth substances and minerals. Since relatively small amounts of free lactic acid inhibit

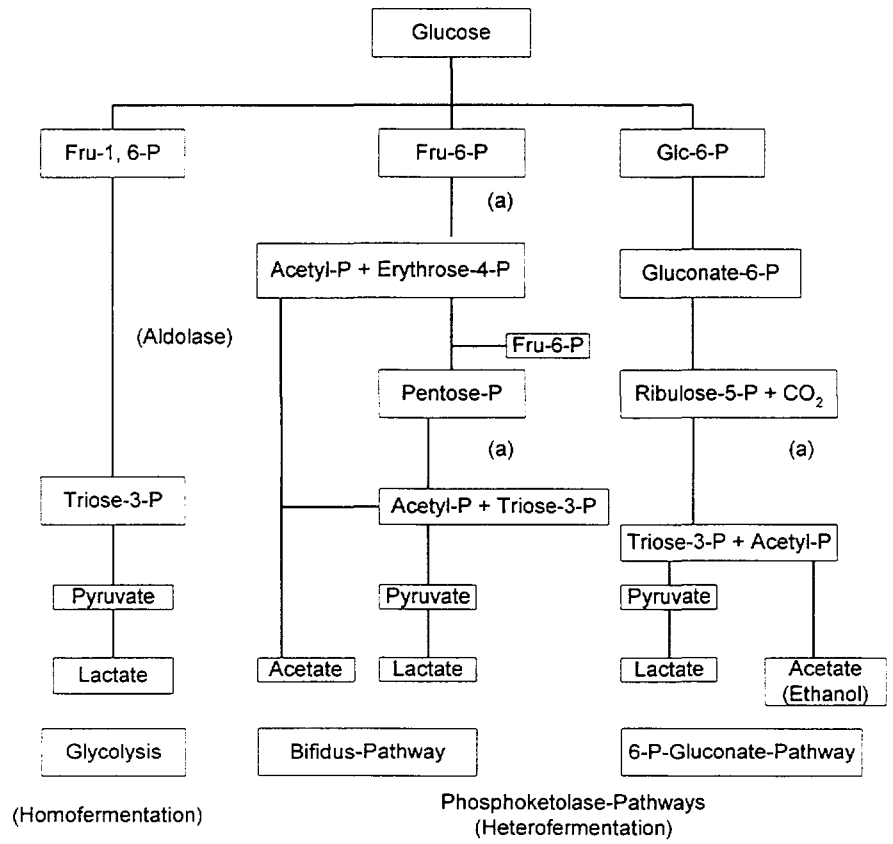


Figure 1: The different metabolic pathways of glucose in lactic acid bacteria – (a) Reaction catalyzed by phosphoketolase[2]

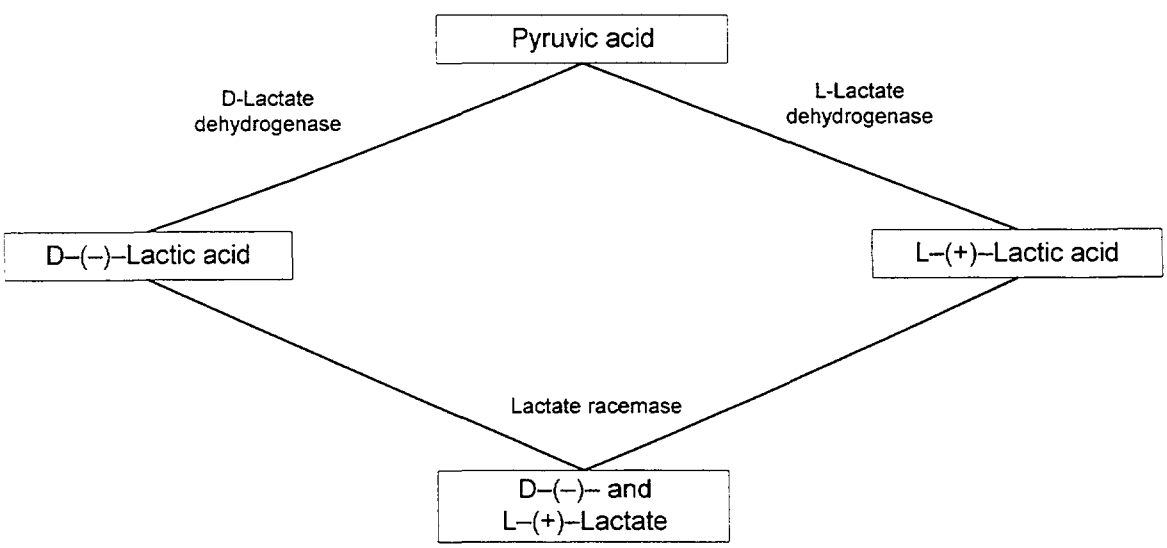


Figure 2: Mechanism of formation of optically active and racemic lactic acids

growth, the presence of a buffer is necessary to maintain the culture. The optimal pH range of lactic acid bacteria is between pH 5.5 and 6.0, but the minimum pH level for growth varies widely between species. Lactic acid bacteria are classified as thermophilic or mesophilic, and Table 3 shows the temperature optima for growth in the genus Lactobacillus.

Table 3: Temperature Optima for Growth in the Genus Lactobacillus[2]

	Homolactic Type				**Heterolactic Type**	
Thermobacterium		Streptobacterium			Betabacterium	
L.caucasicus		*L.casei*	28–32^{0}C	*L.brevis*	28–32^{0}C	mesophilic
L.lactis	37 – 45^0	*L.plantarium*		*L.buchneri*		
L.helveticus		*L.leichmannii*		*L.pastorianus*		
L.acidophilus						
L.bifidus				*L.fermenti*	35 – 40^{0}C	
L.bulgaricus	45 – 62^{0}C					thermophilic
L.thermophilus						
L.delbrueckii						

The Products

Lactic acid bacteria play an important role in many traditional food preparations in different regions of the world. Besides yoghurt and cheese, lactic acid fermentation is important for the preparation of sour-bread and pancakes, starchy porridges and soup bases, alcoholic or non-alcoholic drinks, vegetable pickles and for fish and meat preservation.

Steinkraus[4] classified the indigeneous lactic acid fermented products into acid-fermented vegetables, acid-leavened bread and pancakes, acid-fermented cereal gruels, and acid-fermented milk and milk/cereal foods. Besides these, lactic acid fermentation is important for the fermentation of traditional alcoholic beverages.

Acid-leavened bread and pancakes

Lactic fermentation of bread dough improves the keeping quality and flavour of the baked products. It also enhances the flavour acceptability of the bread made from low grade flours and under-utilized cereals. The traditional European sourdough breads, for example, the acid fermented bread of England using barm as the leavening agent, are largely replaced by the breads leavened with yeast only. The Danish rye bread is an exceptional case in Europe, in that acid-leavened bread is more popular than white yeast-only bread. Acid-fermented breads and pancakes are still important staple foods

for the people in Afirica and some parts of Asia. Table 4 shows some important sourdough bread and pancakes used in different regions.

Table 4: Examples of Acid-leavened Bread and Pancakes used in Different Regions of the World

Product name	Country of use	Major ingredients	Microorganisms	Appearance/ useage
Sourbread	Germany	Wheat	Lactic acid bacteria yeast	Sandwich bread
Ryebread	Denmark	Rye	Lactic acid bacteria	Sandwich bread
Idli	India Sri Lanka	Rice Black gram	*L.mesenteroides* *L.faecalis*	Steamed cake
Puto	Philippines	Rice	*L.mesenteroides* *L.faecalis* Yeast	Steamed cake
Kichudok	Rep. of Korea	Rice		Steamed cake
Injera	Ethiopia	Tef or other cereals	*L.mesenteroides* *P.cerevisiae* *L.plantarum* *S.cerevisiae*	Pancake
Kisra	Sudan	Sorghum millet	*Lactobacillus* sp. *Acetobacter* sp. *S.cerevisiae*	Pancake
Kishk	Egypt	Wheat plus milk	*L.casei* *L.brevis* *L.plantarum* *S.cerevisiae*	Pancake
Hopper	Sri Lanka	Rice plus coconut water	yeast Lactic acid bacteria	Steam-baked pancake

The Indian *idli* types (*idli, dosa, dhokla, khaman*) are important staple foods of Indian and Sri Lankan people, constituting their breakfast and supper, and these are consumed three or four times a week regardless of economic or social status. *Idli* is a small, white, acid leavened, and steamed cake made by bacterial fermentation of a thick batter made from rice and de-hulled black gram. Figure 3 shows that *Leuconostoc mesenteroides* and *Streptococcus faecalis* are responsible for the fermentation which reduces the pH from 6.5 to 4.7. The heterolactic *L. mesenteroides* is responsible for the production of CO_2 and acetic acid, besides lactic acid, which is also formed by *S. faecalis.*(5) Although *P. cerevisiae* appears in the 30 hour-old fermentation batter, it does not exert influence on the product because the batter is usually steamed within 24 hours of fermentation.

Similar products are made in the Philippines (*Puto*) and in the Republic of Korea (*Kichudok*), but they are made from rice only. *Puto* is specialized by using year-old rice and neutralizing the batter in the middle of fermentation.(6) As shown in figure 4, *L. mesenteroides* grow rapidly again by neutralization with NaOH, while yeast *S. cerevisiae* also contributes to the fermentation.

Injera is a fermented sour leavened pancake consumed as a staple food in Ethiopia. It is made from tef, corn, barley, millet sorghum or wheat. The flat loaves are usually 55 to 60

cms. in diameter and weigh between 250 and 700 g. Adults consume two or three *injera* per day, generally one at every meal with meat, vegetables, or legume stew.[7]

Figure 5 shows the rise and fall of microorganisms in *injera* fermentation.[8] *L.mesenteroides* and *S. faecalis* are the major organisms at the early stage of fermentation, but dominated by *P. cerevisiae, L. brevis* and *L. plantarum*, which reduce the pH down to 4.0 at the later stage. Yeast *S. cerevisiae* becomes the major organism at the end of the batter fermentation.

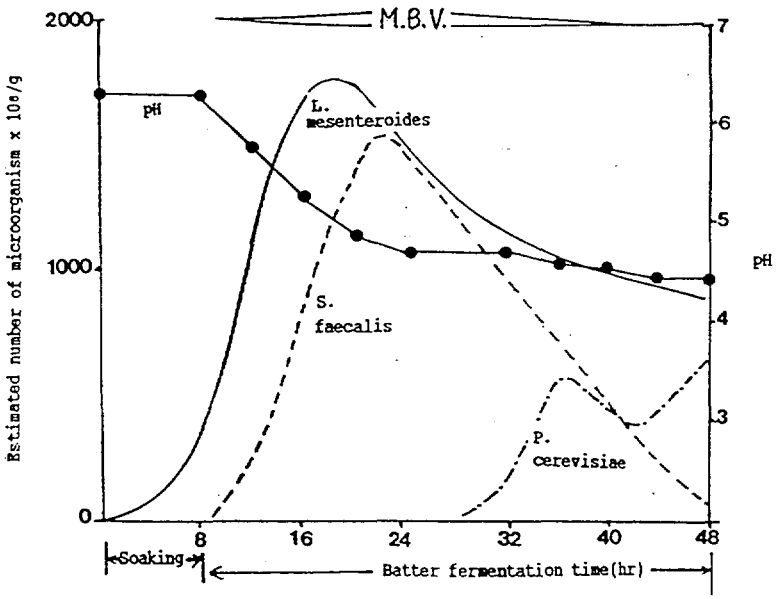
Similar products are found throughout the African continent with different raw materials and with different names. Sudanese *Kisra* is made from sorghum or millet and the major microorganisms are *Lactobacillus* sp., *Acetobacter* sp. and *S. cerevisiae.*[9] Egyptian *Kishk* is made from wheat and the major microorganisms are known to be *L. casei, L. plantarum, L. brevis* and yeast *S. cerevisiae.*[10] In Sri Lanka *hopper* is made from acid fermented dough of rice or wheat and coconut water. In *hopper* fermentation, a very large inoculum of baker's yeast or coconut toddy, which include acid producing bacteria, is added.[11]

Acid-fermented cereal gruels and beverages

Acid porridges prepared from cereals are eaten in various amounts in different parts of the world, particularly on the African continent, where it may represent the basic diet. Nigerian *ogi*, Kenyan *uji* and Ghanaian *kenkey* are examples of these porriges prepared by the acid fermentation of maize, sorghum, millet or cassava, followed by wet-milling, wet-sieving and boiling (table 5).

Table 5: Examples of Acid Fermented Cereal Gruels and Non-alcoholic Beerages Produced in Different Regions of the World

Product name	Country	Major ingredients	Microorganisms	Appearance/ usage
Ogi	Nigeria	Maize, sorghum or millet	*L.plantarum*, *Corynebacterium* sp., *Acetobacter* Yeast	Sour porridge, baby food, main meal
Uji	Kenya, Uganda, Tanzania	Maize, sorghum, millet or cassava flour	*L.mesenteroides, L.plantarum*	Sour porridge, main meal
Mahewu	S.Africa	Maize plus wheat flour	*S.lactis, Lactobacillus* sp.	Sour drink 8–10%
Hulu-mur	Sudan	Red sorghum	*Lactobacillus* sp.	Clear drink
Busa	Turkey	Rice, millet	*Lactobacillus* sp.	



M.B.V = Maximum Batter Volume

Figure 3: Bacteriological and pH changes in idli batter

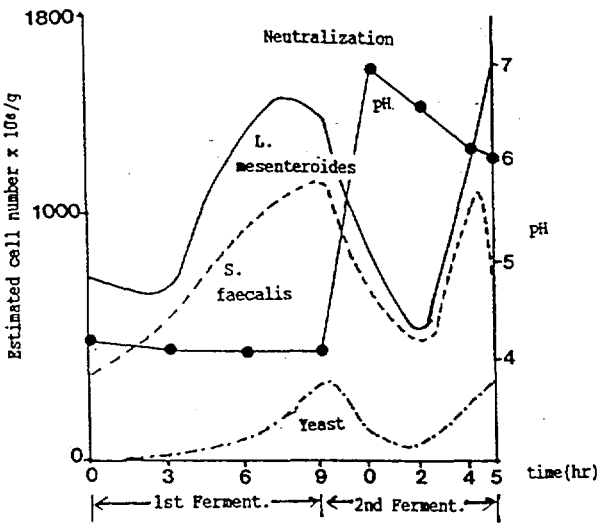


Figure 4: Microbiological and pH changes in puto fermentation

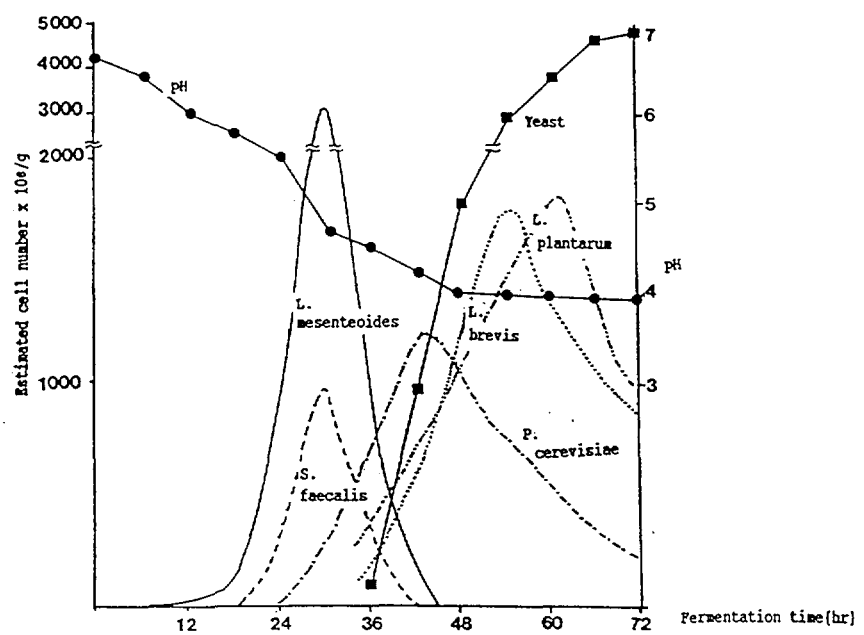


Figure 5: Microbiological and pH changes in tef injera fermentation

The processing of cereals into a porrige without fermentation results in bland-tasting materials. The main objective of fermentation is to develop an acceptable flavour similar to that of yoghurt.

Ogi is prepared from maize, sorghum or millet and consumed as a daily meal by Nigerians, and it is also an important weaning food for infants. The fermentation takes place without the intentional addition of any inoculum. The predominant organism in the fermentation is *L. plantarum* and *Corynebactetium* sp., *Acetobacter.* Yeast *S. cerevisiae* and *C. mycoderma* are also identified. *L. plantarum* produces the highest amount of acid, probably because it is able to utilize the dextrins of corn after the depletion of fermentable sugars. *Corynebacterium* is said to hydrolize the starch of maize to form organic acids, while *S. cerevisiae* and C. *mycoderma* contribute to flavour acceptability. Table 6 shows the organic acid composition of various types of *Ogi.* The highest preference test score was marked to the product containing 0.65 per cent lactic acid and 0.11 per cent acetic acid.

Table 6: Principal Organic Acids in Various Types of *Ogi**

***Ogi* type**	**Lactic acid (%)**	**Acetic acid (%)**	**Butyric acid (%)**	**Ratio of acetic : lactic**
Maize	1.06	0.18	0.01	0.18
Sorghum	1.18	0.21	0.04	0.19
Millet	3.53	0.21	0.02	0.06

*Averages of two or three samples, except butyric acid.

Uji is an important component for both breakfast and lunch, as well as a weaning food for the people of Kenya, Uganda and Tanzania. It is made by suspending maize, millet, sorghum or cassava flour in water, either fermented before or after cooking.[13] In a typical spontaneous *Uji* fermentation, the *coli* formed dominate the fermentation for the first 16 to 24 hours. Then the lactobacilli become dominant producing sufficient acid to cause a decrease in the numbers of coliform. If *Uji* is inoculated with *L. mesenteroides,* the undesirable coliforms are restricted from the start. Also, inoculation with mixed lactobacilli isolated from the late stage of *Uji* fermentation results in very rapid acid production with inhibition of coliforms. It was reported that *L. plantarum* accounted for 72 per cent of the total lactobacilli in fermenting *Uji.* During fermentation the pH falls to between 3.5 and 4.0 in 32-40 hours, and the total acid reaches from 0.55 to 0.62 per cent.

Mahewu is a traditional sour, non-alcoholic maize beverage popular among the Bantu people of South Africa,[14] while *hulu-mur* and *abre* are Sudanese lactic beverages made from sorghum flour, malt and spices.[15]

Sweet/sour alcoholic fermented porridges, which are fermented further to alcohol formation, are also utilized by many people as their staple food. Kenyan *Busaa,* Egyptian *Bouza,* South African *Kaffir* or Bantu beer, Sudanese *Merrisa,* Korean *Takju,* Philippino *Tapuy* and Indonesian *Tape Kekan* are the examples.[4] Lactic acid bacteria play an important role in securing the supression of the growth of putrefactive and

harmful microorganisms at the initial stage of these alcoholic fermentations. However, the growth of lactic acid bacteria is soon supressed by the alcohol from yeast.

Acid-fermented starch ingredients

Food starches with extended shelf-life, having resistance to infectious microorganisms and acceptable flavour, are produced traditionally by acid-fermentation in different regions of the world. Nigerian *Gari,* Ethiopian *Kocho*, Chinese mung bean starch and Mexican *Pozol* are important acid fermented starch ingredients used for the preparation of various dishes, such as porridge, steamed cake, paste, noodles, soups and beverages (table 7).

Table 7: Examples of Acid-fermented Starch Ingredients Produced in Different Regions of the World

Product name	Country	Major ingredients	Microorganisms	Usage
Gari	Nigeria	Cassava	*Leuconostoc Alcaligenes Corynebacterium, Lactobacillus*	Staple/cake, porridge
Mungbean starch	China, Thailand, Rep. of Korea, Japan	Mung-bean	*L.mesenteroides L.casei L.cellobiosus L.fermenti*	Noodles
Khanom jeen	Thailand	Rice	*Lactobacillus* sp. *Streptococcus* sp.	Noodles
Pozol	Mexico	Maize	Lactic acid bacteria *Candida*, moulds	Porridge
Me	Viet Nam	Rice	Lactic acid bacteria	Sour food ingredient

Gari is a granular starchy food made from cassava by fermenting the grated pulp, followed by semidextrinizing, drying and grading. About 10 million tons of *gari* are produced per annum in the southern part of Nigeria. Substantial quantities of *gari* are also produced along the coastal regions of West Africa. *Leuconostoc, Alcaligenes, Corynebactetium, Lactobacillus* and *Candida* were isolated, and *Leuconostoc* sp. appeared to be the dominating organism, as shown in Figure 6.[16] Among the microorganisms isolated, *L. plantarum* produced the most *gari*-like flavour.[17] The raw cassava has pH of 6.2 and is reduced to pH 4.0 after fermentation.

Most of the countries in Asia produce mung bean starch, which is used in making noodles, and is staple in the diet of the Chinese. The process for manufacturing mung bean starch involves an acidic bacterial fermentation.[18] The mung beans are hydrated by soaking in water inoculated with a 12-hour steep water from a previous fermentation to ensure acidification of the beans. The principal microorganisms found in steep-water are *L. mesenteroides, L. casei, L. collobiosus* and *L. fermentum.* The lactic fermentation which reduces the pH to about 4.0 protects granules from spoilage and putrefaction that would otherwise occur in ground bean slurries.

Thai rice-noodles, *Khanom Jeen*, is also made from acid fermented raw rice.[19] Soaked rice is drained and fermented for at least three days before grinding, and *Lactobacillus* sp. and *Streptococcus* sp. are involved in the acid fermentation.

The microbial composition of *Pozol*, a Mexican starch food ingredient, is quite different from *Gari* and mung bean starch. *Geotrichum candidum, Trichosporon cutaneum* and various species of Candida are always associated with *Pozol*, and moulds such as *Cladosporium cladosporioides, Monila sitophila* and *Mucor rouxianus* are also common in Pozol.[20] During the first stages of fermentation, bacteria outnumber the yeasts and mould, and are probably responsible for most of the acid production during the first 24 hours. During this time, the pH drops from 7.5 to 5.0, and reaches pH 3.9 on the eighth day of fermentation. The essential changes that occur in the maize dough during *Pozol* fermentation are the development of acid flavour and a characteristic aroma that gives *Pozol* its refreshing properties when ingested.

Acid-fermented vegetables

Extensive studies on microbiological and biochemical changes of *sauerkraut* fermentation have been reported (table 8). The fermentation of sauerkraut by a sequence of flora has been confirmed. The earliest stages of the fermentation are dominated by *L. mesenteroides* and completed by *Lactobacillus brevis* and *L. plantarum.*[21] In abnormally high temperatures or salt concentrations, two other species *Streptococcus faecalis and Pediococcus cerevisiae* are also involved. A similar microbial pattern is also found in *Kimchi*, as shown in figure 7.[22]

The difference between *sauerkraut* and *Kimchi* is the preferred end points of fermentation. The best taste of *Kimchi* is attained before the domination of *L. brevis* and *L. plantarum* with the optimum product pH of 4.5. The domination of *L. brevis* and *L. plantarum* deteriorates the product quality. The differences of the two fermentations is manipulated by salt concentration and temperature. The optimum range of salt concentration in *sauerkraut* is 0.7 to 3.0 per cent, while that of *Kimchi* is 3.0 to 5.0 per cent.

As shown in Table 9, fermentation is very slow, at a low temperature of 7.5^0C.[4] *L. mesenteroides* grows slowly, attaining an acidity of about 0.4 per cent in about ten days and an acidity of 0.8 to 0.9 per cent acid in a month. The genera *Lactobacillus* and *Pediococcus* cannot grow well at this low temperature. *Kimchi* has traditionally been produced in winter, and therefore *L. mesenteroides* is the responsible microorganism for this product. On the other hand, for *sauerkraut*, *L. mesenteroides* function as a spearhead for the subsequent growth of *Lactobacillus* and *Pediococcus.*

L. mesenteroides has been found to be important in initiating the fermentation of many vegetables, i.e. cabbages, beets, turnips, cauliflowers, green beans, sliced green tomatoes, cucumbers, olives and sugar beet silage. *L. mesenteroides* initiates growth in vegetables more rapidly over a wide range of temperatures and salt concentrations than any other lactic acid bacterium. It produces carbon dioxide and acids that quickly lower the pH, thereby inhibiting the development of undesirable microorganisms and the activity of their enzymes, which may soften the vegetables. The carbon dioxide produced replaces air and provides an anaerobic condition favourable for the stabilization of

the ascorbic acid and the natural colour of the vegetable. The growth of this species modifies the environment, making it favourable for the growth of other lactic acid bacteria in the bacterial sequence. The high acidity produced by itself and other subsequently growing lactic acid bacteria, inhibits the growth of L *mesenteroides*. *L. mesenteroides* ferment glucose to about 45 per cent levorotatory D-lactic acid, 25 per cent carbon dioxide, and 25 per cent acetic acid and ethylalcohol. Fructose is partially reduced to mannitol and is more readily fermented to yield equimolecular quantities of lactic acid and acetic acid. The combination of acids and alcohol are conducive to the foliation of esters that imparts desirable flavours.

Table 9: Effect of Temperature on the Microbial Growth in *Sauerkraut*

Temperature	Days	Acidity (%)	Responsible microorganism
7.5^0C	30	0.8–0.9	*L.leuconostoc*
18^0C	20	1.7–2.3	*L.leuconostoc* *L.brevis*
23^0C	10	1.0–1.5	*L.brevis* *L.plantarum*
32^0C	8	1.8–2.0	*L.plantarum* *P.cerevisiae*

While the importance of *L. mesenteroides* has been stressed in *Kimchi* fermentation, the roles of other lactic acid producing species in sequence, *L. brevis, P. cerevisiae* and *L. plantarum* are also important to other acid fermented vegetables such as sauerkraut. *L. brevis* is heterofermentative, producing DL-lactic acid and gas from glucose and fructose. *L plantarum* is homofermentative, and is the highest acid producing species of this group, yielding three or four times more DL-lactic acid than *Leuconostocs*. *P. cerevisiae* ferments sugars to the inactive DL-form of lactic acid, and up to 95 per cent of the sugar fermented may be recovered as lactic acid and will produce about twice as much titratable acid as the *Leuconostocs*.

Acid-fermented fish and meat

The storage life of perishable fish and meats has been extended by acid-fermentation with added carbohydrates and salts. Rice, millet, flour and even syrup or sugar, are used as the carbohydrate source. Table 10 shows the acid fermented fish and meat products of different countries.(23) The amount of added carbohydrate and salt concentration primarily control the extent of acid fermentation and the keeping quality. Figure 8 shows the microbial and biochemical changes of a typical lactic fermented fish product incubated at 20^0C. The pH decreases rapidly during the first three to five days from 6.5 to below 5.0 and softening of the texture takes place three to four days after fermentation. The amino-N concentration increases steadily up to 14 days and this coincides with the attainment of the optimum taste. The number of lipolytic bacteria decreases rapidly during the initial stage of fermentation and the proteolytic bacteria increases until 12 days of fermentation and thereafter decrease rapidly. The acid forming bacteria increase rapidly and become the dominating microorganisms in one week of the fermentation and reaches its maximum at 16 days of fermentation. In this case, the taste deterioration is associated with the maximum growth of yeast and acid forming bacteria.(24)

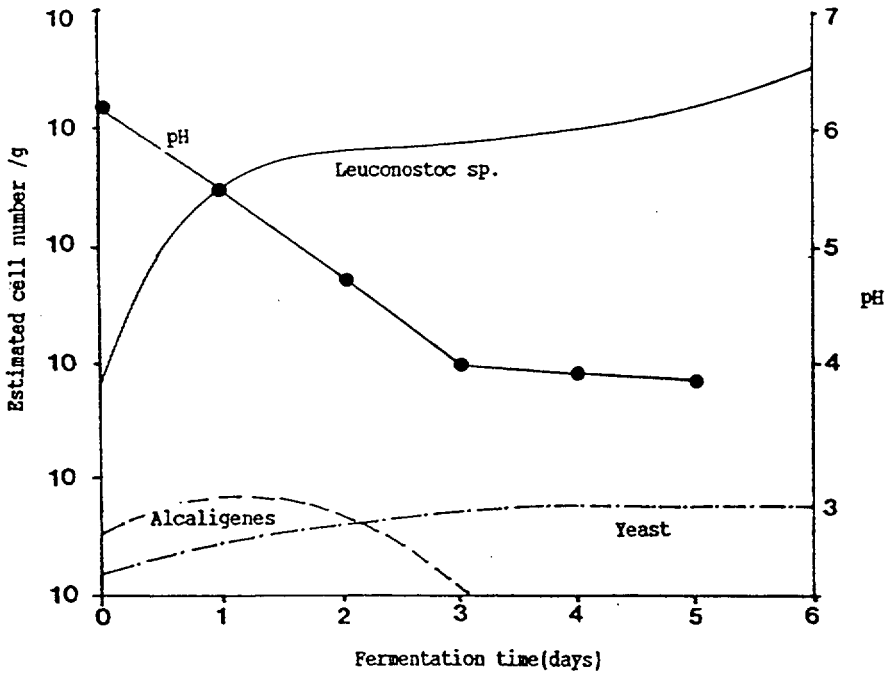


Figure 6: Microbiological and pH changes in gari fermentation

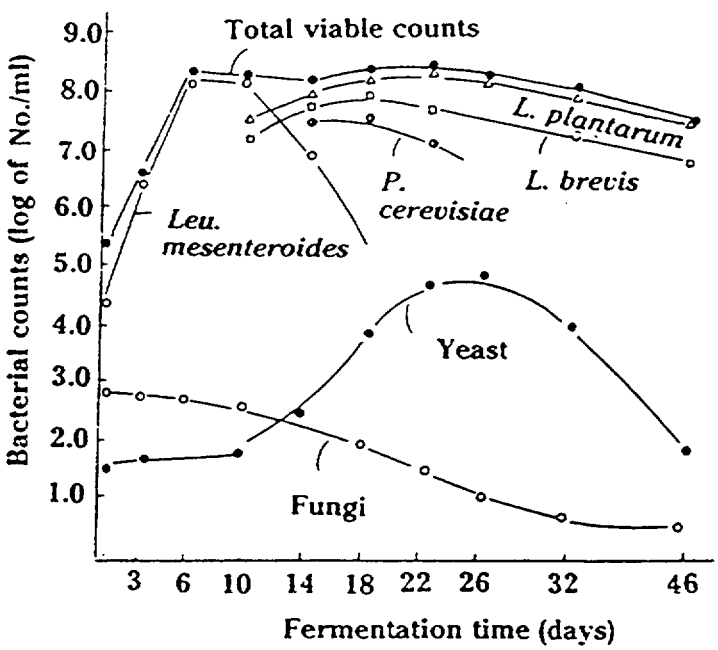


Figure 7: Changes in microflora during kimchi fermentation at 14^0C (3.5% NaCl)

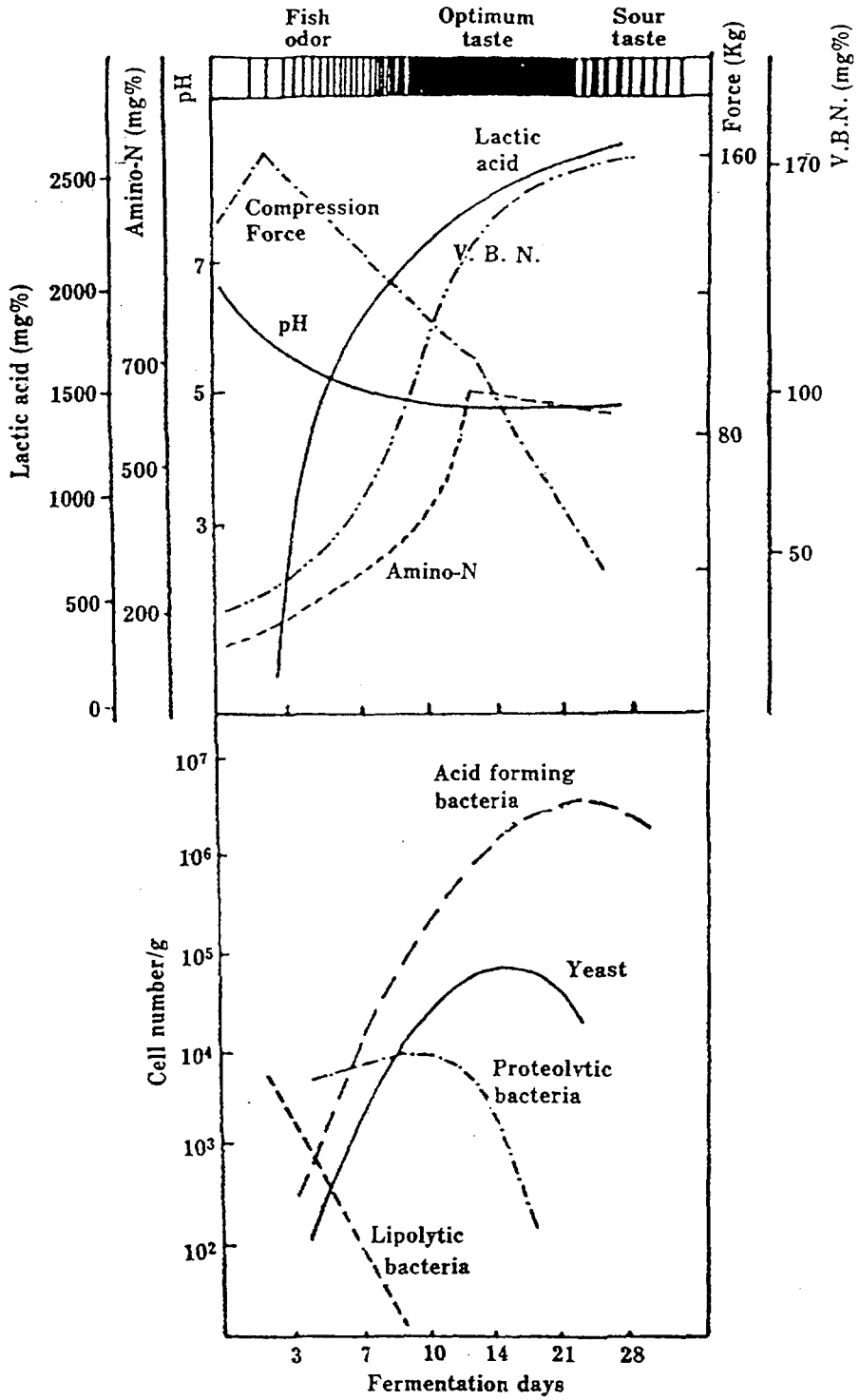


Figure 8: Microbial and biochemical changes during the fermentation of a lactic fermented fish product – sikhae

Table 10: Examples of Acid-fermented Seafood, Cereal and Meat Mixtures

Product name	Country	Major ingredient	Microorganism	Usage
Sikhane	Korea	Seawater fish, cooked millet, salt	*L.mesenteroides* *L.plantarum*	Side dish
Narezushi	Japan	Seawater fish, cooked millet, salt	*L.mesenteroides* *L.plantarum*	Side dish
Burongisda	Philippines	Freshwater fish, rice, salt	*L.brevis,* *Streptococcus* sp.	Side dish
Plara	Thailand	Freshwater fish, salt, roasted rice	*Pediococcus* sp.	Side dish
Balao-balao	Philippines	Shrimp, rice, salt	*L.mesenteroides* *P. cerevisiae*	Condiment
Kungchao	Thailand	Shrimp/salt, sweetened rice	*P.cerevisiae*	Side dish
Nham	Thailand	Pork, garlic, salt, rice	*P.cerevisiae* *L.plantarum* *L.brevis*	Pork meat in banana leaves
Sai krok prie	Thailand	Pork, rice, garlic, salt	*L.plantarum* *L.salivarius* *P.pentosaccus*	Sausage
Nemchua	Viet Nam	Pork, salt, cooked rice	*Pediococcus* sp. *Lactobacillus* sp.	Sausage
Salami	Europe	Pork	*Lactobacillus* *Micrococci*	Sausage

The important bacteria for the lactic fermentation are identified as *Leuconostoc mesenteroides* and *Lactobacillus plantarum.*(25) The role of these acid forming bacteria for the preservation of fish is apparent, but a more important aspect is their ability to produce acceptable flavour during the fermentation.

Fermented pork, *Nham*, is a popular food in Thailand. It consists of fresh pork meat that is trimmed, minced, mixed thoroughly with salt, rice and seasoning, and traditionally wrapped into small banana leaf packets. Similar to Western fermented sausages, like salami, *Pediococcus* sp. *(P. cerevisiae)* are the main microorganism, while *Lactobacillus plantarum* and *L. brevis* have also been identified.(26)

CONCLUSION

The microbial patterns of different types of non-dairy acid fermented foods illustrate that there is a general trend of sequence for the microbial growth and fall. *Leuconostoc mesenteroides* seems to be a spearhead in the series of most cereal and vegetable fermentations where free sugars are available. The heterofermentative production of acetic acid and CO_2, besides lactic acid, develops characteristic flavour and leavening action. In many products, for example *Kimchi*, cereal fermentation starters, both alcoholic or non-alcoholic and some sourdough products, termination of fermentation at the stage of the maximum growth of *L. mesenteroides* is desired. The rapid growth at neutral pH and rapid acid production of *L. mesenteroides* provide a suitable environment for the growth of *Streptococcus, Pediococcus* and *Lactobacillus.* In many of the starch-rice

substrates, like cereal dough, *Streptococcus faecalis* grow concomittantly with *L. mesenteroides,* followed by *Pediococcus cerevisiae.* In the case of vegetables, *Lactobacillus brevis* and *L. plantarum* appear after *L. mesenteroides,* and it inhibits the growth of the latter by producing more acid at a low pH. The growth of *Lactobacillus* is suppressed by yeast in some cases, such as alcoholic fermentation and fish fermentation. The subsequent growth of yeast is desired in sourdough preparation and alcoholic fermentation, but in many cases it is detrimental for the product quality. The domination of yeast in fermented fish products results in a serious deterioration of product quality.

The microbiological characteristic of non-dairy lactic acid fermentation among the others is the importance of *L. mesenteroides* as the spearhead infantry of the microbial troop. The use of *L. mesenteroides* as a starter and control of subsequent growth of other species of microorganisms can improve the product quality of many indigenous fermented foods. The controlled growth of *L. mesenteroides* and other microorganismsm, such as *Streptococcus, Pediococcus* and *Lactobacillus,* in sequence, will be another type of fermentation technique for non-dairy lactic acid fermented foods.

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Food Fermentation – Traditional Low-Cost Food Preservation Systems

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INTRODUCTION

Lesser developed countries require food processing technologies that are technologically appropriate, suitable for tropical regions and that are affordable in rural and urban economies. Fermentation is one such technology that has been developed indigenously for a wide range of food commodities. These include cereals and legumes, root crops, fruit and vegetables, dairy products, fish and meat. This paper considers the advantages of fermentation as a processing operation in developing countries, gives examples of food fermentations that demonstrate these advantages, considers the role of fermentation technologies and fermented foods in development, and examines recent developments in biotechnology that can facilitate the wider application of these technologies.

The term fermentation is often used with imprecision when referring to foods (Adams, 1990). Strictly, it describes the type of energy yielding anaerobic metabolism in which an organic substrate is incompletely oxidised and an organic compound acts as an electron acceptor; examples are the production of ethanol by yeast and production of organic acids by lactic acid bacteria. However, in more general use the term fermentation is applied to any foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification of the food (Campbell-Platt, 1987). Foods such as *tempe,* involving mould growth, and *dawadawa,* involving growth of the *Bacillus* spp., are therefore considered as fermented foods, although the metabolism of the organisms involved may not be strictly fermentative. Several excellent reviews exist which describe and catalogue the production and use of fermented foods worldwide (Steinkraus, 1983; 1989; Wood, 1985; Campbell-Platt, 1987; Lee, 1991).

Advantages of fermentation

As a unit operation in food processing, fermentation offers a large number of advantages. These are: food preservation, improved food safety, enhanced flavour and acceptability, increased variety in the diet, improved nutritional value, reduction in anti-nutritional compounds and in some cases improved functional properties. Each of these advantages will be discussed, together with relevant examples. Often, a particular type of fermentation offers more than one advantage and the advantage under which it is discussed may not be the primary reason for carrying out the fermentation; for example, the lactic fermentation of fish may offer a means of preservation, but it also imparts desirable flavours and aroma to the products.

Low-cost preservation techniques

Lactic fermentation is a common means of preservation in tropical countries (Cooke *et al.*, 1987). Preservation techniques such as refrigeration, freezing, canning or modified atmosphere packaging are expensive, particularly in communities with low levels of disposable incomes; and limited infrastructure available in the food processing industry greatly restricts their use in developing countries. The high ambient temperatures of the tropics heighten the need for low-cost preservation methods such as salting, drying and traditional fermentations.

There is a wide range of perishable substrates that rely on lactic acid fermentation as a means of preservation. These include meat, *(nham* from Thailand, *chorizo* from Mexico), fish *(burong isda* from the Philippines; *pla-som* and *som-fak* from Thailand), leafy vegetables *(kimchi* from Korea) and cassava *(akpu* from Nigeria). Cereals and legumes can also be lactic fermented but preservation is not the primary reason for fermentation.

The dominance of lactic acid bacteria in food fermentations is a consequence of their ability to proliferate in a carbohydate-rich anaerobic environment and their rapid suppression of food spoilage and pathogenic bacteria. The ability of lactic acid bacteria to suppress growth of other microorganisms can result from organic acid production, with a reduction in pH of the food substrate, hydrogen peroxide production, carbon dioxide production, nutrient depletion or production of antibiotic-like substances (Andersson, 1986).

Lactic fermented seafoods are an example of fermentation as a low-cost preservation technique. Fish are highly perishable proteinaceous foods which spoil rapidly when exposed to tropical ambient temperatures. The keeping time of fresh fish which are stored under these conditions is less than one day (Barile *et al.*, 1985 a, b). In many developing countries, particularly in rural coastal areas, usual techniques for extending the shelf-life of fish such as chilling, freezing and canning are too expensive for domestic markets. In many poorer communities, fish processing depends entirely on traditional processing techniques which are low-cost and rely upon the reduction of water activity (salting, drying and smoking) or a fermentation process. Traditional fermented fishery products are popular in South-east Asia and are reviewed by Lee *et al.*, (1991).

The term fermented fish commonly applies to two categories of products (Adams *et al.*, 1985); fish and salt formulations such as fish sauce; and fish, salt and carbohydrate mixtures, which involve a lactic fermentation. These products are not well defined and there are many variations in processing methods and raw material used (Lee, 1990). Manufacture of fish sauce occurs on a commercial scale and although these products could be described as fish hydrolysates, bacteria which can tolerate high salt concentrations seem to be involved in flavour and aroma development (Thonghtai *et al.*, 1990).

In a second category of products, lactic acid fermentation occurs and contributes to an extended shelf-life. There are many examples of this type of product where the storage life at ambient tropical temperatures is extended by a combination of increased acidity and the addition of salt (Lee *et al.*, 1991).

The lactic fermentation involves a succession of bacteria where lactic streptococci and leuconostocs dominate in the early stages (one to three days). These eventually give way to homofermenters (pediococci and lactobacilli) which are more acid tolerant (three to ten days) (Solidum and Acedevo, 1983). The heterofermenters produce both acetic and lactic acids. Production of acetic acid, which has a greater antimicrobial effect (Adams 1990), is important in the early stages of fermentation to ensure the inhibition of competing spoilage on pathogenic bacteria. Lactic fermented seafoods are produced mainly by women on a household scale as an income-generating activity. They are consumed as main courses, rather than as condiments or sidedishes, as is the role of fish sauces and fish paste.

Improved food safety

Food safety is an important issue in developing countries. Processing techniques that ensure food safety are required at both the rural and urban levels, particularly in view of the frequently poor sanitary conditions and high ambient temperatures. In addition to its role as a food preservation technique, lactic fermentation can render foods safer to eat. Lactic acid bacteria have been reported to inhibit the growth of foodborne pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *Staphyloccus aureus* (Nout *et al.*, 1989; Mbugua and Njenga, 1992) in a range of fermented foods used for weaning infants.

It was estimated that based on 1980 data, 4.6 million deaths of children under the age of five years occurred in developing countries as a result of acute diarrhoeal disease. In the light of this, weaning foods have been a particular focus of attention for research on the beneficial effects of fermentation (Mensah *et al.*, 1991, Nout *et al.*, 1989). Lorri and Svanberg (1992) reported on the use of fermented weaning gruels in villages in Tanzania for reducing the incidence of diarrhoea in children. Alnwick *et al.*, (1988) suggests that there is strong experimental evidence that lactic fermentation inhibits the proliferation of bacterial pathogens, extending the time during which the lactic fermented foods can be safely stored.

Improved flavour, variety and acceptability

Fermentation is well known for its ability to improve the flavour and acceptability of food commodities in developed countries, for example the production of wine from grape juice, cheese and yoghurt from milk. Similar improvements have been reported for food commodities in developing countries where bland raw materials are turned into foods with enhanced flavour. With the imparting of flavour, it is also possible to introduce variety into the diet.

For instance, cassava, which is an important staple root crop for many people in sub-Saharan Africa, stores poorly after harvest and has to be processed into a form which can be stored. Many different fermented foods can be produced and hence fermentation serves as a means of introducing variety into the diet. The Natural Resources Institute (NRI) in the United Kingdom has contributed to the recent collaborative study of cassava in Africa (COSCA), which is a study of all aspects of cassava production and processing in six African countries (Nweke, 1990). Information obtained at village level (233 villages) on the classification of 488 processed cassava products showed that more than 90 per cent were fermented. This shows the importance of fermentation as a processing method of cassava in Africa. Figure 1 shows how fermented cassava foods can be divided into a number of different classes based upon the primary method of fermentation (Westby, 1991).

Grated acid fermented products include roasted granules such as West African *gari*, steamed products such as Ivorian *attieke* and Ghanian *yakayaki,* and fermented pastes such as Ghanian *agbelima.* Products prepared by soaking roots in water include acidic pastes such as Zairian *chickwangue,* Nigerian *fu-fu* and *akpu,* and dried products such as Nigerian *lafun.* Air fermented cassava products, where fresh cassava can be covered with leaves for a number of days to encourage mould to grow prior to sun drying, include products such as dark moulded cassava flour (Essers and Nout, 1989) and Tanzanian *ugada.* The other class of air fermented products become mouldy as a result of long drying times and include products such as Ghanian and Ivorian *kokonte.*

Reduction in anti-nutritional factors

Anti-nutritional factors are of concern with the consumption of certain foods, for example lactose in milk, cyanogenic glucosides in cassava, phytate in cereals and legumes, and oligosacharides in legumes. Fermentation has been reported to be responsible for improving nutritional quality by increasing food palatability and digestibility or increasing nutrient availability by destroying anti-nutritional factors or minimizing their effects.(Adams, 1990; Liew and Buckle, 1990).

Although fermentation has been associated with the removal of anti-nutritional factors, there are examples of food where fermentation is not directly responsible for the observed phenomenon. The hydrolysis of cyanogenic glucosides during the processing of grated cassava products has been attributed to fermentation. Recent work at NRI has shown that the release of an endogenous enzyme during grating is responsible for the

hydrolysis of the glucoside (Vasconcelos *et al.*, 1990). The level of endogenous enzyme activity and the efficiency of grating are therefore key parameters in glucoside hydrolysis (Westby and Twiddy, 1992a).

Improved nutritional value

There are many examples of fermentation improving the nutritional value of foods. Chevan and Kadam (1990) have reviewed the situation in relation to cereals. Improvements include reduction in anti-nutritional factors such as tannins leading to better protein digestibility (Svanberg and Lorri, 1992) and hydrolysis of phytate leading to improved iron availability. Vitamin production during some fermentations has been reported (Djurtoft and Nielsen, 1983). The role of fermentation in reducing a range of anti-nutritional factors in *Uji,* a fermented product prepared from a range of raw materials including root crop flours and cereal flours, has been reviewed by Mbugua (1988).

Modified functional properties

Fermentation can bring about changes in functional properties of foods. Fermented cassava starch is produced in South America which has modified functional properties, the most important of which is expansion during cooking that allows the production of textured products. The technology for processing fermented starch has been described by Cereda, (1987). Starch is extracted from the cassava root using water. After removal of the cellulosic fibre, the starch is sedimented and transferred into tanks to ferment under water for between 20 and 40 days. The product is then sun dried. The mechanism by which the change in functional properties is brought about is not or has not been elucidated, but fermentation and sun drying are key factors.

The future role of food fermentations

The nature of food processing and marketing chains in rural and urban populations in developing countries is related to the stage of development, levels of income and sociocultural characteristics of different population groups. It is therefore difficult to make generalisations on the future role of fermented foods in development.

Rural urban migration is one of the major problems facing many countries, particularly in Africa. Over 40 per cent of the world's population will live in urban environments by the end of the century. Traditional food processing systems will have to adopt to the consequences of urbanisation. The type of adoption necessary will be dictated by the nature of the food raw material; for example commodities that can be stored, such as maize and rice can be transported to urban centres and processed, whereas root crops, such as cassava are difficult to transport and store poorly, and as such are better processed close to the area of production.

People in urban centres still demand traditional foods made from locally grown crops. Such consumption patterns should be encouraged as they are sustainable and avoid dependence on imported materials. The processing of many traditional foods involves

fermentation and urban demand may result in increased industrialisation of traditional food processing. Industrialisation has many advantages such as standardised products, safer foods, longer storage life due to packaging, and convenience instant foods. Some indigenous fermented foods such as soya sauce, fish sauce, *gari* and sorghum beer are produced on an industrial scale. With increased urbanisation, consumer demand will probably result in a requirement for foods of a consistent quality that are more convenient to use in the home. The economics of modified processing systems to meet these requirements will have to be carefully evaluated to ensure products are competitive in the market place.

In cases where rural production of traditional food supplies urban areas, the income generated may help stem migration to urban centres. In such cases, the needs of rural processors, who are predominantly women, need to be addressed. This may involve reducing their workload or increasing turnover of materials and releasing finance by, for example, speeding up fermentations.

Contribution of fermentation to food safety

Food safety problems are usually associated with the contamination of raw materials by pathogenic or toxin forming bacteria or fungi. Equally important are the preparation and storage of foods in the home, where poor standards of hygiene and sanitation may exist, and water supplies may be contaminated. Fermentation is clearly one low-cost, low technology processing operation that can contribute to food safety.

In future, it will be important to identify situations where fermentation may be successfully used to contribute to safe food delivery. The ability of lactic acid bacteria to inhibit the growth of food spoilage organisms and foodborne pathogens has been discussed. However there is still insufficient information on the role of fermentation in preventing contamination of weaning foods and a role of fermented foods in the management of diarrhoea.

Role of starter cultures

The provision of starter cultures is an issue commonly raised in relation to fermented foods in developing countries. There are three main simple, low-cost, technologies: natural inoculation, transfer of an old batch of fermented product to a new batch (back-slopping), and indigenously derived starter cultures. Some fermentations do not currently need starter cultures because the processing environment provides a good natural inoculum, for example *gari* processing (Westby and Twiddy, 1992b). Higher levels of technology should only be used for these products if the need can be demonstrated. Several indigenous starter cultures exist, such as those for *tempe* and *tape* (Steinkraus, 1983).

The development of specific starter cultures with desirable properties should be combined with the development of appropriate technologies for disseminating and propagating cultures. Desirable properties include antagonistic activity, production of vitamins, removal of antinutritional factors, improved temperature tolerance, enhanced enzyme production and improved amino acid profiles.

Research needs on fermented foods

Research work on indigenous fermented foods has concentrated on the characterisation of microbiological, chemical and biochemical changes that occur during fermentation. This work has provided a strong basis on which to build, and clearly more work has to be done. To be effective from a development standpoint, however, research will have to focus more on addressing the needs of developing countries, both in terms of indigenous processes and future developments in biotechnology. Indigenous fermented foods are an application of biotechnology at its most basic level. Industrialization and commercialization of traditional fermented foods will be facilitated by advances in biotechnology. New starter cultures can be generated by recombinant DNA technology, which not only maintain a typical characteristic of the natural culture, but enhances the ability to utilise substrates more rapidly. For example, strains of *Aspergillus niger* have been isolated which have improved amino acid profiles and are temperature sensitive, asporegenous mutants (Glenn and Rogers, 1989). These are in use in a solid substrate fermentation process for production of animal feed supplements from cereals and root crops. The wider application of more advanced forms of biotechnology such as production of enzymes, protein enrichment of starchy staples, utilization of process waste and production of food components will all have a wider application in the future.

Information on relevant NRI training activities

The Microbiology and Fermentation Group at the National Research Institute, which is an institute of the University of Greenwich, which in turn validates all training programmes and issues the academic awards, provides individually tailored professional development training in food fermentation for visiting workers. The Group has many years experience in research and developmental work related to the microbiology of food in tropical climates. Consequently, it is also able to offer a Basic Food Microbiology training course over three months at the NRI that focuses on: the role of micro-organisms in the spoilage of tropical foods; utilization of micro-organisms in low-cost preservation systems; hygiene and quality assurance in the food industry; design and operation of a food microbiology laboratory; rapid detection methods for food-borne pathogens. Broader aspects of food microbiology are included in the four month annual courses for the Postgraduate Diploma courses on Grain Storage Management and Post-Harvest Horticulure. Short courses at NRI provide means of entry to MSc programmes.

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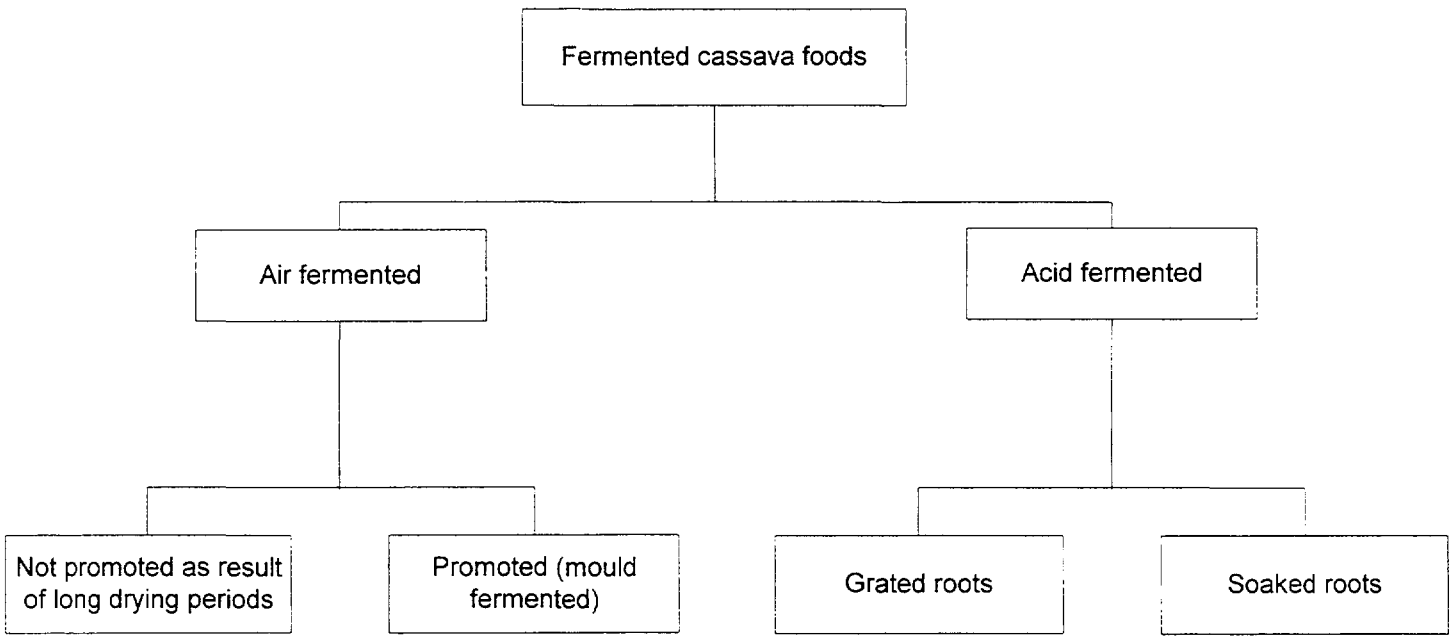

Figure 1: Classification of fermented cassava foods, based on primary (first) method of fermentation

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Natural Resources Institute Training Opportunities

The Microbiology and Fermentation Group at the Natural Resources Institute provides individually tailored professional development training in food fermentation for visiting workers. The Group has many years experience in research and developmental work related to the microbiology of food in tropical climates. Consequently, it is also able to offer a Basic Food Microbiology training course over three months at the NRI that focuses on:

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- design and operation of a food microbiology laboratory;
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Where there is sufficient numbers of students, NRI frequently conducts its training programmes in developing countries.

NRI is an Institute of the University of Greenwich, which validates all training programmes and issues the academic awards.

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Preparing Starters for Lactic Fermentations at the Processing Site: The Canadian Experience in Cheese Production

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INTRODUCTION

Only 60 years ago cheesemaking in Canada was carried out by allowing spontaneous fermentation of raw milk. This practice has now virtually disappeared and we are now mostly using pasteurized milk and inoculating selected starters prepared at the plant. The evolution from spontaneous fermentations to the present situation has involved a few steps, and it is my hope that information on this process could be of use to African scientists and processors.

The aims of this presentation are therefore:

1. To describe the steps that characterized the evolution from spontaneous fermentation of the raw material to highly controlled starter preparation at the processing site and try to apply them to an African context.
2. To show how many mistakes can be made during the production of starters at the processing site, and provide tools to help prepare starters of high and constant activity.

My approach will be to describe the situation in Canada and provide suggestions which, in my view, could extend to the African context.

Evolution

Raw material safety

Paramount to all is the safety of the food.

Raw milk is a very rich food which can unfortunately carry pathogenic microorganisms. Episodes of tuberculosis and throat infections were traced to raw milk and government authorities decided to act. Pasteurization (73^0C/16 sec) was ordered to all milk products that were to be consumed fresh, including fermented products. It is believed in Canada that the pathogens must be exposed for a certain period of time to the acidity of the product so that their complete deactivation may occur. Thus production of cheese with unpasteurized milk is only allowed if the product is stored for 60 days at a temperature greater than 2^0C.

It must be stressed that meat fermentations do not follow this scheme. Raw meats can be used as substrate for fermentations, but in addition to the lactic acid bacteria (LAB), salt and spices are added. Furthermore, the fermented sausages are subsequently dried, cooked or smoked.

In Canada it is thus believed that lactic fermentation in itself, although very helpful, does notuseful enable the production of COMPLETELY safe FRESH foodstuffs if it is not combined with other processing tools (salt, spices, storage, drying, smoking).

A first suggestion for the consideration of African scientists is thus: for *a given food product it could be determined how some additional processing steps can complement lactic fermentation in ensuring safety.* For the dairy industry in Canada this is pasteurization or 60-day storage, while for meat products a combination of salt, spices and drying is applied.

What LAB are involved?

The technological step used to improve the safety of milk had an important side effect: pasteurization of milk destroyed the indigenous LAB. Therefore, spontaneous fermentation could not be achieved, and the application of this heat treatment forced the processor to add a LAB starter. The obvious question was therefore: what culture is the most appropriate? For our particular cheeses, it was determined that strains of *Lactococcus lactis and Lactococcus cremoris* were indicated. This might not completely extend to African products. In practice, cultures ideal for dairy are not appropriate for meat or plant products.

A second corollary to the African context I suggest is thus: *determine what are the LAB species involved in the specific product you are working on.* This can be carried out by either isolating cultures from the highest quality products, or evaluating the effectiveness of cultures used in very similar products.

An interesting evolution of strain preferences has emerged in the last 10 years between Canada and the United States of America (USA). In the USA, there seems to be a preference for the more rapid strains of *Lc. Lactis,* while in Canada we prefer *Lc.*

cremoris, which is a slower acidifying culture. The Canadian processors accept this situation because of the different flavours that the cultures impart, especially on ripened cheeses. This is simply a question of taste.

A third corollary to the African context I suggest is thus: *you can expect regional differences in the starter compositions that will be used, due to consumer preferences or culture.*

Lastly, with respect to the cultures used, we are presently witnessing a shift towards the use of thermophilic cultures. This might be something worth considering in Africa as well. It has the dual advantage of faster fermentation times and prevents the development of mesophilic contaminants.

A fourth suggestion for the consideration of African scientists is thus: *examine the possibility of using thermophilic cultures, but avoid fermentation temperatures around 37^0C which are favourable to pathogenic bacteria.*

Bacteriophages

The best laid plans sometimes go awry! This is what happened in the early stages of the introduction of starter cultures. Pure or multiple cultures were selected and inoculated in milk for cheesemaking but they eventually were attacked by bacteriophages. Now, many actions are taken to prevent phage attack. As related to dairy cultures themselves, we have seen (1) the introduction of mixed-strain cultures, typically four to six strains per starter culture, and (2) culture rotations, typically five to ten different culture mixtures.

The phage problem is very important in the dairy industry. The wine industry also seems somewhat affected by phages, but this does not seem to be the case in the meat industry. My personal opinion is that fermentation of liquids is more subject to phage problems than that of particulate foods. Therefore some food fermentations might be more susceptible to phage attack than others.

In my view, the potential disruptive effect of bacteriophages must be examined. A fifth corollary to the African context I suggest is thus: *determine if phage attack might be a problem in a given food fermentation process. If this is the case, the isolation and selection of the LAB strains to be used in the fermentation process should be carried out so as to enable the preparation of many different mixed cultures.* This is not an easy task, since strain compatibilities and phage sensitivity patterns must be known.

Starter suppliers

The necessity to maintain many mixed cultures was a burden to small cheese manufacturers.

Only very big cheese manufacturers maintained their culture collections. Therefore, we have seen the emergence of companies whose purpose is to provide cultures for the processors. In Canada, three such suppliers exist. There are many in the USA and in Europe. Examples include Rhône-Poulenc/Marshall-Miles, Hansen, Visby, Elf-Sanofi, Rosell and Equipharm. Even the largest dairy processors have abandoned the maintenance of mother cultures.

If you isolate or select original and valuable cultures for your fermentation process, I do not think you want the plant personnel to have the burden of maintaining them. A sixth corollary to the African context I suggest is thus: *in addition to the food fermentation industry itself, it should be examined if the creation of African culture suppliers is warranted.* They could provide the cultures specific to the African products. There might be a niche for specific African-designed cultures that are not marketed by European or North-American suppliers.

If it is decided by the plant to use cultures purchased from a supplier, their form must be determined. In North-America, frozen starters are more popular than the freeze-dried (FD) ones because they start acidification faster. However, such cultures might not be appropriate to the African context as they are expensive to ship and require – 40^0C freezers at the processing plants.

Therefore, as a seventh suggestion for your consideration: *determine if it seems more appropriate for suppliers to provide frozen or freeze-dried starters to the processors.* Although FD starters do lose viability if stored at room temperature, they can still be successfully used even after one week of exposure to room temperature. They are very stable at 4^0C in an ordinary refrigerator. FD cultures would appear more convenient, especially at small, local production sites.

Preparation at the processing site

Preparing bulk starters at the processing site requires that you identify critical points in the procedure and that you provide control methods that enable the technicians to verify if a mistake has been made. Critical points that I have identified are the following:

1. Selection of the milk substrate for specific use in starter propagation;
2. Rehydration of milk powder to a specified solids level;
3. Heat treatment applied to the starter milk; take into account time to reach the holding temperature as well as time to cool to the incubation temperature;
4. Precision and maintenance of incubation temperature;
5. Inoculation level;
6. Incubation time; not fixed, depends on acidity developed;
7. Cooling temperature of the ripe starter.

Numerous mistakes can be made in the bulk starter preparation process. A concise review of such occurrences is found in "Factors other than bacteriophage that affect lactic starter activity" (**Food Research International**, 25: 309-316, 1992). Obviously, they do not apply specifically to original African fermented foods. However the principles that are presented are universal: careful selection of substrate for starter preparation, control of process parameters, quality evaluation of the starter, verification of equipment malfunctions.

This brings me up to an eighth corollary to the African context: *determine the critical points in the specific bulk starter preparation process and provide your technicians with analytical tools to immediately determine if a mistake has been made.* For example, in the case of dairy starters, the milk solids contents can rapidly be estimated, prior to the heat treatment, by the determination of titratable acidity or determination of ^{0}Brix with a pocket refractometer. Such tests are not as precise as oven, Karl Fisher or NMR determinations, but do serve to approximate the situation and prevent major deviations to the established procedure.

When unexpected results occur

Since African specialists are better placed to determine the critical parameters of their fermentation processes, I will not venture further in this area. However I hope to be useful by presenting my investigative approach when a starter does not perform as expected.

My first reflex is to verify the equipment: pH probes, NaOH solutions, temperature gauges. For example, we once discovered that a temperature gauge on the starter vat was not providing the adequate reading. This faulty thermometer generated three mistakes in the starter preparation procedure: faulty pasteurization temperature, faulty incubation temperature and faulty refrigeration of the ripe starter.

My second reflex is to ask if something new has been introduced: new raw material, fresh NaOH standards (titratable acidity), new lot of mother cultures, new lot of cleaning or sanitizing products.

The third step is to ask if something unusual occurred: repairs on the equipment, modifications in personnel or production schedules, fluctuations in steam pressure, ventilation breakdown (affects room temperature).

Finally ask if the technician was disturbed during his shift and was pressed to do some operation more hastily. In one case I was told, the technician was asked to perform some unexpected duty and fell back on his schedule. To save time, he then decided to thaw a frozen culture in 55^{0}C water rather than the usual 20^{0}C. Needless to say the mesophilic culture was scorched!

And there will be times when the people simply do not respect orders. Once, in the night before Christmas, the personnel responsible for cleaning and sanitizing the starter tanks decided to combine the acid and alkali treatments, instead of carrying them out sequentially, so that they could get home earlier. Obviously the acid and afkali neutralized themselves and the cleaning treatment was totally ineffective. This example serves to illustrate the need for selection of personnel. The person responsible for starter preparation must be dedicated, precise and reliable.

Improvement of the Manufacture of Traditional Fermented Products in Morocco: The Case of *Jben* (Moroccan Traditional Fresh Cheese)

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Milk production and consumption in Morocco

Milk production in Morocco (25 million inhabitants), which was estimated to be approximately 7.3 x 10^8 litres in 1985, reached a level of 9.5 x 10^8 litres in 1994. Per capita consumption of fluid milk was 33.3 litres in 1985 versus 39.9 litres in 1990 and reached the level of 41.7 litres in 1992 (Ministry of Agriculture, 1993). This is very low when compared to that of advanced countries such as Iceland, UK and Spain, where the per capita consumption of fluid whole milk was estimated to be 209 litres, 134 litres and 108 litres, respectively (IDF, 1986).

The proportion of milk received and processed by the dairy plants increased from 10.9 per cent in 1970 to 52.6 per cent in 1994. This increase is due to the installation of many new milk collection centres (20 in 1972 to 554 in 1994) as well as processing dairy plants. Currently, there are 34 dairy processing plants in Morocco compared to five in 1972. The non-industrially processed milk (about 47.4 per cent in 1994) is used for auto-consumption or marketed in the cities by hawkers as fresh raw milk.

About 87 per cent of the milk received by the dairy plants is pasteurized and used as fluid milk and the remainder (13 per cent) is converted mainly into fermented milks (11 per cent). The industrial production of butter, cheese and other dairy products represents only 2 per cent of the total milk processed.

Although Morocco does not import fluid whole milk, the national milk production meets only 54 per cent of the population's needs, the deficit of 46 per cent is compensated by imports of butter (35 per cent), powdered milk (9 per cent) and condensed milk and cheese (2 per cent).

Cheese production and consumption

Moroccan cheese production by industrial dairy plants increased from 99 tons in 1970 to 4,500 tons in 1982 and 8,600 tons in 1992. This production is represented mainly by spread cheese; the quantities of fresh cheese made by these plants are very small and variable (723 tons in 1988, only 84 tons in 1989 and 358 tons in 1990). The cheese paste used for making processed spread cheese is imported from European countries and represents the major part of Moroccan cheese imports (1,010 tons of cheese paste and 331 tons of ripened cheese in 1993).

On the other hand, per capita consumption of cheese did not increase much from 1985 to 1990 in Morocco (247 g in 1985 versus 314 g in 1990). These values are insignificant when compared to those reported by the IDF (1986) for France (20.4 kgs), UK (6.9 kgs) and Chile (2.8 kgs). This low level of cheese consumption in Morocco is mainly due to the high market prices of cheese, which are related directly to high import costs and low national cheese production.

As mentioned above, the industrial production of fresh cheese is very low (358 tons in 1990). Most Moroccan fresh cheese is produced by farmers and urban dairy shops. This sector handles a considerable amount (20 to 30 per cent) of the total milk produced in Morocco for the manufacture of traditional Moroccan dairy products including *Jben.*

These fermented dairy products originally made by farmers for their auto-consumption during the periods of surplus milk production, have become popular in recent years in the cities. The number of urban dairy shops preparing and selling these traditional fermented products is increasing.

Although it is well known that a considerable amount of the raw milk is handled by the traditional sector, the exact quantities of the dairy products made by this sector are unknown. This is due to the small-scale production and the absence of an official well organized system of control.

Procedure of *Jben* making

Among traditional dairy products, *Jben* is the most popular because of its gustatory quality. The techniques of *Jben* making vary from region to region. The principle of its preparation, however, is roughly the same (Hamama, 1989). *Jben* is a fresh white cheese with lactic characteristics. It is basically prepared by allowing cow's or goat's milk, or a mixture of both, to spontaneously ferment at room temperature for a period of two to three days, depending on the seasonal temperature. The coagulated milk is then placed in a cloth or mat to drain at room temperature. The curd is kept tightly inside the cloth, which is squeezed several times in order to spread the curd and enhance the whey draining. The cloth would normally be hung from its two ends and kept in that position

for two or more days until the desired texture of cheese is obtained. Once the cheese is cured, the *Jben* is either surface salted or put briefly into brine. This traditional procedure of *Jben* making is commonly used in rural areas and is generally intended for local consumption (Hamama, 1989).

In recent years, however, and concomitantly with the increase in the number of dairy farms and urban dairy shops specializing in *Jben* making for commercial purposes, the preparation of this cheese has been modified to shorten the time of preparation. Raw milk is first filtered to get rid of gross contamination and then coagulated with a rennet solution. After milk coagulation, which is obtained within one to two days, the curd is poured into aluminium or plastic moulds. During the draining time (24 to 48 hours) the curd is regularly pressed in the mould to help remove the whey and obtain the desired texture. Finally, *Jben* is cut into suitable pieces of 120 to 250 g and wrapped in waxed paper. Often producers market their products before complete whey draining. This fresh cheese is sold and consumed within a week from the date of its production although no expiration date is indicated on the cheese label (Hamama, 1989).

Composition of *Jben*

According to a study by Hamama and Bayi (1991), *Jben* obtained from the market has the following average chemical composition: pH: 4.1; titrable acidity: 1.04 per cent lactic acid; total solids: 37.5 ± 6.8 per cent; fat: 16.5 ± 3.7 per cent; proteins: 15.8 ± 3.2 per cent; lactose: 4.1± 0.1 per cent; ash: 1.3 ± 0.3 per cent; chloride: 0.5 ± 0.4 per cent. In general there are large variations in composition between *Jben* samples. This is due to the absence of a standardized manufacturing procedure.

Microbiology of *Jben*

As shown by Hamama and Bayi (1991), the microbiology of *Jben* (table 1) is predominated by lactic acid bacteria (10^8–10^9 UFC/g). Fungi are also present in high numbers (10^6 UFC/g) as well as microorganisms of faecal origin (10^5 total coliforms; 10^4 faecalcoliforms and 10^5 enterococci/g). This high level of faecal contamination shows the poor sanitary conditions used in *Jben* preparation and/or the use of raw milk of doubtful quality (Hamama and El Mouktafi, 1990). Different and varying levels of microbial pathogens have been documented in *Jben*. Of the samples examined by Hamama and Bayi (1991), 17.4 per cent had *Staphylococcus aureus* and those samples tested by Hamama in 1989 showed a 6.6 per cent incidence of contamination by staphylococcal enterotoxin type C. The *S. aureus* isolates were predominately of the human type as indicated by the study of their biotype and phage-type (Hamama and Tatini, 1991). *Salmonella* sp., *Yersinia enterocolitica,* and *Listeria monocytogenes* have also been detected in 10 per cent (Hamama, 1989), 4.1 per cent (Hamama *et al.*, 1992) and 18.1 per cent (El Marrakchi *et al.*, 1993) of the *Jben* samples tested, respectively.

Standardization of *Jben* making procedures

To remedy these problems of non-uniform composition and poor microbiological quality that are linked to the traditional preparation of *Jben*, a study was inititated in

1991 to see if it was possibile to standardize preparation procedures. This study financed by the International Foundation of Science (IFS) had as a major objective the improvement of the traditional processing of *Jben*. The study included the following comparisons to the traditional processing of *Jben*:

1. Pasteurization of the raw milk;
2. Addition of selected lactic starters;
3. Addition of rennet to milk when acidity reached 2–25^{0}D;
4. Moulding of curd at pH levels 4.4 – 4.6 for 24 to 30 hours at 15–20^{0}C; and
5. Addition of 1.5 per cent salt as a surface application for salted products (figure 1).

Standardization Results

In general, the results of this experimental work showed that *Jben* made from pasteurized milk was more acceptable to the sensory panel than commercial *Jben*. The best sensory scores were regularly obtained with products using starter cultures composed of an equal ratio of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* var. *diacetylactis* and *Lactobacillus casei* subsp. *casei*. By contrast, commercial *Jben* had an inconsistent sensory quality and in the taste tests and showed more organoleptical defects (Mahi, 1992). The *Jben* made from pasteurized milk using the improved processing procedure had a very consistent chemical composition and good microbiological populations (absence of faecal coliforms, *Staphylococcus aureus* and *Salmonella* sp., and low counts of moulds, yeasts and enterococci). These data (table 1) indicate that improved technology can lead to the standardization of methods and procedures for quality control in *Jben* production.

Possibility of use of recombined milk for *Jben* making

In addition to *Jben* quality, the producers and the dairy industry are faced with other problems that relate specifically to the seasonal character of Moroccan milk production. The use of recombined milk from powdered skimmed milk and butter oil for making *Jben* appears to be a very interesting alternative to solving these problems, since the key ingredients are relatively less expensive than fresh milk and are available all year round. Owing to the availability of raw materials, dairy plants can operate at maximum capacity and at a profit, thus ensuring *Jben* production in sufficient quantities to meet the concomitant increase in demand.

Although powdered skimmed milk was first used in the manufacture of cheese by Hansen and Theophilus in 1930, the use of 100 per cent recombined milk was not common until research in the late 1950s had established the necessary technology. The cheese making properties of recombined milk differ from fresh milk in that the rate of coagulation is slower, the coagulum strength is reduced, and the rate of syneresis of the curd during manufacture is slightly decreased. To compensate for these differences,

resulting from the powder manufacturing process, minor modifications in the cheese making procedures are required (Gilles and Lawrence, 1982).

In general, acceptable quality cheese can be made from recombined milk using the same principles and standards that apply to the production of cheese from fresh milk. According to Gilles and Lawrence (1982), fat and casein losses during cheese manufacture are no greater for recombined milk than for fresh milk provided that a stable emulsion is achieved by efficient homogenization of the basic ingredients.

Some developing countries have succeeded in producing local varieties of fresh cheese using recombined milk exclusively or a mixture of fresh and recombined milk. Brito and Buhler (1987) have noted three different types of fresh cheese in Chile (quesillo, cottage and mozzarella) with 40 per cent of powdered skimmed milk substituted for fresh milk. The cheeses obtained have similar quality characteristics to the cheeses made from 100 per cent fluid milk.

In Syria, the so-called "white cheese" has been made from recombined (75 per cent) and fresh (25 per cent) milk with satisfactory results (van Middendorp, 1982). Adding fresh milk was considered necessary for the development of the taste.

Regarding the manufacture of *Jben* from recombined milk, it has been attempted by Hamama *et al.* (1995) using low heat skimmed milk powder and pasteurized butter. For this preparation, recombined milk was pasteurized and cooled overnight at 6^0C to improve the powder rehydration. Then milk was inoculated with 3 per cent of a lactic starter composed of *L. lactis* subsp. *lactis, L. lactis* subsp. *lactis* var. *diacetylactis* and *L. casei* subsp. *casei,* and kept at 30 to 37^0C to activate fermentation. At pH 5.0 to 5.5, the rennet was added and moulding was realized at ambient temperature when the pH reached a value of 4.3 to 5.0. Standardization of the processing gave fresh cheese with a regular chemical composition and a satisfactory microbiological quality. The sensory evaluation of fresh cheese showed that the products made with 100 per cent recombined milk were less appreciated than those prepared from recombined milk with 20 to 25 per cent fresh milk.

Possibility of using nisin-producing starters

Another concern regarding *Jben* processing is the microbiological quality. Previous studies on this product have shown that acidity alone is not sufficient to assure *Jben* safety (Hamama, 1989; Hamama *et al.*, 1992; El Marrakchi *et al.*, 1993). Pathogens such as *Salmonella* sp., *Staphylococcus aureus, Yersinia enterocolitica* and *Listeria monocytogenes* have been recovered from *Jben* having a low pH (4.1) and a high lactic acidity (104^0D). Although the level of cheese-borne illnesses in Morocco is unknown, these pathogens have been associated with several explosive outbreaks of illness worldwide due to cheese consumption (Fontaine *et al.*, 1980; Todd *et al.*, 1981; Wood *et al.*, 1984; Recourt, 1989). The use of pasteurized milk, however, instead of raw milk, for *Jben* production, along with improved manufacturing practices were very effective in reducing hazards along with hazards from these pathogens (Mahi, 1992).

In the manufacture of *Jben* from recombined milk special attention should be paid to certain microorganisms usually associated with milk powder and in particular the spore-forming pathogens. These Gram-positive bacteria *(Bacillus cereus, Clostridium perfringens)* have been the causative agents of several food poisoning outbreaks linked to consumption of reconstituted milk and infant formulae (Golpfert *et al.*,1972; Johnson, 1984; Rowe *et al.*, 1987). Since *Jben* is primarily targeted for a vulnerable population (childern and mothers) it should be totally free from Gram-positive (*Listeria monocytogenes, Bacillus cereus,)* and Gram-negative *(Salmonella* sp.) type pathogens.

To achieve this objective, starter cultures will be selected that are capable of producing high levels of acid and other antimicrobial agents for both *Jben* fermentation and control of pathogens. Production of acid in addition to pasteurization of recombined milk should assure the control of most pathogens. For Gram-positive pathogens, however, particularly spore-forming bacilli such as *Bacillus cereus* and *Listeria monocytogenes,* certain strains of *Lactococcus lactis,* will be used to exploit their ability to produce the specific antimicrobial nisin. This important bacteriocin has a broad spectrum of activity and inhibits Gram-positive bacteria (Mattick and Hirsh, 1949). Nisin is bactericidal to different pathogens including *Bacillus cereus* spores and *Listeria monocytogenes* particularly at low pH levels.

Currently, we have several three nisin-producing Lactococci cultures which have been successfully used to manufacture Cheddar cheese (Roberts et al., 1992) and Camembert cheese (Maisnier-Patin *et al.*, 1992). These studies also demonstrated control of pathogens. These cultures, plus others from our stock of lactic cultures, will be examined for their sensory attributes in *Jben* made from recombined milk.

CONCLUSION

The manufacture of *Jben* from raw materials that are more readilyavailable and less expensive than fresh milk is imperative in order to ensure sufficient production throughout the year and to make this nourishing product regularly available and affordable to low income populations suffering from malnutrition. The manufacture of *Jben* from powdered skimmed milk and butter oil has been shown to be possible, but adjustments to its traditional processing must be developed to simulate the texture and flavour of the traditional product. Use of nisin-producing starters should ensure both acceptable fermentation and control pathogens.

Table 1: Composition and Microbiology of *Jben* (average values)

Parameter	Traditionally made *Jben*	*Jben* made with improved technology
1. Composition (%)		
Lactic Acid	1.04	0.97
Total solids	37.5	36.7
Fat	16.5	18.5
Proteins	15.8	15.9
Lactose	4.1	3.9
Chloride	0.50	0.34
Ash	1.26	1.29
pH	4.1	4.2
2. Microbiology (per gram)		
Total aerobic flora	8.2×10^8	2.1×10^8
Lactobacilli	3.2×10^8	9.1×10^8
Lactococci	5.1×10^8	1.3×10^9
Leuconostocs	2.6×10^8	1.2×10^6
Moulds	1.3×10^6	6.3×10^2
Yeasts	3.4×10^6	2.3×10^4
Total coliforms	4.3×10^5	1.1×10^2
Faecal coliforms	2.7×10^4	$\angle 1$
Enterococci	2.4×10^5	1.7×10^2
Staphylococcus aureus (% positives)	17.4	0.0
Salmonella (% positives)	0.0	0.0

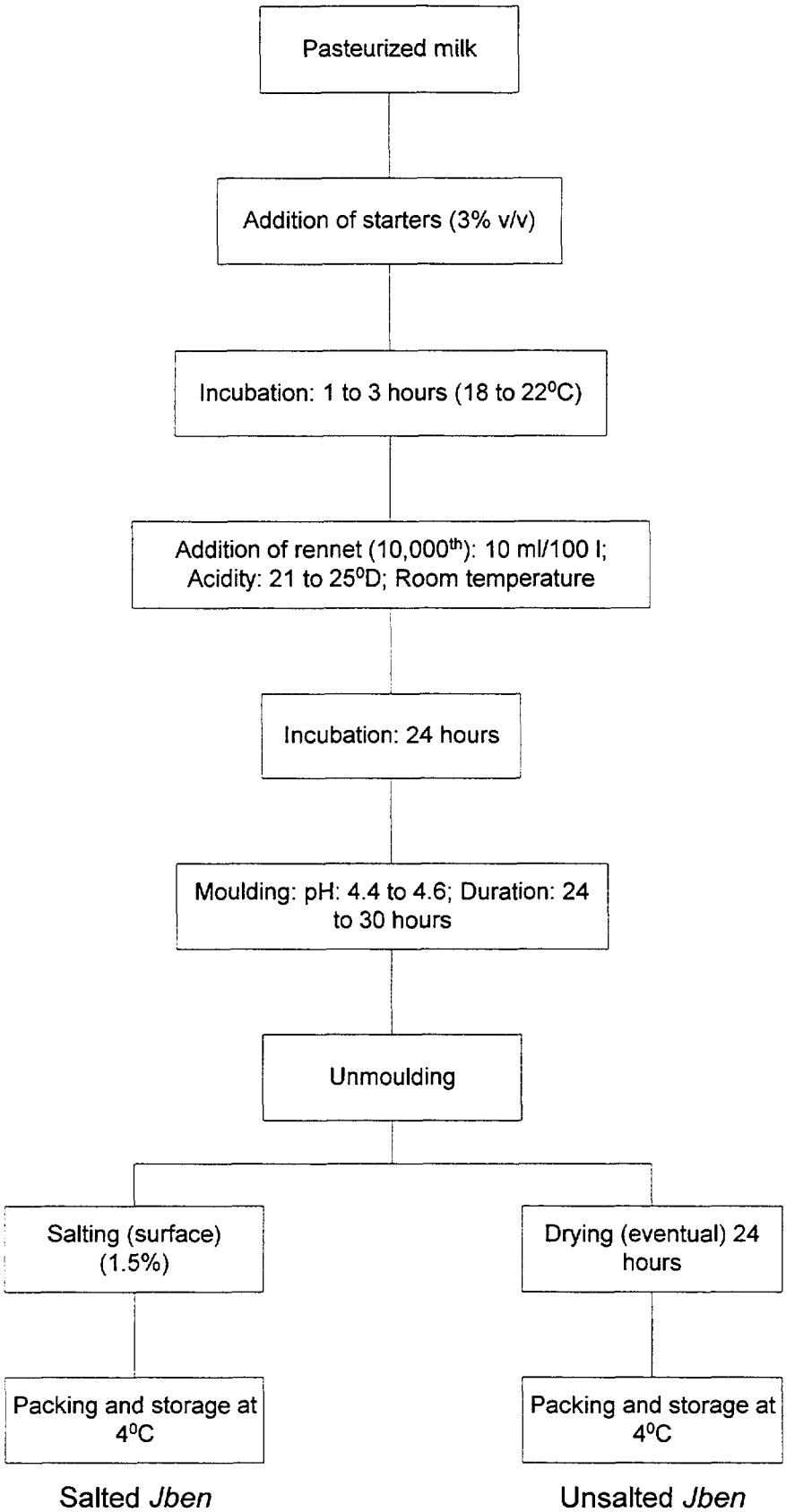

Figure 1: Manufacture of *Jben* from pasteurized milk

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Two Indigenous Ethiopian Lactic Acid Fermented Foods: *Tef Injera* and *Kocho*

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Abstract

Tef injera, a pancake-like fermented bread prepared from tef dough, is the traditional staple food of Ethiopia.

Fermentation of tef is initiated by members of Enterobacteriaceae. Their activities during the 18 hours of fermentation reduces the pH of the dough from 6.3 to 5.8. They are then succeeded by Leuconostoc mesenteroides and Streprococcus faecalis. As the pH is further reduced to about 4.7, Pediococcus cervisiae, Lactobacillus brevis, Lactobacillus plantarum and Lactobacillus fermentum become the predominant flora and remain so until the fermentation is terminated at 72 hours. These lactic acid bacteria are responsible for the acidic characteristic of the dough. The lactic acid is the major organic acid produced and is mainly responsible for the fall in pH and the sour taste of injera.

Kocho, the main fermented product from the pseudostem, corm and tubers of ensete (Ensete ventricosum), is an acidic starchy food. The microorganisms which initiate ensete fermentation are Leuconostoc, Streptococcus and Pediococcus species. Their activities reduce the pH from 6.5 to 5.6. They are then succeeded by homofermentative bacteria, Lactobacillus coryneform and Lactobacillus plantarum. Through the activities of the Lactobacillus species the pH further drops to 4.2. Spore formers are present in fairly high numbers during the first 15 days of fermentation.

INTRODUCTION

Ethiopia is basically an agricultural country. About 90 per cent of the total population live in rural areas, being engaged in agricultural activities. Agriculture is the major source of food, industrial raw materials and export items. About 70 per cent of the population is directly dependent on agriculture, producing 70 per cent of the total

national income.[1] The major food crops of the highland areas include tef, wheat, barley and pulses. The major crops of the lowland areas include sorghum, maize and millet. Ensete, one of the major root crops, is also a major food crop in the midland and highland areas of the southern and south-western parts of the country.

There are many indigenous fermented foods In Ethiopia that are derived from cereals, tubers and dairy products. These fermented foods are prepared by traditional methods and are popular and well liked by the general population, and constitute the major portion of the daily diet. This popularity is due to the characteristic flavour of these fermented foods . The processing methods are traditional and do not require sophisticated equipment. Among these fermented foods, *tef injera* from the fermented cereal tef, and *kocho* from the tuber ensete are two common indigenous lactic acid fermented foods which have been studied to a certain degree.

The lactic acid fernentation

Fermentation is one of the oldest known methods of preparation, processing and preservation of foods. It may be defined as a complex biochemical transformation of organic substances brought about by enzymes originating either from the food or microorganisms associated with it. Raw foods harbour a heterogeneous group of microorganisms. The degree of contact of different foods with the air, soil, water, animal bodies, equipment and other foods as well, determine the nature of the contaminating flora.

Basically, fermented foods are agricultural products that have been converted into desirable products by the enzymatic activities of the microorganisms. They are of a diverse nature; some result from lactic acid bacteria fermentation; some from mould fermentation; yet others from alcoholic fermentations by yeast; and many by a combination of these fermentation processes. Fermented foods are generally considered more attractive and desirable than the unfermented raw materials from which they are prepared. Fermentation may contribute to the flavour and aroma in the fermented food products. Fermentation also destroys undesirable factors, such as trypsin inhibitors, phytates, etc., and makes the nutrients more available.

The lactic acid-producing bacteria are, with some exceptiors Gram-positive, catalase negative, non-spore-forming spheres and rods. All require carbohydrates for energy source, are unable to synthesize amino acids and growth factors for reproduction and also produce lactic acid from sugar. These bacteria thrive mostly in microaerophilic to anaerobic conditions. Their rapid production of lactic acid is known to inhibit the growth of many bacteria.

The lactic acid bacteria include species of *Lactobacillus, Leuconostoc, Pedicoccus* and *Streptococcus.* Among these, species belonging to *Streptococcus* and *Leuconostoc* produce the least acids; the homofermentative species of *Lactobacillus* produce the greatest amount of acid. Heterofermentative *Leuconostoc* and *Lactobacillus* species convert glucose to about 50 per cent lactic acid, 25 per cent acetic acid and ethyl alcohol, and 25 per cent CO_2. This is important in flavour development and in the leavening of

certain bread-like fermented foods.[2,3] In addition to giving the desired aroma, texture and flavour, better palatability and physical appearance, the lactic acid bacteria are known to produce various metabolites that preserve foods. This is directly related to the production of antimicrobial substances other than acids.[4,5] These are usually antibiotics, which are either bacteriostatic, or bactericidal in action.[6] The action of these antibiotics is species-specific in some cases and of broad spectrum in others.[7] The antimicrobials so far isolated from lactic acid bacteria includes Lactocins, Lactacins, Helveticins, Diplococcins, Leucocins, Pediocins and Plantaricins.[8,9] The antimicrobial behaviour of lactic acid bacteria fermentation therefore appears to be due to the cumulative effect of the acids, H_2O_2, CO_2, (creating anaerobic environments) and antibiotics produced during the fermentation process. Some of these compounds are volatile or are readily oxidised, while others are stable for short periods during the fermentation process.

Ethiopian fermented foods

Several indegenious fermented foods and beverages are known in Ethiopia. Most of these fermented foods are mainly derived from cereals like tef *(Eragrostis tef)*, sorghum, barley, maize, wheat, or from tubers like ensete *(Ensete ventricosum).* The two most common and very popular acidic fermented foods which are unique to Ethiopa are *tef injera* and *kocho*. They are derived from tef and ensete, respectively. In this paper, the nature of fermentation of these two foods will be presented.

Tef (Eragrostis tef)

Tef is one of the important cereal crops which is widely grown and used in the preparation of staple food in Ethiopia. It is a fine millet-like grain indigenous to Ethiopia, where it is extensively cultivated for human consumption. The genus *Eragrostis tef* is a member of the tribe *Eragrosteae*, sub family *Eragrostoidae,* of the family *poaceae*. The grain, which is about the size of a pin head, is either white, brown or red in colour. The seeds are oval in shape, generally 1 to 1.2 mm in length and very light, with a mean individual mass of about 0.6 mg. So far there are 35 cultivars of tef that are known, of which 21 are white seeded and 14 brown or red seeded. However, all of these cultivars are known to have similar amino acid compositions.[10]

Tef is first milled and the flour used to make a variety of food items, such as *injera* (a leavened pancake-like bread), porridge, *tella* (a traditional local beer) and *Katikalla* (traditional spirit) , etc. However, it is mainly used to make *injera*. *Injera* is an Amharic word for a fermented and baked product made from the major cereal grains in Ethiopia such as tef, maize, barley, sorghum, wheat or a mixture of these. Of these cereals, tef is reputed to make the best quality *injera* and the preference of tef *injera* is higher than that of any other source in the country.

Tef fermentation

The preparation of *injera* is a traditional art which does not require sophisticated equipment and is therefore an inexpensive, culturally accepted practice. The processing of tef grain for the making of *injera* requires several stages. Initially, tef is cleaned by

winnowing and sifting to remove foreign material and the cleaned seeds are ground into flour in mills. The flour is first mixed with *irsho* (a thin paste saved from the previous fermentation) and kneaded by hand. It is then thoroughly mixed with water in a proportion of about two parts flour to three parts water to make a dough and kept in an opaque container (made from clay, wood or metal) with a cover and left to ferment at room temperature. The fermentation period, however, may vary from 48 to 72 hours, depending on the altitude of the area, the concentration of *irsho* and the type of container used.[11]

Tef fermentation is subjected to two stages. The first stage is marked by the complete separation of solid and liquid parts. At this stage, the liquid part is decanted and discarded. It is believed that this practice reduces the sourness of the bread. During the secondary fermentation, a portion of the fermented dough, usually about 10 per cent, is mixed with three parts of boiling water and heated further for about 10 to 15 minutes. This boiled fraction is called *absit* and is then mixed back with the fermenting dough in the container. Enough water is added to make a thin paste, which is left to ferment for a further 30 minutes to two hours. During this process, the dough begins to rise and settles, which signifies the end of secondary fermentation. The aim of adding *absit* is to provide a clean looking inviting appearance. In the absence of *absit*, an unleavened product with a powdery look is produced. When excess *absit* is added the product will have very tiny honeycomb-like eyes, becomes sticky and difficult to remove from the baking object.

Major events in tef fermentation

Bacteria and yeasts are predominantly involved in the fermentation of tef. The Gram-negative Saccharolytic bacteria, including certain members of the family Enterobacteriaceae are responsible for initiating the fermentation, dough rising and the reduction of pH from 6.3 to 5.8. Leavening and the evolution of gas in the dough are the major characteristics resulting from bacterial activity during the first 18 hours of fermentation. As the pH drops to 4.7, the rising dough begins to settle and a substantial reduction of gas evolution is observed, with a liquid layer appearing on top of the dough. During this event, the *Lactobacillus* species begin to appear in large numbers. At 48 hours fermentation, there is a complete solid-liquid layer separation, the dough settles completely and a fair amount of gas evolves. The pH also drops to about 4 and the *Lactobacilli* become the single most abundant fermentative bacteria, and the yeast populations also increase. At 72 hours fermentation, the pH drops further to 3.8, the lactic acid bacteria still remain as the dominant bacteria, and there is a confluent growth of film yeasts on the liquid layer.

The activity of lactic acid bacteria as a whole continues for almost 54 hours (18 to 72 hours) of fermentation with a reduction of the pH in the dough by a unit of two (5.8 to 3.8). The bacteria are responsible for bringing about the desired acidity in tef fermentation. The chemical analysis of fermentation products also reveal that the lactic and acetic acids are the major organic acids produced, are responsible for the fall in pH during fermentation and for the sour taste of *injera*.[12]

Ensete (*Ensete ventricosum*)

A wide range of root and tuber crops are cultivated in the southern and south western parts of Ethiopia.(13) These crops, which form the major component of the local population's diet, are grown in small plots, often near homesteads. Among these, ensete is by far the most important staple food crop for more than 10 million people in the country.

Ensete belongs to the family Musaceae, to which abaca *(Musa textillis)* and banana *(M. paradisiaca)* also belong. There are six species of ensete distributed within Africa and Asia. In Ethiopia, *Ensete ventricosum* represents about 70 per cent of the total ensete cultivars. It is only in Ethiopia that ensete is cultivated as a food crop. It is a perennial root crop which grows at altitudes between 1,500 and 3,000 metres above sea level.(14) In Ethiopia, the cultivation of ensete is mainly confined to Sidamo, Western and Southern Showa, Gurage, Kambata, Hadiya, Gamo Gofa, Wolayita, Kaffa and Il-lubabor. An estimated area of 67,000 sq. kms. are used for its cultivation. Regions where ensete is cultivated as a staple diet are among the most densely populated in the country. The plant reaches a height of 5 – 6 metres and it takes 6 – 8 years to mature and be harvested for food. An average family cultivates 200 – 400 plants in a small plot and the consumption per person varies from 20 – 40 plants per year. A mature plant is estimated to yield from 26 – 42 kgs. of food.

Ensete fermentation

The two main ensete products utilized as food are locally known as *kocho* and *bulla*. *Kocho* is a non-dehydrated fermented product from the pulp of the pseudostem and corms. *Bulla* is a dehydrated juice collected from the pulp of the pseudostem. The processing of ensete fermentation to produce *kocho* and *bulla* is laborious and time consuming. First, the whole plant is loosened with the help of a special knife, lifted from its hole and transported to the place where it will be processed. The parts of the plant which are fermented consists of the inner non-pigmented portion of the trunk, stem and corm. A wooden plank is kept at an angle of about 40^0 against a pole on which the leaf sheath is scraped with a large bamboo splinter. The stem and corms are pulverised with a long wooden pestle whose ends are carved into short multi-pronged comb-like protrusions. The liquid is allowed to run down and settle in a small pit lined with fresh ensete leaves in order to give *bulla*. The major part of the product is placed in a one square metre sized pit whose inner part is lined with fresh ensete leaves, covered, and left to ferment for about five days. The root is also covered with ensete leaves and left to decompose before being crushed and mixed with the scraped pulp. Locally, this is thought to initiate the desired fermentation process. After about a week, it is then properly mixed, kneaded, pressed by hands or feet and covered with fresh ensete leaves. Heavy stones are then laid on top of the mantle. The whole process of pressing the scraped and mashed ensete, layering it and placing heavy loads on top of it is thought to ensure that airtight conditions are created in the pit. The length of fermentation time, however, varies from a few weeks to several months. In colder regions, it can be kept in a pit for years. In such regions, the quality is said to increase with increasing fermenta-

tion time. In the warmer regions, fermentation is rapid and the process is completed within one to three months. In such regions if fermentation time increases, the *kocho* becomes very acidic and occasionally blue, black and brown discoloration develops.

Microbial dynamics of ensete fermentation

During the initial stage of ensete fermentation, the pH is about 6.5 and a diverse group of microorganisms, such as aerobic and anaerobic spore formers, Gram-negative bacteria, including members belonging to the Enterobacteriaceae, lactic acid bacteria and yeasts, are present.[15] Among them, yeasts are the most abundant, followed by *Streptococcus faecalis* and *Leuconostoc mesenteroides.* However, the *Leuconostoc mesenteroides* sustains the highest growth rate and is the most abundant microorganism during the first week of fermentation. It is considered to be the initiator of ensete fermentation. Thereafter, however, it remains the second most abundant microorganism. Even though it is not a strong acid producer, *Leuconostoc mesenteroides* is mostly responsible for lowering pH. *Streptococcus faecalis* is the second most abundant during the first-week of fermentation and disappears altogether as the pH becomes acidic, i.e. 4.4. The Lactobacilli exhibit a fast growth rate after the first week of fermentation. They remain as the single most predominant group till the end of fermentation.[16] The isolates were found to be *Lactobacillus coryneformis* and *Lactobacillus plantarum.* During this fermentation process, the *Lactobacillus* species succeeded the *Leuconostoc* spp. as the most abundant microorganisms. Through the activities of the *lactobacilli*, the pH of the fermenting ensete is reduced from 5.6 to 4.2. *Pediococcus cervisiae* is also present during the fermentation process but is less active, probably due to the low temperature (14 to18^{0}C) fermentation of ensete.

CONCLUSION

Most staple foods of Ethiopia are fermented before they are consumed. As the practice of fermentation is a traditional art passed on generally from mother to daughter, it is not standardised. All our fermented foods are prepared by spontaneous or natural fermentation but specific microorganisms predominate. So far, our research work focused on the understanding of the processes involved in traditional fermentation, isolation, characterisation and understanding the role of microorganisms involved during the fermentation process, thereby collecting information on our traditional fermented foods. Even then, the research and development of fermented foods in Ethiopia is meagre. Much basic research is needed to develop starter cultures, means and ways of shortening the fermentation period, improving the art of traditional processes, adopting and improving technology to reduce nutrient losses during fermentation, and standardization of the fermented foods.

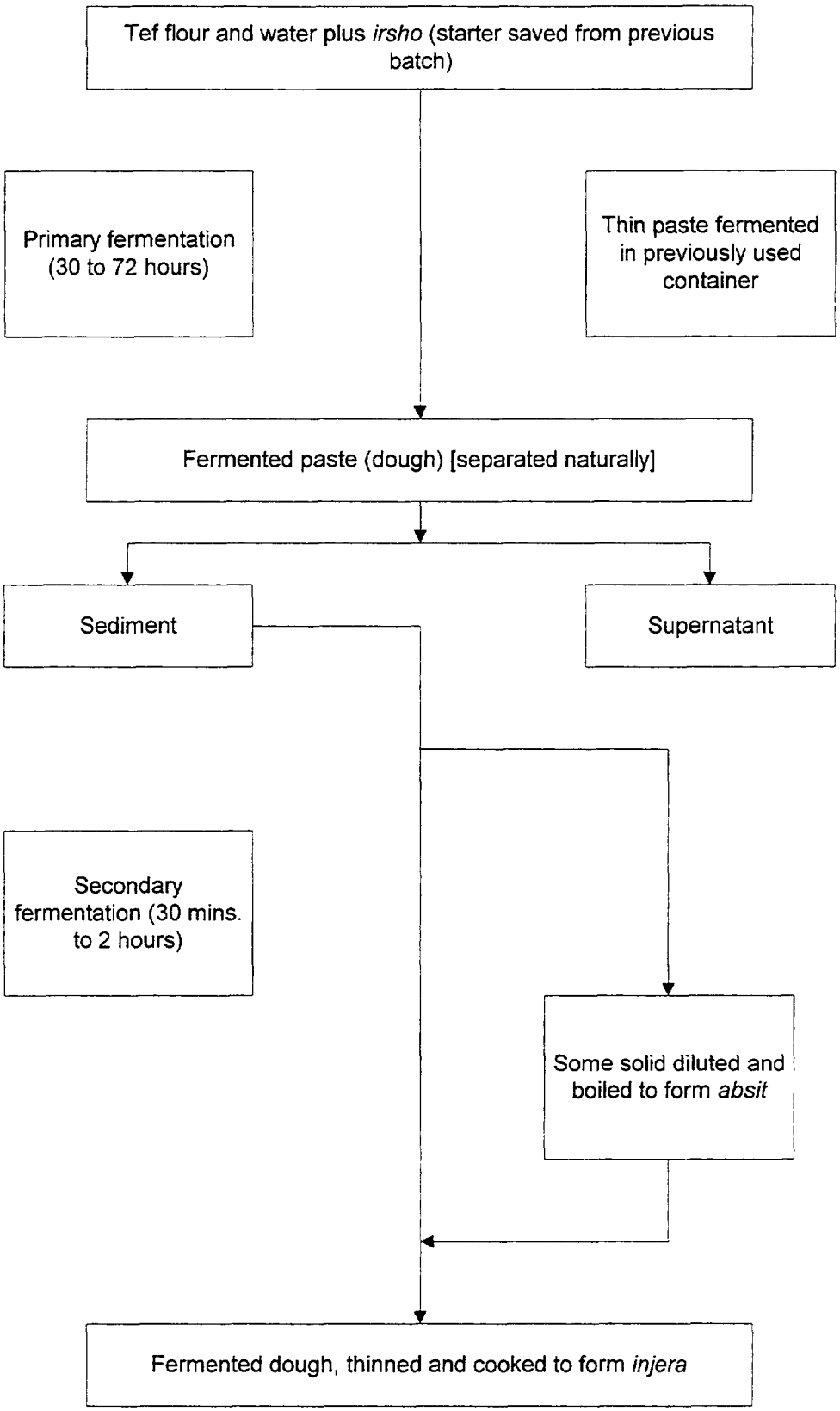

Figure 1: Traditional method of preparing *tef injera*

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Lactic Acid and Lactic Acid Bacteria in African Indigenous Knowledge

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INTRODUCTION

How long have Africans known lactic fermentation is something beyond our present knowledge. However, in spite of the meagre research carried out on the continent concerning the evolutionary track of human communities, we do have some indications that could shed some light on the history of lactic fermentation in Africa. Judging by our present knowledge of indigenous food practices, we may logically assume that the history of lactic fermentation is the history of food fermentation in general, but more so the history of cereal and dairy processing.

Archaeological findings have revealed that ancient human communities flourished on the site of present-day Khartoum in Sudan some 9,000 years before present (BP). These communities primarily depended on Nile fish for their livelihood primarily, but they also gathered some wild seeds (Arkell, 1949). These Mesolithic communities of Early Khartoum were replaced 5,000 years BP by Neolithic communities who raised cattle and either practiced intensive seed gathering or even cultivated sorghum and millet (Krzyzaniak, 1984; Klichowska, 1984; Doggett, 1988).

Ancient languages spoken some 6,000 years BP in Nigeria, Cameroon and Chad had words for sorghum, millet, flour, porridge, goat, sheep and cow (Ehret, 1984). Contemporary languages that were spoken in southern Sudan likewise had words for weed, cultivate and herd. Recently, sorghum and millet grains that are 8,000 years old have been found on the Sudanese-Egyptian border (Wendorf *et al.*, 1992), reversing a long-standing view that the development of African plant foods was linked to the development of wheat and barley in Asia.

It is very hard to believe that those ancient African communities who were well aquainted with cereals and milk did not know lactic fermentations. More recent, but still ancient history, brings us to the Kingdom of Meroe that flourished in the Central Nile Valley in Sudan some 2,000 years BP. We have well-documented proof that the people

of the kingdom practiced lactic fermentations. A wall graffito depicting two men drinking sorghum beer (Shinnie,1967) from a typical African clay pot has been left for us by the people of Meroe (Hintze, 1979). Also a drawing of a procession of dancers ladling wine from earthenwares has been found on a Meroitic jar, and actual wine presses have also been unearthed that belonged to the Kingdom of Meroe (Woolley and Randall-MacIver, 1910; Adams, 1966). Further, a complete cow milking seance with men handing over milk to a queen-like woman has been found carved on a Meroitic bronze bowl. The woman has five containers placed on the ground near her feet, which to us may indicate the partitioning of the milk for various purposes of which lactic fermentation would be impossible to avoid. For these historical drawings the reader is referred to Dirar (1993).

Further proof that the Meroites practiced lactic fermentation has been given to us by classic Greek and Roman writers who wrote about the Kingdom of Meroe, mentioning something about its foods. Strabo (7 BC), for instance, wrote that the people of Meroe (referred to as Ethiopians by classic writers) made cheese and butter, and ate sorghum, from which they also made a drink *(merissa)*.

The symbol of food abundance known to the ancient kingdoms of the Old World was the so-called Table of the Sun. This table was found in Africa, in Meroe. It had been believed that a table existed in an extended meadow on the outskirts of the city of Meroe which the magistrates of the Kingdom replenished with fresh food every night. During the daytime, all people were welcome to eat freely of the food. According to Herodotus (in his Histories, 430 BC), King Cambyses (ca. 522 BC) of Persia sent envoys to Meroe to gather information on the Table of the Sun. If the Meroites knew beer, wine, cheese and butter, one wonders how much lactic bacteria had contributed to the food entries of that table!

Fermented foods and beverages are well documented in the relics of Ancient Egypt (Ghalioungui, 1979). Many historians, however, believe this civilization had its roots deeper in sub-Saharan Africa, in particular in Meroe (Diop, 1974; Williams, 1987).

In conclusion, it could be said with confidence that lactic fermentations have been with the Africans for thousands of years. During this period a great mass of knowledge must have been accumulated by women who preserved it and handed it down from mother to daughter. This knowledge, like so much information on other African aspects of life, has not been documented. Accordingly, no single researcher can claim to know much more than part of what is known in his or her own country. Research in lactic fermentations could be envisaged as making much use of indigenous knowledge in this area. Indigenous knowledge-generated research could prove to be more relevant to the actual needs of the poor African communities. As much local information as possible must be gathered first before planning any improvement or modernization research of an indigenous lactic fermentation process.

This paper is only an attempt to give examples of indigenous knowledge pertaining to lactic acid and lactic acid bacteria in Africa, by necessity with great emphasis on Sudan.

Lactic acid

The applications of lactic acid in the African home employ, of course, lactic acid-bearing materials rather than the pure chemical as we know it in the laboratory or the industry. The acid-bearing material most commonly used is either a cereal or a dairy product wherein lactic acid has been produced *in situ* by spontaneous fermentation by a group of lactic acid bacteria.

Lactic acid and thirst quenching

A number of authors have observed the more than usual liking by Africans of lactic acid foods and beverages, but gave no definite answer as to the reasonfor this liking.

Cailliaud (1826) mentioned that all Sudanese had a great liking for that which was acidic; in his own words: "Tous ont un gout decidè pour ce qui est acide." Hesseltine (1979) noted that lactic acid fermentation seemed to be the rule throughout Africa. Ko (1982) stated that fermentation of starch-rich substrates by bacteria into fermented foods was practiced mainly in Africa. Novellie (1977) mentioned that Africans seemed to be especialy fond of the sharpness given to foods and beverages by the presence of lactic acid. On another occasion, Novellie (1986) wrote: "One is struck, when studying African fermented foods and beverages, by the prevelance of lactic fermentations. The pleasantly sharp sourness of these edibles seems to suit the African palate; certainly the sourness is refreshing in the usually hot climate."

This predeliction for lactic acid foods and drinks by Africans could possibly be explained on the premise of a possible thirst quenching property of the acid. It may not be as serious as hunger, but thirst is a daily suffering of many people in Africa. The hottest regions of sub-Saharan Africa are the lowland savannahs and the semi-arid Sahel regions extending from the western rim of the Ethiopian plateau to the shores of the Atlantic, in addition to those parts of southern Africa with hot Mediterranean-type summers.

Many a time one hears of individuals that have died of thirst. This is particularly true with certain sectors of the population such as hunters, wood collectors, shepherds and travellers who go astray.

It is not surprising therefore, that communities in the hotter regions of Africa have developed ways and means whereby the impact of thirst is mitigated. For example, some techniques have been developed to encourage individuals to drink more and more water whenever they find it. One ingenious method involves the chewing, just before drinking, of the swollen root of a wild plant called in Sudan, *kurdala (Maerua pseudogetalosa).* The root causes a special sweetish (soft drink-like) sensation on the tongue at the time of drinking so that the individual is enticed to drink more and more water. The root itself is also employed as a clarifier of muddy water (Jahn and Dirar, 1979).

Another indigenous approarch to alleviating the feeling of thirst by the African is the development of thirst-quenching foods and drinks. Although certain bulbs and tubers of wild plants are used (Larken, 1927), the primary thirst quenchers of Africa are fermented

foods. The common denominator of these products is that they all have lactic acid. This may explain the observation made by Muller (1981) that porridges did not appear to be common in Central and Eastern Africa, but they were very popular in West Africa. Perhaps this is so because they are more needed in the warmer West Africa than in the cooler East and Central Africa because of their thirst-quecching properties.

Let us now give some examples of thirst quenchers in Sudan. It must be mentioned at this point that the whole group of these products is ill-defined and no studies have been made on them. However, one can discern two sub-groups: mild thirst quenchers and effective thirst quenchers. Of the first group, a very thin slurry of the stiff porridge *aceda* is made *(tau-okara)* and is used as a light thirst quencher. Another example comes in the form of a smooth, thin, sour porridge *(nasha)* which usually forms part of the drinks of Ramadan fasters. In yet a third example of mild thirst quenchers the flakes of the sour sorghum bread, *kissra*, are soaked in water and strained. The filtrate called *movat-kissra (kissra* water) is used as a thirst quencher.

However,, there are two major, presumably very effective, thirst quenchers in Sudan: *ghubasha* and *abreh.* The first is obtained by diluting *rob* (sour buttermilk) with two to three volumes of cold water. This is one of the most widely used thirst quenchers in the country. Interestingly, undiluted *rob* is believed to aggravate the sensation of thirst rather than mitigate it. This phenomenon of a chemical causing one effect at low concentrartions and the opposite effect at higher concentrations is commonplace in biology. In the Middle East there are many diluted sour dairy products similar to *ghubasha,* which are also used as thirst quenchers. Examples are: *laban-zeer* and *kishk* of Egypt (Khalafalla, 1985), *leben* of Iraq and vicinity (Abo-Elnaga *et al.* 1977) and *lassi* of India (Vedamuthu, 1982). The famous medieval Arab traveller, Ibn-Batutah, who visited the Sahel in 1374, reported on a product called *dagnu* in that region of Africa consisting of (sour) milk and millet that was used as a thirst quencher (El-Kitani, 1975). An identical product called *ajinat-um-jingir* or *deshisha* now exists in western Sudan and makes part of the evening break fast of the fasters of the holy month of Ramadan.

The hypothesis that lactic acid has something to do with thirst quenching is corroborated by the existence of a food product in Sudan that is specifically prepared and consumed to fulfil the sole function of thirst quenching. That product is *abreh* which is only prepared in Ramadan to make part of the fasters' drinks. What makes this even more supportive of the said hypothesis is that the fermentation procedure followed in this product is geared for maximizing lactic acid production.

In the preparation of the product either whole pearled grain or grain meal is soaked in water overnight followed by a series of wet millings, which may amount to four times, using a saddle stone or a quern. This process takes about four days during which time fermentation continues, resulting in the production of more and more lactic acid. Next, about two thirds of the dough are cooked into a thin porridge. The porridge is allowed to rest for 24 hours before mixing back with the remaining mother dough, which in its turn acts as a starter for the second leg of fermentation. In this second fermentation, which also extends for about four days, whole black cumin seeds are added. The function of the spice is not known, but it may help mask the strong acid flavour of the product.

The batter is now thinned with water and strained to remove the spice as well as other granular impurities. The strained dough is baked on a hot plate into extra-thin, dry, almost transparent flakes that constitute *abreh*.

The basic idea behind the cottage technology of *abreh* is to produce as much lactic acid during fermentation as possible and to administer this into the stomach of the thirsty person in such a way that the strong acid concentration by-passes the taste buds of the buccal cavity without being detected.

The maximization of the lactic acid yield is achieved by coaxing the bacteria to continue metabolizing for the maximum period possible. The continuous wet milling, which extends for a number of days, perhaps acts as an expedient of mixing the dough now and then and providing more readily accessible nutrients during the first stretch of the fermentation. The cooking of the bulk of the dough helps expell most of the toxic substances, such as volatile fatty acids and alcohols that have accumulated during the first stage of fermentation. Moreover, the lactic acid which is not removed by cooking reacts in the presence of high temperatures with the starch to produce more sugars, thus replenishing the supplies of this substrate which may have been depleted in the first stage. All this would help produce more acid in the second stage.

The product comes in the form of very thin whitish flakes that become extremely smooth, almost mucilaginous, when wetted. The product is consumed as a suspension in cold water which the consumer swallows without chewing. The flakes slip down the throat without being felt as particulate matter. In effect, *abreh* is an acid pill or capsule designed to quench thirst.

To encourage the consumer to take more of the product, women add a touch of beauty to it. *Abreh* must have a very clean whitish colour and this aesthetic look has been augmented in recent years by making use of modern food colouring matter so that now *abreh* comes in yellow, pink, and greenish colours in addition to the natural creamy white colour.

It is assumed that *abreh* was invented by African woman following the Islamization of Sudan some 700 years ago, when confronted with a hungry and thirsty Ramadan faster. She invented *abreh* to quench his thirst, and another product — *hulu-mur* — to quickly replenish his blood sugar level. The foods and drinks of Ramadan fasters in the hot Sahelian countries of Africa provide a model for studying the group of thirst quenchers. Sometimes the lunar month of Ramadan falls in the extremely hot months of May and June and the suffering experienced by fasters as a result of thirst is unbearable. A thirst-quenching item in the evening break fast is very helpful, otherwise the faster continues to drink water throughout the night while consuming very little food.

It should be mentioned that other acids such as tamarind soaked in water, lime or lemon juice, or *gungpleis* (the fruit of the baobab tree, *Adansonia digitata)* although refreshing, are not considered thirst quenchers. In fact, lime or lemon juice is believed to augment the feeling of thirst. Only lactic acid-bearing material could be a thirst satiator.

Cosmetic and medicinal uses of lactic acid

Sometimes women in Sudan are seen rubbing their faces with sour sorghum dough and then covering the face with a smear of the dough for some time before removing it. This is believed to help remove blemishes and pimples and to give the face a healthy-looking complexion.

But sour dough is most widely used as a cosmetic preparation in the form of a mixture of dough and sesame oil to give a stiff paste called *um-lakhokha*. This preparation is used to rub and massage the whole body, specially that of young brides-to-be in preparation for marriage. Such girls (aged between 13 and 16 years), particularly those in nomadic communities, are usually rough-skinned and carry bruises. Lactic acid-bearing sorghum dough applied repeatedly for several days is said to heal the bruises and to result in a skin with vitality and a healthy appearance.

Lactic acid-bearing materials or foods are also put to use in the treatment of a number of illnesses. Burns, for instance, are treated by applying sour sorghum dough. Sore throats, pharyngeal pains and inflammations, and other ailments of this general area of the body are treated by applying sour dough to the outer surface. The treatment is called *labkha*.

When young children play bare-foot in the hot sun of the summer on hot sands and soils they are said to develop a malaise called *ramdah* (hot sands) which manifests itself as anal itching. The child is seen scratching its anus, particularly in the evening at bedtime. Women treat this, most likely parasitic or protozoal irritation, by the anal application of sour dough.

The best example of the use of lactic acid-bearing materials as medicine in Sudan comes in the form of a fermented camel milk product. This spiced fermented camel milk is used to treat the often fatal disease called *kala-azar*, or visceral leishmaniasis (Maegraith, 1976). The outward manifestation of the disease is a large distended belly.

Camel boys who roam far away grazing lands with their camels sometimes depend totally for months on a fermented camel milk product called *gariss* (Arabic for 'pinching', implying high acidity). These boys are said not to contract *kala-azar* at all, thanks to the protection provided by the sour milk. *Gariss* is fermented in large skin bags hoisted on the saddle of a camel where it undergoes fermentation under more or less shaken conditions as the camel goes about its usual business of grazing day and night.

When an adult male contracts the disease back in the family camp he is sent off with the camel boys to live on *gariss* alone for months. It is said that such a person would come back to the camp as 'slim as a whip'.

When a woman, a child or an elderly person becomes sick with *kala-azar* in the camp, he or she obviously cannot be sent off with the camels. Such individuals are treated with camel milk prepared at home in a special manner. In other words, this milk is made for the specific purpose of being used as a medicament. Camel milk is placed in an earthenware jar and an assortment of 12 spices are ground and mixed into the milk. For each five litres of milk, about 40 g of each of the following spices are used: ginger, galangal, cinnamon, cumin, black cumin, black pepper, coriander, garlic, fenugreek,

cloves, arjel and onions. The earthenware is then covered securely and completely buried in a pit in the ground covered with soil. The content undergoes lactic fermentation for three days.

When the earthenware is dug out after this incubation period the milk will have soured up and will have separated into a gas impregnated floating curd *(kushkush)* and a bottom whey *(safwa)*. The patient is asked to eat from the curd and drink from the whey to the exclusion of all other food for 12 days. After this period the patient is kept on another lactic acid bearing food, *nasha,* for three days. If by this time the patient has not gained full health the treatement is repeated, but this time for only seven days.

Lactic acid-bearing materials are also used to treat bruises on domestic animals. At one time in a village in eastern Sudan, people complained that the milk they bought from Fulanis (originally of West African origin) soured up faster than milk bought from other milk vendors. An illiterate amateur 'scientist' decided to investigate the problem. He discovered that the Fulanis rubbed the cow's udder with sour milk at the time of milking. They claimed that sour milk healed the bruises inflicted on the udder while the cows roamed the thorny acacia grazing grounds. However, during the act of milking a few drops of the sour milk inadvertently find their way into the fresh milk and thus hasten its souring by acting as a lactic inoculum.

Lactic acid as an industrial chemical at the cottage level

In modern brewing technology, the wort in which the yeast grows to produce alcohol must have an acidic reaction. The lowering of the pH is achieved through either the addition of pure lactic acid to the wort or the growing of a suitable strain of lactic acid bacteria in it during the first stage. The lactic bacteria convert part of the sugar of the wort into lactic acid, which would drop the pH to the required level so that the yeasts can grow in the second stage. The bacteria are then destroyed.

In the African cottage, where the brewing of local beer is very common, pure lactic acid is of course not available. Women resort to the second option, harnessing the powers of lactic bacteria to produce lactic acid *in situ* and thus drop the pH for yeast growth. A good example to illustrate this point comes from the brewing of Sudan's opaque sorghum beer, *merissa*. Incubation conditions are so manipulated by the brewer that they become quite conducive to the growth of the homo-fermentative lactobacilli (Dirar, 1976; Dirar, 1978). Within 36 hours the required amount of acid is produced in the sour dough. Now the lactic acid bacteria are destroyed through a tedious toasting and scorching process of the dough in a hot hollow iron container. The resulting intermediate-moisture, granular product is for all practical purposes devoid of living things. This bitter, scorched substance, called *surij,* is inedible but forms an excellent growth medium for yeasts as it has the correct pH value. The major function of *surij* is to act as a carrier of lactic acid besides acting as the source of sugar later when malt is added to it. Yeasts are grown in this substance not to turn it into beer, but to activate the yeast starter. The product of this second fermentation, called *deboba*, is too bitter to be consumed by humans, but it contains vigorously growing yeast. It has to be further processed to give the final beer in the third fermentation stage.

Here then we have a kind of lactic fermentation process, in which lactic acid bacteria are used to produce the acid which is used for an industrial purpose in the cottage commercial brewing process.

Another example of a lactic fermentation process, where the acid is used for an industrial end and is not meant to produce a sour food, can be found in the cottage-level starch or *jir* production. The starch can be put to many uses, including the making of special candies and the cooking of a stiff porridge *(jiriva)*. This last product is meant to have no taste — a virtually bland food item. The souring process is therefore essential for the production of the starch itself and is not meant for the production of a sour food. In the preparation procedure, dehulled whole grains are soaked in copious amounts of water in an earthenware and fermented for up to 15 days. The grains are then sun-dried and milled into fine flour, which is next suspended in a large quantity of water and allowed to stand overnight when the starch would precipitate. The supernatant containing the lactic acid and the other solubles is siphoned off. The starch, which may be washed again, is then sun dried. In the related West African *ogi*, the acid is still there in the starch (Bascom, 1951).

The functions of lactic acid in this process is probably related to the release of starch from the binding components of the grain, such as the protein matrix in which the starch granules are embedded. Lichtenwalner *et al.* (1979) suggested that in cases like this, proteases of the grain itself break down the proteins. But the fact that lactic acid in *jir* fermentation is washed away points to its possible use as an industrial chemical intended to facilitate the release of the starch.

Lactic acid bacteria

Here again, when we talk about bacteria in the context of the African home, obviously we are not talking about pure microbial cultures. Rather, mixed microbial populations of unknown makeup, carried usually in a fermented food material are meant. The presence and predominance of lactic acid bacteria is indicated in such cultures by the presence of lactic acid as verified by taste and odour. Working in close proximity with African women during the preparation of lactic fermentation foods one is tempted to think that these women are aware of the presence of some living thing in the cereal mash and that surely they know the basic properties of that living thing which produces the required acidity in the food.

Lactic acid bacteria and fermented foods

A thorough treatment of African fermented foods involving lactic fermentation is outside the scope of this paper. For a greater coverage of the subject the reader is referred to Odunfa (1985), Odunfa (1988), Muller (1981), Muller (1970), Nout (1981), Novellie (1977), Novellie (1982a, 1982b), Novellie (1986), Dirar (1992a,b) and Dirar (1993).

Opaque beers such as *kefir beer, burukutu, pito, merissa, busa*, as well as clear beers such as *assaliva, dolo, chaknalo, amgba* and *otika* all have lactic acid bacteria as major components of the microbial consortia responsible for their production. Similarly, sour porridges such as *mahewu, ogi, kenkey, tuo-zaafi, aceda., nasha*, etc., all involve lactic

fermentation. African dairy products which undergo lactic souring include *maziwa lala, mlbanick, amaas, rob,* etc. (Nout, 1981; Ndir, 1985; Golberg *et al.*, 1945).

In Sudan, lactic acid bacteria have been isolated from a number of fermented foods. The sorghum dough fermented for the making of *kissra* bread has been found to contain *Pediococcus Pentosageus, Lactobacillus confusus, L. brevis* and other lactobacilli (Mohamed, 1992). In the fermentation of *hulu-mur* (spiced malt-containing dry flakes), members of *Lactobacillus, Streptococcus* and *Leuconostoc* have been isolated (Marhoum, 1987; Bureng, 1979; Agab, 1985). Members of *Lactobacillus* have also been isolated from the dough from *abreh* (El-Sharif, 1993). In the Sudanese fermented dairy products, *rob, Lactobacillus fermentans, Streptococcus lactis,* and other lactic acid bacteria have been isolated (El-Mardi, 1988; Saeed, 1981). Mirgani (1993) and Dirar (1993) have isolated *Lactobacillus helveticus* and *L. delbrueckii* from the fermented camel milk, *gariss*. In the yoghurt-like *zabadi,* the predominant microorganisms are *L. bulgaricus* and *Streptococcus thermophilus* (Suleiman, 1982). Date wines in general contain *Lactobacillus, Streptococcus* and *Leuconostoc*, depending on the type of wine (Ali and Dirar, 1984). Even fermentations undergoing proteolytic breakdown have lactics among their microflora. For instance, *kawal* has *Lactobacillus plantarum* (Dirar *et al.*, 1985) and *siqda* has *Streptococcus* and *Pediococcus* (Elfaki *et al.*, 1991).

In conclusion, it is very hard to find a fermented food product in Africa without the involvement of lactic bacteria.

Management of lactic cultures at the cottage level

In some instances the African woman needs to encourage the growth of lactic acid bacteria, whereas in other instances she would want to suppress their growth. In both fields she had admirably succeeded in achieving her goal within the limits of the cottage givings.

Stimulation of lactic cultures to grow

In the preparation of the sour dough in *merissa* brewing, the brewer wants to encourage lactic acid bacteria to grow. To do this she would create the right conditions which favour the growth of these organisms over other microorganisms. She "knows" that lactic acid bacteria are microaerophilic favouring little sugar (compared to yeasts) and would grow at an initial pH value of around 6.0. She adds very little water to the flour to ensure microaerophilic conditions, while avoiding the use of malt at this stage of brewing which produces more sugar and thus favours the growth of yeasts. The pH of the unfermented sour dough is conveniently around 6.0. She uses no starter or back slop from a previous batch, as she would do in the case of the fermentation of sour doughs for other food products. It has been observed that women use back slops in fermentations involving no metabiosis (succession of microbial species). In the latter case, fermentation is left to proceed spontaneously. This technique produces ample amounts of lactic acid within 36 hours (Dirar, 1978).

Even specific lactic cultures are raised by African women for particular fermentation processes. In doing so they rely heavily on the enrichment technique that students of

modern microbiology know very well. The technique requires ample knowledge of the properties of the microorganism to be enriched for. For instance, in the fermentation of *duma,* a honey wine from southern Sudan, a certain starter culture preparation called *ival-duma* (*duma* seeds) is used. Each family of brewers has its own strain of culture which she keeps as a guarded secret within the family. If one brewer loses her starter culture, which has been handed down from mother to daughter to grandaughter, then a new culture has to be raised through enrichment from the wild. Preliminary research (Dirar, 1993) has shown that the culture consists of a chain-forming lactic acid bacterium and two kinds of yeast. These organisms are all thermotolerant as well as osmotolerant. Women use the roots of the *deleib* palm *(Borassus aethiopum)* as the source of the culture to be enriched for. The enrichment technique quite logically calls for using a warm diluted honey medium of high sugar concentration. The roots are immersed into this medium and incubated for a few days, after which the supernatant is decanted and thrown away. The process is repeated a number of times until a visible whitish paste, representing the culture, appears. More growth of this would result in obtaining a sizeable microbial biomass, which can then be used in the cottage commercial process.

This is actually a highly advanced practice and is right-away biotechnology. Unfortunately, because of the civil strife in the southern part of the country most of the old cultures that have been acquired by families over a long time have now been lost. Women often say that these cultures no longer exist in the wild, indicating that through long use, the domesticated cultures have somehow undergone a degree of improvement over their wild bretheren. It would be very rewarding to carry out research on this very interesting culture of rural people's biotechnology.

Another example of an interesting lactic acid culture prepared by women through the enrichment technique comes from the far north. The culture is called *berbassa* or *khamirat-laban* (milk starter) and is used in the production of a wheat bread called *gergosh.* To prepare the starter, milk is first overboiled to a concentrated state and then a few washed beans of any legume such as broad beans, lentils, pigeon pea, white beans or even coffee beans are added to the hot milk in a can such as that in which powdered milk is packed. The can is then securely covered and buried in a heap of grains in a barrel or a sack. The initial heat of the milk and the warmth provided by the live respiring grains keeps the temperature of incubation warm enough so that within 20 hours a frothy fermentation of the milk results and the starter is now ready for use. There is no research on this culture but it is almost sure to contain lactic acid bacteria. Gaseous fermentation in this starter preparation sometimes results in the cover of the can being blown off. This may also indicate the presence of yeast.

Temperature controls for incubation at the cottage level have also been dealt with by the African woman. In most cases in the Sahel, warm temperatures are sought after during the winter. In the example of the *berbassa* starter given above, use is made of the warmth generated by the respiring cereal grains. In the case of *duma* grains, warm water is used, but as southern Sudan falls in a region that is warm throughout the year there is no problem in providing warm incubation conditions. Problems of incubation at warm temperatures are felt more in areas bordering the Sahara Desert, such as northern Sudan, in the winter time. For example, minimum daily temperatures in the winter commonly

drop to as low as 3^0C in such towns in northern Sudan as Fasher and Dongola. It is in these areas that warm incubation is needed. In addition to the incubation examples given above, some women use a mass of wool as a warm incubator. Others more commonly bury the fermentation vat in a pit in the ground. It was found that ground temperatures undergo negligible temperature variations below the depth of 50 cms., whether between day and night, summer or winter. It was also found that fermentation jars that are buried in the ground for incubation purposes have at least their bottom at that depth.

But a most surprising kind of warm incubation that we have only recently stumbled upon is that which is resorted to in the fermentation process of a certain millet or sorghum product called *hussuwa*. Many a time one observes women cooking food on the usual open fire where the cooking pot is raised on three stones. But what one does not see is the large jar of fermenting *hussuwa* buried in the ground far beneath the fire. An outsider would not know of the contrivance even if he or she lives on the premises for more than two months because the jar once buried is only dug out after three months. The cooking of the three daily meals will ensure a continuous renewing of the warmth of the soil throughout the period of incubation. Both lactic and ethanolic fermentation take place during the process, which finally gives a sweet-sour product.

Suppressing the growth of lactic acid bacteria

In the preparation of *surij* (see above) it has been mentioned that the roasting of the sour dough aims at destroying all lactic acid bacteria in the dough as they are not desired in the next stage of brewing. It has also been mentioned that *surij* is scorched and has visible burnt portions. Unconfirmed data (Dirar, 1978) show that *surij* has an inhibitory or even lethal influence on lactic acid bacteria but not on yeasts, for example. It would be very interesting to check on the effect of burnt sorghum grains on these microorganisms. It is well known in the Sudan that the soaked, roasted or burnt sorghum grains, as a preparation called *galiva* is used as a cure for certain stomach ailments.

But the need for the suppression of lactic acid bacteria is manifest in two distinct areas of traditional food processing in Sudan. One is the technology aimed at producing *assal* (sirup) from sorghum or millet malt. Here the major enemy is the growth of lactic acid bacteria in the material being prepared. Women resort to certain techniques to suppress this growth. The process itself calls for the extraction of sugar from the malt through a laborious procedure involving boiling and cooking for hours and hours. This simultaneously ensures the "sterility" of the product itself. But contamination would come from the utensils and from added water. The procedure is very long and may take two days. At one point along the road the addition of cold water is strictly prohibited. Concerning the utensils, first, in spite of the fact that most fermentation processes are traditionally carried out in calabashes and clay pots, the process of *assal* making is carried out using metal utensils such as oil drums and petrol tins, and that is done for a specific purpose. These metal utensils are amenable to both scrupulous scrubbing and washing and to "sterilization" by solar energy. Every now and then, whenever the container is empty, it is scrubbed, washed and placed in the scorching sun. The temperature of the drum would probably reach 70–80^0C and remains at that value for hours. The preparation method of *assal* is probably the cleanest of all the food preparation methods in rural Sudan. The

major aim behind the cleanliness is to check the growth of lactic acid bacteria. The resulting sweetish extract may either be given to children or more commonly used to produce the clear beer or sorghum wine called *assaliva.*

The second distinct area where lactic acid bacterial growth must be checked is the area of dairy utensils. Traces of milk are extremely stimulatory to the growth of these organisms. Where fresh milk is needed women must find a way of suppressing the growth of these bacteria. The major source of contamination here would be the dairy utensils, in particular the milking pail. The time-honoured milking utensil of Sudan is the *omra* (also called *kahal* and *talamul).* This type of milking pail is thousands of years old. The drawing on the bronze bowl dating back to the ancient kingdom of Meroe, alluded to above, has a clear sketch of the *omra* as we know it today. Shinnie (1967) thought that the *omra* in that drawing was carved of wood. Paul (1950) thought the *omra* of today was smeared on the inside with clay and described it as the most insanitary looking container among the paraphernalia of the nomadic Sudanese women.

Both these authors were wrong. First, the *omra* is actually a basket woven of palm leaves. It is the ingenuity of women that made it possible to hold milk. A thin plastic layer is developed on the inner surface of the utensil by the following technique. The newly woven container is first washed with milk and then turned over a smoking fire for half an hour or so. The smoke chemicals and the milk proteins and lipids, in the presence of heat, undergo chemical reactions that produce a dark "plastic" layer on the inner surface. The process of washing with milk and smoking is repeated until the container becomes impervious. It is this "plastic" layer that Paul (1950) mistook for a clay layer. Now, for years to come whenever the *omra* is to be used for milking it has first to be smoked. When the container is taken off the fire it gives off a hissing sound, which emanates from the numerous small craters that errupt in the plastic layer due to heating. Hot molten fat is also seen on the plastic layer. The wood used to generate smoke preferably comes from the *sarob (tundub)* tree *(Capparis decidua).*

Lactic acid bacteria and other microbes are destroyed or suppressed by the numerous anti-microbial smoke chemicals impregnating the "plastic" layer and by the high temperature of the *omra,* and are effectively fried alive in the hot oil of the layer. The *omra* is thus a most sanitary utensil and is, as Paul himself mentioned, "one in which milk is said to remain sweet longer than any other". The *omra* is never washed with water and when not in use, is kept out in the sweltering heat of the sun on a raised support.

In many parts of Africa milk utensils are often rinsed with cow's urine (Miracle, 1965). Some authors thought that consuming copious draughts of urine-reeking milk by some African tribes was meant to make good the deficiency in table salt (Jack, 1923; Jackson, 1955; Bloch, 1963). But it is very likely that the treatment of milk utensils with urine is meant to hamper the growth of lactic acid bacteria. In line with this, Evans-Pritchard (1937) mentioned that urine is used by the nilotic Nuer of southern Sudan for certain technological purposes such as butter improvement and preservation, which most likely meant control of microbial growth, in particular lactic acid bacteria.

Lactic acid bacteria in relation to food spoilage

Whatever devices women resort to in order to encourage the growth of lactic acid bacteria, occasionally the growth of these bacteria fail and the fermentation is high-jacked by other microbes that cause spoilage. In the fermentation of *kissra* sour dough *(ajin)*, for instance, a defect called *zobara* is known to sometimes take place. The dough develops a bad flavour with some proteolytic smell. The defect is similar to that called *zapatera* in the lactic fermentation of Spanish olives (Smyth., 1928; Fleming, 1982; Vaughn, 1954). Both defects are probably caused by spore-forming proteolytic soil bacteria belonging to the genus *Bacillus.* When this happens in the case of *ajin*, the fermentation jar is scrubbed and washed many times before a new batch of *ajin* (mixed with a fresh starter) is fermented.

In a warm country like Sudan, coliform bacteria sometimes outgrow the lactic acid bacteria in the souring fermentation of milk and bring about a frothy kind of fermentation of a lesser acidic nature, dropping the final pH to about 5.6. The most important coliform bacterium here is *Aerobacter aerogenes (Klebsiella pneumoniae)* (Dirar, 1975).

Coliform bacteria also take over from lactic acid bacteria in the semi-modern cottage cheese industry (a low-input private sector business). These bacteria cause a defect manifesting itself as numerous tiny eyes in the cheese (AOAD, 1983).

The milk of modern farms near Khartoum often does not spoil by natural souring as happens in the case of vendor's milk in the same area. It frequently spoils by sweet curdling caused by a yellow pigmented *Micrococcus* sp. (Dirar, 1975). Apparently the use of detergents and antibiotics select for these organisms while weeding away the lactobacilli and the streptococci.

The growth of lactic acid bacteria themselves in situations when fresh milk is desired, is of course a case of spoilage. A dehydrated milk plant (the Babanousa factory) was erected in Sudan in the late 1960's in an area of nomadic tribes where plenty of milk was produced. During the collection of the milk and its transportation to the factory in bulk, as much as two thirds of the milk went sour before reaching the plant due to the growth of lactic acid bacteria. When artificial cooling was introduced as a remedial measure the loss in milk was reduced to one third, but the cost of production was increased (Dirar, 1985). In a very successful field trial, hydrogen peroxide was used, in accordance with FAO recommendations (FAO, 1957), to collect milk and transport it to the factory with neither loss in the milk nor any mentionable added cost (Dirar, 1975; Dirar and Abdel Gadir, 1976). Unfortunately, no use was made of the results in actual production. At present, the factory is not processing milk, being completely crippled by the souring of milk caused by lactic acid bacteria.

Reflections on modernization and the role of biotechnology

In food fermentations, the metabolic activities of lactic acid bacteria are not confined to the production of only lactic acid through glycolysis. At relatively high sugar concentrations the homolactic fermenters produce lactic acid from hexose sugars, gaining two

moles of ATP. At low sugar concenterations they are able to secure up to three moles of ATP under anaerobic conditions using half of the pyruvate generated through glycolysis as a hydrogen acceptor, and to gain up to four moles of ATP under aerobic conditions via the route of acetyl phosphate, producing in the process much acetic acid and carbon dioxide (Dirar and Collins, 1972; Dirar and Collins, 1973). Lactic acid bacteria are also able to produce compounds other than organic acids. Most important among these in the area of foods are the flavour compounds such as diacetyl (Collins, 1972; Dirar, 1972). The heterolactic fermenters produce lactic and acetic acids and carbon dioxide through the pentose phosphate pathway, and gain one mole of ATP from hexoses (Tortora *et al.*, 1992).

From an African perspective, one should pay ample attention to the importance of the various metabolic end products and secretions that lactic acid bacteria release in the final food. These bacteria might be producing vitamins and minute quantities of health protecting secondary metabolites, such as antibiotics, which have been shown to be produced in other fermented foods of the world (Van Veen and Steinkraus, 1970; Hesseltine, 1965). When El-Tunisi (1850) asked the inhabitants of Darfur of western Sudan why their foods were either bitter (sour) or foul-smelling, they told him that was so because he who did not eat that kind of food in their land should expect disease.

An important point to remember is the fact that lactic acid bacteria in indegenous fermentations are never alone in the fermenting milieu; other microbes, particularly yeasts, are always there. These eukaryotic organisms are usually present in milieus that are primarily undergoing lactic fermentation; conversely, lactics are always present in fermentations that are dubbed alcoholic. It has been observed that often there is a symbiotic relationship between these two groups of microorganisms in cereal fermentations, such as those of the San Francisco sour dough and the Scottish Parisian barm (Wood *et al.*, 1975; Wood and Hodge, 1985; Sugihara, 1985).This situation might exist in many African cereal and dairy fermentations. The container *(khummara)* in which *ajin* for *kissra* is fermented, is often more than 10 years old, during which period it is rarely washed. The starter *(khammar)* that apparently comes to a balanced state through the years is mostly anchored to the tiny crevices and hidden niches of the inner surface of the earthenware jar. Both yeasts and lactic bacteria are found in this starter, perhaps in a symbiotic state. This relationship may prove beneficial to the well-being of the African from a health-promoting standpoint.

One other point is the fact that no pure culture is used in indigenous fermentations in Africa and there is no incubation at a strictly constant temperature in most cases, since incubation is usually at ambient temperatures. Temperatures could vary within 24 hours in Sudan from 20 to 40^0C. It is very important to study the effects of these variables on the various activities of microorgnisms in African fermentations and their nutritional and health implications.

It is, therefore, essential that indigenous knowledge-generated research in the field of biotechnology takes into cosideration all these ramifications of food processing. In developing starter cultures for rural African foods, for instance, a particular emphasis should be placed on the role of these cultures in improving the nutritive value of the final

product. In the make-up of the starter culture no organism should be left out which, under cottage conditions, contributes to nutrition. This would aggravate a nutritional situation that is already precarious.

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II. CASSAVA FERMENTATION

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Traditional Cassava Fermentation

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Cassava, a plant with high energy potential

Cassava *(Manihot esculenta* Crantz) is the staple food for more than 500 million people. It is **the** typical crop in developing countries. Cassava gives a reasonable harvest (9 to 10 tonnes per hectare) and will grow under the broad range of climatic conditions, soils (including the poorest and most acid) and cultural constraints which are characteristic of large parts of tropical Africa, Asia and America (Cooke and Cock, 1989).

Cassava is considered to be one of the most reliable, efficient and economical converters of solar energy (Cooke and Cock, 1989). Cassava requires no cultivation or any particular care until it is harvested. It can be kept in the soil for two years after maturity, thus protecting consumers from the uncertainties of harvests and famines.

Cassava is thus considered to be a key component in famine prevention in African countries in spite of a certain degree of toxicity caused by the presence of cyanogenic glucosides (mainly linamarin) and the highly perishable nature of the roots after harvesting. Native populations have empirically developed several procedures to stabilise cassava and reduce its toxicity. Even though they had no knowledge whatsoever of the biochemical principle of linamarin, they knew that the "bitter juices" had to be removed during pressing to make cassava edible. The fermentation, which is part of almost all these processes, is an important stage in obtaining the required sensorial qualities and for detoxification.

From cassava to *Gari, Chikwangue, Fufu,* etc.

Although all these traditional procedures (Figure 1) use the same raw material and include a fermentation stage, clearly different foodstuffs are produced. The range results from the great variability of the main fermentation parameters:

* the substrate (cassava) is used in different forms: peeled or unpeeled whole roots (*chikwangue, fufu, lafun*, etc.), pulp (*gari*) or raw starch (sour starch); fermentation time varies from a few hours for *attieke*, to two to five days for *gari, fufu* and *chikwangue*, and three weeks to a month for sour starch. The environmental conditions of fermentation are very varied: ponds, rivers, barrels of rain water (*fufu, chikwangue*), canvas sacks (*gari*) or concrete tanks (sour starch).

The major problem in all these artisanal procedures is that the quality of the resulting foodstuffs fluctuates considerably. Fermentation takes place spontaneously through the development of epiphyte microflora and can give results that are undesirable from the organoleptic, microbiological or toxicological points of view. Measures to reduce this variability require, above all, a comprehension of the biochemical, enzymological and microbiological phenomena involved. This should then make it possible to control the fermentation stage and standardize the quality of the products obtained.

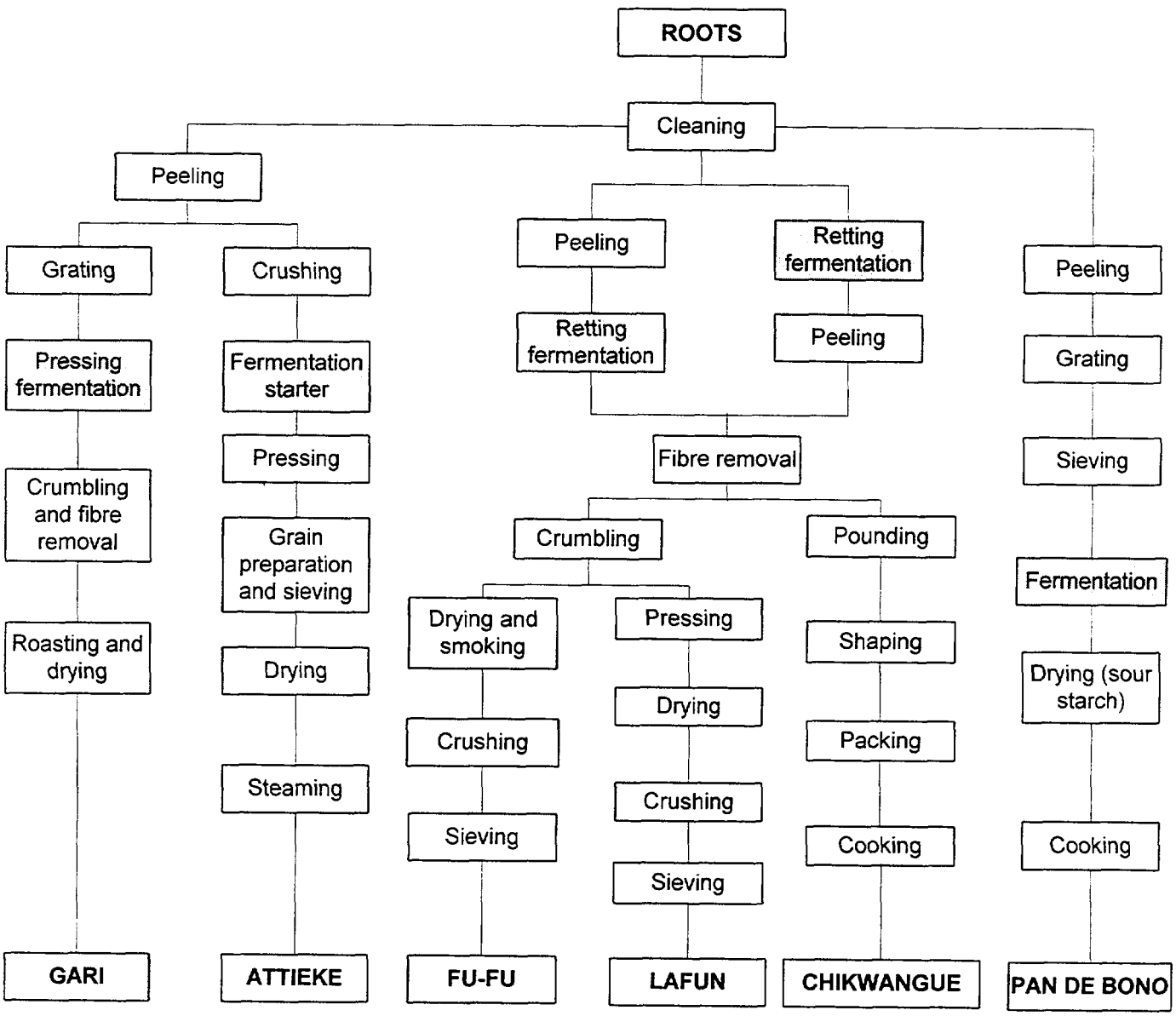

Figure 1: Local cassava processing technologies (a few examples)

Lactic acid bacteria: a dominant microflora

Gari, the fermented cassava product best known to scientists, receives most coverage here. Several microbiological studies have been performed on this fermentation, but authors are far from unanimous on the type of the microflora present:

Collard and Levi (1959) put forward a theory according to which fermentation takes place in two stages:

1. During the first two days, the bacterium *Corynebacterium manihot* develops and hydrolyses a fraction of the starch, producing organic acids including lactic acid. The resulting fall in pH enables spontaneous hydrolysis of the cyanogenic glucosides and release of HCN.

2. In the second phase proposed, conditions become favourable for the development of a fungus *(Geotricum candida)* when sufficient organic acids have been produced. This would produce a variety of aldehydes and ketones characteristic of the taste and aroma of *gari*.

Akinrele (1964) reported that temperature movement was biphasic during fermentation and attributed this to the two fermentation stages previously described by Collard and Levi *(op. cit.)*. He also noted that lactic acid was the main organic acid produced and that there was a direct correlation between the final pH of the fermented pulp and the flavour of *gari*.

In 1977, Okafor contradicted Collard and Levi's theory *(op. cit.)*. He was only able to isolate *Corynebacterium* on one occasion from various samples and in such a small quantity that the bacterium could not be responsible for fermentation. However, he observed predominance of lactic acid flora *(Leuconostoc* sp. (mainly) and *Lactobacillus* sp. and smaller amounts of *Alcaligenes* sp. and yeasts *(Candida* sp.). He concluded that Collard and Levi's results *(op. cit.)* could be attributed to the use of an unsuitable selection medium which excluded lactic acid bacteria. He also suggested that linamarin degradation was probably caused by endogenous linamarase activity rather than activity of the microflora.

Abe and Lindsay (1978) also refuted the findings of Collard and Levi. They observed large quantities of *Corynebacterium manihot,* but showed that the strain developed very slowly and had only a slight acidification property. These authors considered that the dominant microorganism was *Streptococcus faecalis.* This produces volatile compounds, and diacetyl in particular, which account for the odour of the product.

Ngaba and Lee (1979) showed that fermentation of cassava pulp was essentially performed by lactic acid bacteria. *Lactobacillus* species – especially *L. plantarum* and to a lesser extent *Streptococcus* – were responsible for the production of lactic acid and development of the characteristic taste of fermented cassava. Trials on massive inoculation of pulp with isolated *gari* strains were performed and showed that use of *Lactobacillus plantarum* as a starter gave a faster and more substantial decrease in pH. Other research has been carried out on cassava fermentation for

processing into products other than *gari.* The main microorganisms found are listed in Figure 2.

Food Product	Microorganisms Isolated	Authors
Gari	*Corynebacterium manihot, Geotrichum candida*	Collard and Levi (1959)
Gari	*Leuconostoc, lactobacillus, Alcaligenes, Corynebacterium, Candida*	Okafor (1977)
Gari	*Streptococcus faecalis*	Abe and Lindsay (1978
Gari	*Lactobacillus, Streptococcus*	Ngaba and Lee (1979
Fu-fu	*Bacillus, Lactobacillus, Klebsiella, Leuconostoc, Corynebacterium, Candida*	Okafor *et al.* (1984)
Fu-fu	*Lactobacillus plantarum, Leuc. mesenteroides, Lact. cellobiosus, Lact. brevis, Lact. coprophilus, Leuc. lactis, Lact. bulgaricus*	Oyewole and Odunfa (1990)
Fu-fu	*Leuc. mesenteroides, Lact.plantarum, Corynebacterium manihot, Bacillus subtilis, Pseudomonas alcaligenes*	Nwanko *et al.* (1989)
Lafun	*Bacillus, Leuconostoc, Klebsiella, Lactobacillus, Corynebacterium, Candida*	Oyewole and Odunfa (1988)
Sour starch	*Lactobacillus plantarum, Lact. casei, Saccharomyces, Geotrichum candida*	Cardenas and Buckle (1980)
Chikwangue	*Corynebacterium, Bacillus, Lactobacillus, Micrococcus, Pseudomonas, Acinetobacter, Moraxella*	Regez and Schmidt-Lorenz (1988)

Figure 2: The principle microorganisms isolated from different traditional cassava-based food

In spite of the diversity of the results obtained, most authors now agree that there is predominant development of lactic acid bacteria during the various cassava natural fermentation processes. The rapid decrease in pH and the production of lactic acid caused by growth of these bacteria make a considerable contribution to preservation and the organoleptic characteristics of the different foodstuffs. Lactic acid inhibits the growth of many pathogenic microorganisms and has been found to be the main agent

responsible for the flavour of many products. Dougan *et al.* (1983) identified other compounds (aldehydes, ketones, alcohols, etc.), which may contribute to the characteristic flavour of *gari* but stressed that these compounds may either be formed during fermentation or by Maillard reactions during the cooking stage.

One might wonder why there is such a predominance of lactic acid bacteria in all these traditional cassava-based types of fermentation, given the considerable environmental differences between procedures. It could be a result of cyanides in the cassava exerting a selective effect on the development of the various microorganisms during fermentation. While most microorganisms are inhibited by a few ppm of cyanides, it has been shown that lactic acid bacteria can withstand concentrations of over 500 ppm (Giraud, 1993).

Toxicity and detoxification

Cassava toxicity is caused by two cyanoglucosides – linamarin and lotaustralin in the vacuoles; the proportions are generally 96 per cent and 4 per cent respectively (Butler, 1965). According to the theory put forward by Collard and Levi *(op. cit.),* the fall in pH caused by the production of organic acids by the microorganisms during the fermentation stage causes spontaneous hydrolysis of cyanogenic glucosides. However, Wood (1965) disproved this hypothesis by showing that linamarin is particularly stable in a dilute acid medium even at 100^0C. Finally, in 1969, Conn proposed a two-stage linamarin decomposition mechanism (Figure 3):

(a) enzymatic hydrolysis of cyanogenic glucosides with formation of the corresponding cyanohydrins (acetone for linamarin and methyl-ethyl ketone for lotaustralin); and

(b) spontaneous dissociation of acetone cyanohydrin. This compound can dissociate spontaneously at neutral or basic pH and give acetone and cyanhydric acid. The equilibrium pH of the dissociation reaction is about 5.5. Cyanhydric acid (HCN) is a very volatile compound (BP 26^0C) and is rapidly eliminated in the atmosphere.

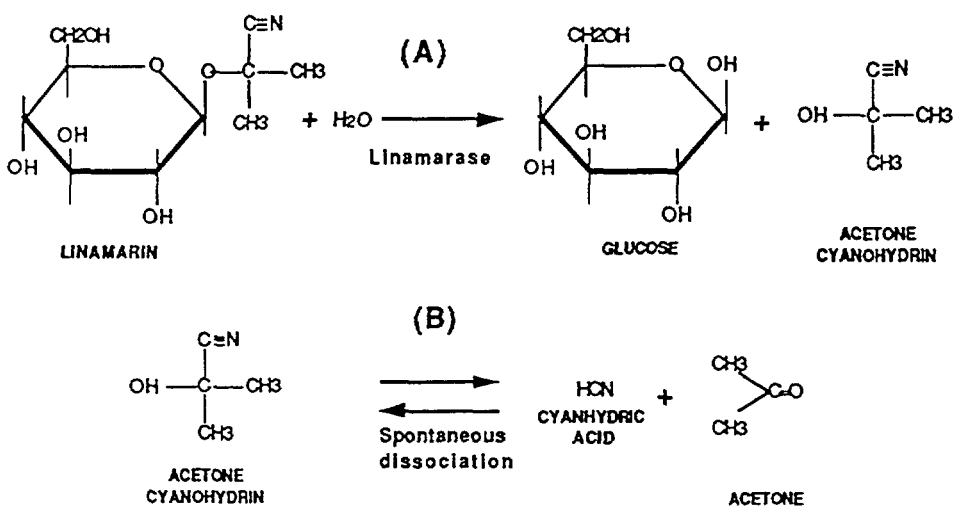

Figure 3: Degradation of linamarin (Conn, 1969)

It thus seems that three forms of cyanide are present in cassava: glucosides (linamarin and lotaustralin), cyanohydrins and free cyanhydric acid. These compounds are eliminated by the combined effects of various enzymatic, microbiological and physical phenomena. The relative effects of the various *gari* processing operations on the elimination of cyanide compounds are shown here (Figure 4).

Two stages seem to be required for satisfactory detoxification (Nambisan and Sundaresen, (1985); Vasconcelos *et al.* (1990)):

(a) grating, in which damage to the plant cell structure releases endogenous linamarase, which can hydrolyse linamarin, producing glucose and acetone cyanohydrin; and

(b) the cooking stage, during which free cyanides (acetone cyanohydrin and HCN) are volatilised.

The fermentation stage causes a rapid fall in pH with various effects on the elimination of cyanide compounds:

- progressive decrease in the activity of cassava endogenous linamarase(Ikediobi and Onyike, 1982a);
- slowing of the rate of dissociation of cyanohydrins into cyanhydric acid when the pH is lower than 5.5 (Cooke, 1978);
- a shift in the equilibrium of ionisation of cyanide into its molecular form HCN. Cyanhydric acid is particularly volatile in this form and thus easily eliminated.

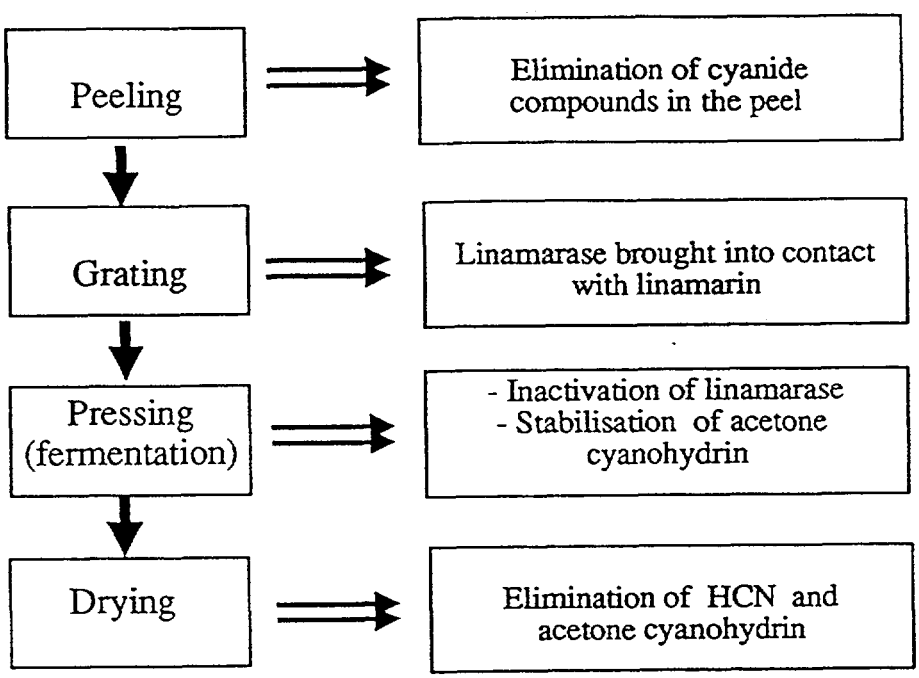

Figure 4: Elimination of cyanide compounds during the various *gari* preparation

Total detoxification of cassava therefore requires the breakdown of cyanogenic glucosides and the dissociation of cyanohydrins, which is unfortunately not often the case. Residual total cyanide contents of some 15 ppm are thus found in *gari*. This average in fact masks considerable dispersion since, for example, the amount in traditional *garis* on markets in Togo and Benin ranges from 10 to 35 ppm cyanides (Chuzel and Griffon, 1989). Local populations thus ingest 5 to 10 mg cyanide daily, i.e. 10 to 20 per cent of the lethal dose.

Human beings – like animals – can detoxify a small amount of cyanide in body tissues. The main process involves an enzyme, rhodanase, and sulphur compounds (sulphates or certain amino acids, especially cysteine and cystine) and gives sulphocyanides eliminated in urine. All tissues contain rhodanase, especially the liver, the main detoxification site. Other pathways are possible, especially that of vitamin B_{12} and the thyroid gland (Oke, 1973).

Thus, depending on the amounts ingested, speed of ingestion and detoxification rate, the amounts of HCN in the body may or may not reach levels causing acute toxicity (50 to 60 mg is a lethal dose for an adult male). Fairly low levels are still dangerous and may cause chronic disorders (Osuntokun, 1972; Delange *et al.*, 1973), such as cretinism, ataxic neuropathy (lack of coordination of movements), goitre (disturbance of iodine uptake by the thyroid gland) and tropical diabetes. It is noted that these problems related to cassava toxicity are only found in very small areas in West Africa where root processing techniques are extremely rudimentary and where the daily intake of iodine, protein and amino acids containing sulphur is very small (Cock, 1982). It would therefore seem that problems caused by cassava toxicity should disappear completely with the extension of appropriate root processing techniques and a suitable complement to the daily diet.

Towards better *gari*

There is apparently insufficient endogenous linamarase to break down the linamarin entirely (Ikediobi and Onyike, 1982 ; Maopoog *et al.* 1989). However, it has been shown (Ikediobi and Onyike, *op. cit.),* that it is possible to reduce toxicity in *gari* by adding supplementary linamarase during the fermentation stage. Okafor and Ejiofor (1986,1990) isolated several microorganisms from cassava pulp that were able to break down linamarin *(Alcaligenes faecalis, Leuconostoc mesenteroides, Saccharomyces cerevisiae,* etc.). They thus suggested that the microflora might also play a role in the cassava detoxification stage and that inoculation with a strain having such a capability would reduce toxicity.

It is noted that use of a microorganism starter had been proposed by Collard and Levi *(op. cit.)* for co-operative societies producing *gari*. They suggested a mixture of pure *Corynebacterium manihot* culture and *Geotricum candida*.

The choice of one strain or a mixture of strains as a starter will depend on the organoleptic qualities required in the foodstuff and the potential of the microorganisms chosen. In the case of *gari,* where detoxification and considerable acidification and high

lactic acid are the main features sought, a set of criteria defining the "ideal" strain could be listed:

- rapid development to outweigh development of the natural microflora and especially that of undesirable microorganisms;
- tolerance to acidity and decrease in pH to 3.9 as rapidly as possible;
- production of a large quantity of lactic acid and mainly isomer L(+) lactate, the only type which can be metabolised by humans;
- hydrolysis of cassava cyanogenic glucosides to reduce toxicity;
- ability to metabolise starch (the main available carbon source);
- genetic stability.

It seems obvious that it is very difficult to find a lactic acid bacterium that meets all these criteria. Advances in molecular biology will doubtless enable progress in this respect. However, inoculation of a non-sterile medium, such as cassava pulp with such a strain, may result in problems of genetic stability. A mixture of several wild microorganisms assembling all these criteria would have more chance of survival under non-aseptic conditions.

It should be noted that Giraud *et al.* (1991 and 1993) isolated a *Lactobacillus plantarum* strain (A6) from fermented cassava, which appears to meet most of these criteria. In addition to its homolactic character and high tolerance to acidity, the strain isolated has considerable capability for hydrolysing linamarin and starch. Inoculation trials were performed on cassava to improve *gari* production. Such inoculation causes change from a heterolactic profile characteristic of natural fermentation to a homolactic profile, more marked and rapid fall in pH (pH 3.8) and greater lactic acid production. Distinct improvement in the acidification profile was also noted; this made it possible to envisage shortening fermentation time to 24 hours. It should nevertheless be noted that no significant improvement in cassava detoxification was recorded; release of endogenous linamarase during the grating phase appeared to be sufficient to achieve total, rapid degradation of linamarin. This result agrees with that of Vasconcelos *et al.* (1990), who observed that 95 per cent of the initial linamarin had been hydrolysed three hours after the grating stage, but differs from those of Ikediobi and Onyike *(op. cit.)* and Okafor and Ejiofor *(op. cit.)*. The differences may be the result of use of cassava varieties containing more or less endogenous linamarase or the use of non-traditional cassava preparation techniques.

Root grating was carried out in some cases with an electric food mill, probably causing complete destruction of the plant structure and enhancing contact between linamarin and the enzyme. The precise influence of inoculation of cassava pulp with a strain containing linamarase activity during the detoxification process should therefore be appraised on the basis of trials performed under real preparation conditions.

Although the use of a strain such as *L. plantarum* A6 appears to be well-suited to *gari* production, it might be debatable for other types of cassava processing. The key points to

be settled for better control of manufacturing procedures are specific to each product. Examples are as follows:

- in the preparation of *chikwangue*, a traditional food in the Congo, the problem of retting cassava roots lies mainly in the break-down of plant cell walls by cellulases and pectinases. The use of pectinolytic or cerulolytic lactic acid bacteria would be more appropriate in this case;
- for sour cassava starch, a common foodstuff in Latin America, the main problem concerns starch structure and characteristics. It can be used for baking after over a month of fermentation. The relation between the fermentation phase and the quality of the product, essentially containing lactic acid as the fermentation product, is not yet known. It is suggested that some strains of *Leuconostoc* might produce polysaccharides which cause physico-chemical changes in the starch and thus make it suitable for bread making.

Towards new foodstuffs

Cassava root contains over 80 per cent starch and gives mainly energising foods. However, the latter contain very little protein as the protein content of cassava roots is only 3.5 per cent. Any diet based mainly on cassava must be complemented with protein-rich foods to prevent nutritional deficiencies. The development of various processes to enrich the protein content of cassava would be extremely beneficial. One way of improving the nutritional quality of cassava would be to use microorganisms *(Aspergillus, Rhizopus,* etc.), which produce protein by developing on cassava carbohydrates thanks to slight stimulation by the addition of inorganic nitrogen (Brook *et al.*, 1969; Raimbault *et al.*, 1985; Daubresse *et al.*, 1987). The most recent work by Soccol (1992) has shown that a final protein content of 12 to 14 per cent could be achieved by fermentation on solid medium using a strain of *Rhizopus oryzae.*

Bread consumption is increasing steadily in many developing countries and they are unable to produce sufficient wheat for their requirements. The sour cassava starch widely consumed in Columbia shows that it is possible – after a long fermentation stage, to obtain products which can be used to bake as bread (*pan de bono, pan de yucca*). It would thus be particularly interesting to develop the use of these cassava flours for bread-making in developing countries.

Acid milk drinks based on lactic cultures are increasingly popular. The nutritional and dietetic merits of starchy beverages in cases of acute dehydration, and especially for cholera, have recently been demonstrated by American researchers. Raimbault (personal communication) suggests the processing of cassava starch suspensions into an acid fermented beverage. Developing countries are short of milk products but possess excess sources of starch and would thus have a new, affordable foodstuff with definite nutritional and dietetic advantages.

Developing new foods is extremely complex, especially if they are for human consumption. They involve work by specialists in different disciplines: nutritionists, microbiolo-

gists, socioeconomists, food engineers, etc. They also require very large financial, human and technical resources. They are subject to numerous safety regulations and are above all open to frequently irreversible criticism by consumers. Traditional fermentation techniques thus still seem to have a substantial future. Even if they can be improved, they form a human heritage, which should be conserved. At a time when modern fermentation processes use increasingly standardized, genetically manipulated strains of microorganisms, traditional fermentation methods may form an excellent "biotope" for the discovery of atypical microorganisms with new potential.

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Application of Biotechnology to Cassava Processing in Africa

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Abstract

Cassava (Manihot esculenta) is one of the most important food crops in Africa and many parts of the tropics. In Africa, fermentation is an important means of processing raw cassava root to food. The roles of various microorganisms in these fermentation processes have been confirmed to include that of detoxification, flavour development and preservation. This presentation reports on our work on the traditional submerged fermentation of cassava and its optimization. The characteristics and roles of lactic acid bacteria in cassava fermentation are also presented while efforts at developing appropriate starter cultures for cassava fermentation are reported. The need to further improve the strains through some biotechnological processes are highlighted while future research needs and further stategies for the biotechnological improvements of cassava processing are presented.

INTRODUCTION

Cassava *(Manihot escutenta* Crantz) is a major source of energy for millions of people in the tropics (de Bruijn and Fresco, 1989) and is also one single crop that is helping to alleviate food crisis problems in many war-torn and drought-ravaged parts of Africa (Hahn and Keyser, 1985). Cassava is currently playing an important role in solving the food insecurity problems in these regions because it has a comparatively high biological efficiency of food energy production and has the ability to survive and grow under very adverse weather conditions, which most other tropical crops cannot resist (Cook, 1985).

In spite of these, cassava is often castigated as an "inferior food crop" (Kwaga, 1986); as "poor peoples' crop" (Hahn and Keyser, 1985) and as a "dangerous crop" (Check, 1978). These myths on the cassava crop were due to some limitations in the crop itself.

The major "limitations" of cassava as a food crop include its potentially toxic cyanoglucoside content, its low protein content and its short shelf-life. Traditionally, the challenges of most of these constraints have been met through processing. Indeed, cassava is not consumed without being processed because processing is known to remove or reduce the potentially toxic glucosides, improve the palatability of the crop as well as serve as a means of preservation (Nambissan and Sundaresan, 1985). Various traditional processing methods are known, which include boiling, smoking, drying and fermentation. Fermentation of cassava is by far the most important and widely used means of processing cassava (Oyewole, 1992).

As of date, the age-old traditional fermentation processing of cassava is still being used. These practices, however, are plagued with so many problems for which modern biotechnology offers the best solution. For example, traditional fermentation processes depend on chance inoculations from the enviroment. As a result of this, the fermentation period is rather long, the quality of the products varies from one processor to the other, or from one production batch to the other by the same processor, and from one season to the other. Improvements in cassava processing should help to reduce the duration of processing to economically viable limits, maximise the detoxifying process for safe products, and improve the physical and nutritional qualities of cassava products.

Biotechnology has been identified as a technology that could be used to meet the current challenges in the traditional fermentation processing of cassava (Bokanga, 1992). This understanding guides our research on cassava fermentation. Our approach to cassava processing research involves investigations into the science of the traditional fermentation process, optimization of the fermentation process, and the improvements of the processes and quality of the products through biotechnological techniques. This presentation will therefore highlight our current understanding on cassava fermentation processes and the important role of biotechnolgy to their improvement.

Cassava fermentation process

The fermentation processing of cassava has been categorized into solid-state and submerged fermentation processes (Oyewole, 1992).

Solid state cassava fermentation

The major feature of the solid-state fermentation processing of cassava is that the cassava root is not soaked in water. There are two major variations of the solid-state fermentation processing of cassava.

The first is typified by the West African *gari* or the Brazilian *farinha de mandioca* production technologies, where peeled cassava roots are grated, packed into polypropylene or jute sacks and subjected to pressure using heavy weights or hydraulic pressure for the three to five days' duration of the fermentation process (Okafor, 1977; Ofuya *et al.*, 1990). The fermented mass is further de-watered after the fermentation process, sieved and roasted [garification] before consumption.

In the second variation of the solid-state fermentation of cassava, the roots are not grated, but cut into pieces or sliced before being spread out in the open air or in the sun (Essers and Nout, 1989). The dried product is then milled to flour and cooked into a stiff dough before consumption with soup.

Submerged fermentation processes

The major feature of this method is that the cassava roots, peeled or unpeeled, whole or cut into pieces, are submerged in water for the duration of the fermentation process (Oyewole and Odunfa, 1989). The duration of soaking varies according to the weather, i.e., where relatively short periods (two to three days) are used during the hot, dry seasons and longer periods (four to seven days) are used during cold, rainy seasons.The fermented products may be wet-sieved and the wet mass cooked in boiling water to a stiff dough, as in Nigerian *fu-fu* (Oyewole and Odunfa, 1989), or subjected to further processing which may include sieving, sun-drying, smoking and milling to flour, which may then be cooked to a stiff dough as in Nigerian *lafun* (Oyewole and Odunfa, 1988) or the Zaïrean *cassettes* and the Tanzanian *makopa*.

Biotechnological investigations

Our works on cassava fermentation have been solely devoted to submerged fermentation processing. Investigations have been carried out on:

i. The traditional fermentation process and its optimizations

ii. The lactic acid bacteria

iii. Starter culture development.

The traditional fermentation process

The submerged fermentation of cassava to *lafun* and *fu-fu* is mainly an acidic fermentation process during which the pH of the cassava root decreases from 6.5–6.9 to 3.8–4.1 after 84 hours of fermentation.

A wide spectrum of microorganisms have been implicated in cassava fermentation. In our report (Oyewole and Odunfa, 1988), we noted the isolation of *Bacillus* spp., *Leaconostoc* spp., *Klebsiella* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Aspergillus* spp., *Candida* spp., and *Geotrichum* spp. A pattern of microbial succession was established to take place during the submerged fermentation of cassava. *Bacillus* spp., *Corynebacterium* spp., and *Klebsiella* spp., which were present at the beginning of fermentation decreased gradually as the fermentation progressed because they could not withstand the increasing acidity of the medium. This first group of organisms however, was found to play an important role in the fermentation process, as most of the strains are capable of producing amylase enzymes needed for the initial breakdown of starch to sugars. The sugar produced is needed for the growth of other microbial groups and for acid production. In the submerged fermentation process, some moulds were oocassionally encountered and, where present, they disappeared after 36 hours of fermentation due to the low oxygen tension which develops in the steeping water. The latter period of fermentation was dominated by the yeasts and lactic acid bacteria.

Similar studies have been carried out on the solid-state fermentation of cassava. The spectrum of microorganisms implicated in the solid-state fermentation of grated cassava root for *gari* production (Okafor, 1977; Abe and Lindsay, 1978; Ngaba and Lee, 1979) were similar to those implicated in the submerged fermentation process with the lactic acid bacteria and some yeasts dominating the latter period of the process. However, the spectrum is different for the solid-state fermentation of ungrated cassava roots. Essers and Nout (1989) reported that moulds predominated in such products, yielding dark-coloured, dry cassava pieces. The moulds implicated include *Rhizopus* spp., *Mucor* spp., *Penicillium* spp., and *Fusarium* spp. The products of such mould-fermented cassava have been confirmed to be safe (Thambirajah, 1989).

The submerged fermentation has effects on the carbohydrate, protein and mineral content of the cassava root (Oyewole and Odunfa, 1989). Fermentation causes a reduction in the starch content. The total soluble and reducing sugar levels increase during the first 36 and 24 hours respectively, but decrease afterwards for the remainder of the fermentation process due to microbial utilization and conversion to organic acids. The fermentation process causes an increase in the concentrations of calcium (+12 per cent) in the cassava root, but effect reductions in the levels of manganese (-53 per cent); potassium (-71per cent); sodium (-68 per cent); iron (-50 per cent); copper (-7 per cent), zinc (-85 per cent); and phosphorus (-67 per cent).

In our investigations on the optimization of the submerged cassava fermentation process through process control (Oyewole and Odunfa, 1992), the size to which the roots were cut was found to affect the rate of fermentation and quality of the product. Also, a temperature range of 30 to 35^0 C was found best for the submerged fermentation process, while a soaking period of not less than 60 hours was found appropriate to obtain a good quality product using the traditional fermentation method. The amylase and pectin-methyl esterase activities were reported to be involved in the cassava root retting of the submerged fermentation process (Oyewole and Odunfa, 1992). Apart from these, we have developed a process for enriching the submerged cassava fermentation with legume protein (Oyewole and Aibor, 1992). The developed scheme, which resulted in the increase of the protein content of fermented cassava from 1.8 to 5.5 per cent with cowpea and 8.2 per cent with soybean involved the addition of 20 per cent of the legume flour to fermenting cassava after 48 hours and their co-fermentation for the remaining period of the fermentation process.

The lactic acid bacteria

Lactic acid bacteria are an important group of microorganisms which have been consistently isolated from fermenting cassava (Okafor, 1970); Abe and Lindsay, 1978; Ngaba and Lee, 1979; Oyewole and Odunia, 1988). The involvement of more than one species of lactic acid bacteria were reported by most of the workers. This necessitated our detailed studies into the spectrum of the lactic acid bacterial flora in submerged cassava fermentation (Oyewole and Odunfa, 1990). Different groups of lactic acid bacteria were isolated from fermenting cassava during *fu-fu* production and they include *Lactobacillus cellobiosus., Lact. bulgaricus, Lact. brevis, Lact. coprophilius, Lact. plantarum* and *Leuconostoc mesenteroides.* A succession trend was also established among the lactic

acid bacteria involved in cassava fermentation in which *Lactobacillus plantarum* predominated in the last 36 hours of the submerged fermentation process (Figure I).

These findings necessitated further studies into the roles and activities of the lactobacilli group in cassava fermentation:

Firstly, the possible roles of the lactobacilli group in starch hydrolysis and the detoxification process were investigated. A total of 43 lactobacilli strains isolated at different times during cassava fermentation were screened for their abilities to hydrolyse starch and linamarin, which is the main cyanogenic glucoside in cassava. Twenty four of the isolates were able to hydrolyse linamarin and most of these (83 per cent)] belonged to the *Lactobacillus plantarum* group. The linamarase enzyme responsible for linamarin breakdown produced by one of our *Lactobacillus plantarum* strains (GL 721) was purified and characterized. The optimal linamarase activities were obtained at pH 5.7 and a temperature range of 30 and 40^0C. The physiological properties of the linamarase elaborated by our *Lact. plantarum* strain was similar to those produced endogenously by cassava plant materials (Yeoh, 1989). Our studies confirmed that the detoxification of cassava during submerged fermentation process, where the roots are not grated, involves enzymes from both the plant material and the microorganisms involved in the fermentation (Oyewole and Odunfa, 1 99 I).

When screened for amylase production, over 80 per cent of the lactobacilli strains were able to hydrolyse starch. Optimal amylase activities of *Lact. plantarum* strain GL 721 was found to be optimal at pH 5.8 and a temperature between 30 and 40^0C.

Because of the detoxifying and amylolytic characteristics of the lactobacilli strains isolated from fermenting cassava, this group of microorganisms was identified as a good candidate for the development of appropriate starter culture for cassava fermentation.

It was envisaged that apart from selecting microorganisms with multiple characteristics for starter culture development, it will be necessary to carry out genetic studies on the chosen strains. Because of the importance of plasmids in the genetic studies of bacteria, the lactobacilli strains were screened for plasmid possessions. Plasmids of various sizes were found to be present in 27 per cent of the lactobacilli strains screened. The sizes of the plasmids range from 2.1 to 52 Kb. However, we are to date not able to correlate the possession of plasmids with the abilities of the isolates to hydrolyse linamarin or starch.

Starter cultures development

In an aspect of our investigation, four different microorganisms (*Bacillus subtilis, Klebsiella* spp., *Lactobacillus plantarum* and *Candida krusei)*, were singly inoculated into sterilized cassava tubers as single inoculum for cassava fermentation (Oyewole, 1990). The effort was useful in identifying the roles of the inocula in the natural fermentation process. While acid production was confirmed in all roots inoculated with the single organisms, highest acid production was recorded for the root inoculated with *Lactobacillus plantarum* while roots inoculated with *Bacillus subtilis* showed the highest rate of retting, although all roots inoculated with each of the organisms were variably retted. The perceived characteristic fermented cassava flavour was noted to be

highest in roots inoculated with *Candida krusei*. The different organisms implicated in the natural fermentation process were confirmed to play specific complementary roles in cassavafermentation

In a similar work, the strain of *Lactobacillus plantarum* (GL 72) was also investigated as a single starter culture for cassava fermentation since it showed good characteristics for cyano-glucoside and starch hydrolysis. (Oyewole and Odunfa, 1991). In this investigation, the rate of acidification was relatively lower than the natural process within the first 36 hours during which the pH remained above 5.0 while it was in the range of 4.4 to 4.7 for the natural process at the same period. Acid production stabilized and increased rapidly to a normal level at the end of fermentation .

Further work still needs to be carried out on the development of appropriate starter cultures for cassava fermentation processes. Starter cultures will help to standardize the processing, optimise the microbial activities in the detoxification and acid production stages to the extent that the duration of processing may be reduced to levels that will meet today's time constraints. There is still the need to develop appropriate carriers for the starter cultures.

Future challenges

In spite of the various work done on cassava fermentation, the age-old method of cassava fermentation is still being practised. The fermentation process is still relatively long and the quality of the products still varies. Our current understanding of the activates of microorganisms still needs to be translated into packages that will benefit local processors.

In our work, we have been able to select a single lactic acid bacterium which has multiple capacities for starch hydrolysis, acid production and detoxifying enzyme production. This is a good candidate for starter culture development. But beyond these, appropriate carriers still need to be found and investigations are still needed on the viabilities of the starter cultures in the various carriers and under different environmental and storage conditions.

It is also not just sufficient to select strains for starter culture development, the strain could still be improved through the various genetic and molecular studies. Cassava-fermenting microorganisms and their enzymes can be engineered to carry out specific desired functions or improved through modern bioteohnological investigations.There is still the prospect to genetically improve the nutritional quality of cassava through the use of protein enriching microorganisms. Also, the problems of cassava processing wastes still need to be challenged with biotechnolgical solutions.

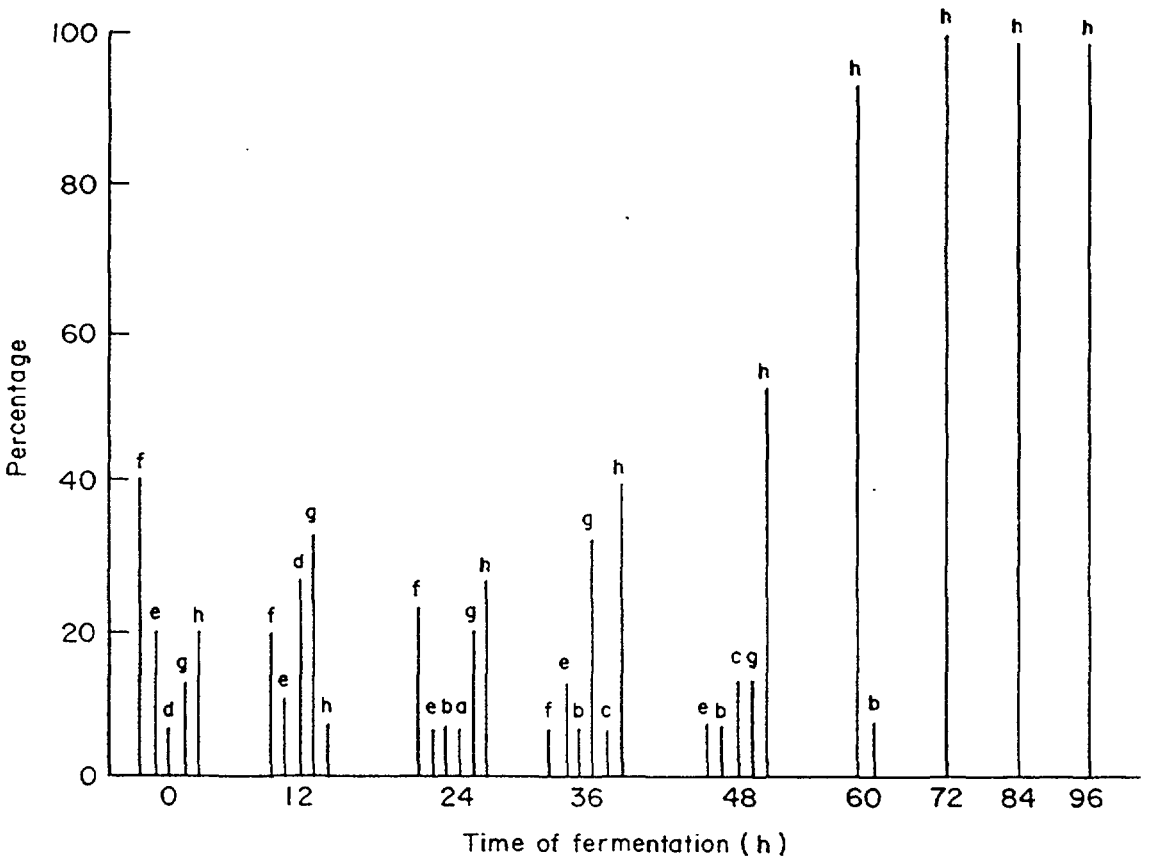

Figure 1: Percentage distribution and succession of species of lactic acid bacteria isolated during cassava fermentation.

a: *Lactobacillus bulgaricus;* **b:** *Lactobacillus lactis;* **c:** *Leuconostoc lactis;* **d:** *Lactobacillus coprophilus;* **e:** *Lactobacillus brevis;* **f:** *Lactobacillus cellobiosus;* **g:** *Leuconostoc mesenteroides;* **h:** *Lactobacillus plantarum.*

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Progress Report on Cassava Fermentation in Zimbabwe

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Targets

The targeting of cassava fermentation is multi-pronged:

- it may be a village level fermentation to produce protein rich palatable food for children;
- it may be targeted at protein enhancement for cattle feed, particularly to help the cattle through a long dry season, as is experienced in Zimbabwe;
- it may be aimed at the production of alcohol (ethanol) for use in motor vehicles or for supplying school and university laboratories, i.e. they can be large or small scale;
- or it may be aimed at deriving butanol and so thinning the diesel in our trucks, buses and tractors.

I am told that in Uganda there are villagers who ferment cassava flour with a locally preserved fungus in a non-sterile process that enriches the protein and produces a very tasty product. All credit to them. This is increasingly a critical component of diet, as often the children get the least nutritious part of the family food and in war-torn situations this can permanently harm the growing youngsters.

I believe a major project in Venezuela, funded by CAFCA, the Andean Development Bank which has strong biotechnology interests (it is encouraging to hear of an all-developing-country-bank that is abreast of the possibilities of biotechnology), is being set up to exploit the enriched-protein-for-cattle idea on a scale of several thousands of hectares of cassava raw material.

Brazil has pioneered the alcohol-for-cars story, with California and other US states taking up the concept in the interests of cleaner air.

As yet I know of no group working on the butanol target, although our Zimbabwean National Programme has some plans and is seeking donor funding.

Progress

At this point I should report on the little we have managed to do so far at the University of Zimbabwe.

Planting material

We have developed *virus-free* material that has increased actual (trials) and potential yields dramatically – from the African average of 6 t/h to a healthy 20 + t/h, the *plus* figure depends upon the rainfall, soil and management available from season to season.

Breeding

We have gone through six cycles of crossing local farmer selections with international (IITA) germplasm, plus trials (measuring and selecting for all kinds of parameters, such as Harvest Index, taste and dry matter content of tubers, tuber shape and angle of descent into the ground as well as various disease tolerances), until we produced six lines that suit several Zimbabwean agro-ecological regimes. The primary selection was however for *yield per hectare* as we were specifically aiming at the cattle fodder and alcohol fermentation aspects of the potential market. As a result we can offer 30 t/h/year in almost any conditions in Zimbabwe, barring the severest of droughts. As you know, in semi-arid areas cassava, like most root and tuber crops, responds in direct proportion to whatever water it receives. Our target has been reached due to a combination of virus elimination, good breeding and the glorious sunshine of Zimbabwe.

Protein content

We have shown that, like others, we can boost protein content of our local lines by the process of dry fungal fermentation in the laboratory, raising protein content to about 17 per cent. This figure is dependent on temperature, pH, and on how long it is possible to sustain the non-sterile process without too much interference from other organisms. We have not solved the problem of how to transfer this technology out of the laboratory to the villages.

Sociology

We have informally surveyed our *rural people* and discovered that the only way they cook cassava is by boiling it and eating it as a side dish. It is not a staple with Zimbabweans, although it is with most of our SADCC neighbours; our *city dwellers* have a rather superior attitude towards cassava, it being beneath their dignity to consume it now that bread and maize-derived *sadza* are readily available all year round; amongst *politicians* there is a widespread fear of being accused of poisoning the voters with cyanide, so there is great caution about this "new" crop, based perhaps on limited information supplied mostly by a poorly informed media that follows tradition and circulation and so highlights problems.

The caution of the politicians is very real and has been a major difficulty to progress. However, circumstances, in the form of the worst drought in our hundred years of meteorologically recorded history, have changed perceptions in that political figures also now wish to find a drought tolerant crop as a food security stand-by. Our President, the Honourable Robert G. Mugabe, in a sense saved our National Programme by reminding the nation in a political speech that cassava was indeed drought-avoiding and a true food security crop that he himself was very fond of, dating from his time in exile in West Africa. This simple political event has encouraged our programme a great deal. I mention it to illustrate that the political and socio-psychological atmosphere circumscribes nearly all our decisions in Africa, if not all over the world. These aspects are often the make-or-break factors in the chain of necessary decisions as to whether or not anything gets done, particularly when dealing with a "new" crop or process.

Cyanide content

Finally, to reassure ourselves and to defuse the above worries, we have measured the levels of cyanide found in *our* cassava, its peel, in the flour we make (via dried chips, which are then put through a maize hammer mill, many of which are locally available), and in the biscuits we can make from the flour. We have also followed the timecourse of cyanide evaporation during boiling to see how long it must be boiled to make it safe.

You can see that here we are basically re-inventing the wheel, but this seems very necessary as politicians and the media are not assured by scientists from other nations, however good and internationally recognised, telling them that all is well based on experiments and results reported from other parts of the world. To cover their fears of losing favour with "the people" they want proof on the ground at home – and someone within their power on the ground at home whom they can blame if things go wrong.

Plans

In terms of plans, Biomass Users Network, an NGO receiving funds from the Rockefeller Foundation through the Commonwealth Science Council, is promising to pursue three of the targets outlined in the first paragraphs: cattle fodder, alcohol and butanol. It should be noted that they are not limited by the constraints of economics and balance sheets: they are donor funded. They in their turn, have held networking workshops and have drawn up a plan where it is clearly shown that all the science, all the methodologies, are currently available.

What is not yet clear is which combinations of technologies and circumstances combine to make **a viable, profit-making operation.** So the issue is now implementation, and whether or not the funding body wishes to leave a functioning, product-producing and profit-engendering plant that will continue to serve the nation(s).

In parallel, I have personally set up a company, Agri-Biotech (Pvt.) Ltd., one of whose targets is to produce planting material profitably to service the above aims. In addition, another target that does not require fermentation is the production of pure starch for industry (paint, textiles, adhesives, food additives). This we see as an important intermediate income generator on the way to more sophisticated fermentation applications.

This comparison may prove an interesting competition between donor and investment funding.

A third proposal has also been made by the fledgling government supported Scientific Industrial Research and Development Centre which proposes to do much the same thing. Funding started in 1992.

Current status

This topic is one where I believe the science has been worked out. There is little further to do. The technology is on the shelf, ready to be transferred, it is available in the scientific literature and it is also there to be viewed and photographed and copied as pilot projects, or in on-going concerns. As yet there is no rush, particularly in Africa, to get the possibilities turned into production. Why not?

Problems?

Firstly, it only appears to be economic in particularly favourable circumstances.

In Brazil the high price of imported oil makes the production of alcohol viable in some years and not so in others, a rather precarious existence for the alcohol producers. The current lower oil prices are forcing the alcohol producers out of production.

For Australia, Greenfield has carried out an economic analysis and come to the conclusion that for Australian conditions in Queensland, where rainfall can come at any time, the energy (money) spent on drying the cassava in order to prevent it from rotting before it dries naturally, renders the sums uneconomic and the proposals non-viable.

In Thailand, with commendable energy a whole industry grew up around the export of cassava chips for use in Europe's pig and cattle industry, to the extent that they were recently shipping ten million tonnes per year. Then EC regulations were passed to erect a trade barrier and the Thai farmers were left to fend for themselves. Again, with courage, their scientists adapted, turning their surplus cassava into starch and into substituted sugars and so forth, and they found more local markets. Just how economically viable this has been has been difficult for me to trace. Again there seems to be some government support for an industry afflicted by the impacts of such regulations.

This example of cassava illustrates the precarious situation that a whole industry can get into if it depends on one buyer, another factor worth thinking about before rushing into fermentation technology and pilot plants.

In South Africa, where a govemment-supported venture, SASSOL, successfully pioneered coal into oil substitution, it was clear that the value fixed by Government on solving their security problem of perhaps being without oil in the event of a successful oil embargo, made it viable in their political judgement to have an (albeit expensive) alternative, which turned out to be coal as a starter, rather than sugar-cane or cassava (both of which were available to the government of the day) to supply starch for alcohol as an alternative oil substitute.

These hard-nosed current decisions should give us grounds for pause before we rush ahead on cassava fermentation for alcohol production particularly.

Is it sustainable, is it viable ?

We believe in Zimbabwe, that we do have special circumstances. We too, are far from sources of cheap oil, and we too have security concerns about running out of oil, and we do indeed already have a successful commercial fermentation plant producing alcohol from sugar-cane resulting in our petrol being currently thinned (20–23 per cent) with alcohol, a situation which has been on-going for the past twenty years, so some of the fermentation technology and expertise is already on the ground.

The question for us is whether or not cassava provides an alternative that is cheaper, more appropriate (from land that does not require irrigation, or high management levels and provides a potential cash crop to resource-poor subsistence farmers), and "doable" (is the the planting material, farming skill, and coordinating, collection and marketing capacity available?).

To answer these questions to the best of my abilities without bothering you with the details because they are only of local interest, I can report that the sums say it is viable, the sociology says it is environmentally appropriate and socially needed.

However, we are at the threshold of finding out if the organisational skills are available.

Organisational capacity

In this context it may be worth discussing how decisions come to be made, how ideas become action, possibilities become products, dreams turn into reality – or to dust. There are a number of models on offer. There is capitalism, socialism and any number of hybrids in between.

The Western, mostly successful, philosophy of controlled capitalism tends to use the "bottom-line" test: does it make a buck? But this is distorted by the "got to keep the voters happy" subsidies. Thus, despite the ideas offered by the World Bank and the International Monetary Fund, the reality lands you back in the harsh world of what suits the power-brokers to keep them in power.

In the case of the West it appears that this process results in farming subsidies, which have the "unfortunate" side-effect that many of the poorer countries, where they can produce food – grain, roots, fruits, legumes, milk and beef – sadly have to do it in the face of competition from subsidised surpluses from the USA and EC. Japan and the former USSR also heavily subsidised their home market, thus protecting them from outside competition where Third World countries could perhaps expect to compete in order to earn foreign exchange with which to pay for their TVs and tractors. This structure distorts and, sadly, totally dominates our economies and therefore our scientific planning. Thus the sums we do to decide on what to pursue concerning cassava fermentation must factor in these international realities.

To put it simply: the world price of corn and the world price of oil are the controlling factors over whether or not cassava fermentation for cattle feed and for oil substitution is viable and worth doing.

Many governments have apparently accepted some of this kind of thinking, e.g. in Zimbabwe in the last two years the government-set price for maize has increased fourfold, as it is thought that cassava is worth about 80 per cent of the going maize price (less, due to its lower protein content, etc.). This then alters the value of cassava fourfold also. How can you plan a five to ten-year Research and Development strategy for cassava fermentation products if the value of your raw material, which is your biggest input, can fluctuate fourfold in two years? The reason behind that sharp rise in our case, was that we were accustomed to producing a maize surplus, so Government chose a price below world prices – like the West, subsidising our urban dwellers – and then the drought forced us to import and pay world prices plus transport costs. So we were forced back to reality, where either the urban consumer or the taxpayer had to pay for the difference.

There is no point in going into details, but I can say that for Zimbabwe, now that our farmers are getting somewhere close to the world price for their maize, it becomes logical in fact to grow cassava because:

a. cassava can outyield maize (only about ten Zimbabwe farmers can exceed ten tonnes/hectare each year and the national average is nearer 6 t/h for big farmers and 2 t/h for small farmers), whereas cassava can be expected to yield 30–36 t/h where 10 dry tonnes of tubers equals 30 "wet" tonnes of lifted tubers;

b. the inputs are less (pesticides, fertilisers, energy); and

c. more labour is needed (planting, weeding, lifting, chipping, drying),

and this can be an advantage in a country suffering from massive unemployment – provided of course that a share of the added value in the crop is passed on to the labourers. (Despite the massive unemployment it is hard to find cotton-pickers in Zimbabwe who consider current wages not worth the pain). So cassava labourers would have to be paid enough to make the work attractive and this may be more than the nation's current minimum agricultural wage. The sums allow for this, and still come out ahead for cassava.

The other competitor, oil, is very expensive in Zimbabwe as the oil price is the world (spot) price plus the transport costs to bring it to our land-locked economic centres: this gives cassava alcohol a margin not enjoyed, in for example, oil-rich Nigeria, and in fact many other developing countries. So the context is again important for the decision makers.

Secondly, there is socialism, command economics, whose advocates are in retreat after the collapse of the Soviet Union and the discovery by the rest of the world of the hidden costs of pollution, suffering and disorganisation. Here the idea is that the needs of the people are met despite "bottom-line" economics, and that the price to square off that

bottom line is to be paid by all the citizens contributing what they are able and wise leaders will make appropritae decisions seeing to social justice.

Most African countries however have decided they want neither naked capitalism nor unvarnished socialism. In between lies some form of responsible economic policy, caring for both people and the environment, that allows "concensus" approaches to problem-solving, with self-sufficiency, dignity and independence as worthy aspirations that are held to be more important for sovereignty than "mere" economics.

Strangely enough, cassava fermentation is a good example to discuss. It is clearly uneconomic in most circumstances, but only just so. Perhaps that should be an end to it and we should not therefore be meeting here. Yet it is potentially a crop and a process that could improve the lot and the life of many a poor farmer and liberate a number of governments from depending on OPEC and oil prices, and to be free of IMF/World Bank "advice" by helping their nations to become more self-sufficient in fuel and food. At this point I cannot emphasise too much that simple low-tech tissue culture is capable of cleaning planting material, particularly of virus, so that the average African yield could be doubled at practically no extra cost. This of course changes all the figures again and makes us all optimists again.

Context and conclusions

Zimbabwe is land-locked and oil-poor, so again alcohol and butanol make a lot of economic sense, which is why we are pursueing it. It also has six months of guaranteed sunshine so we can assume chips can be dried free and so high-energy cattle feed will be marginally profitable and valuable socially. High protein food is worth having, but we have not yet solved the organisational problem of how to "get it together" at the local village level. It may not even be needed as we can get the protein content from soyas. Zimbabwe has another contextual problem, and that is how do we get from small plantings at the rural farm level to the large hectarages near a depot that could sustain an alcohol fermenter and distillery or a cattle feed formulation factory. These plants have to run all year to be economic, so there has to be a reasonably constant throughput every week and the cost of transport to the depots has to be reasonable. With tubers and even chips having a high bulk ratio the fields should not be too far from the factory and some form of transport has to be available to the resource-poor farmer for deliveries. These are not easy problems to solve, but Brazil and Thailand, and I believe India, have solved them, so why not Africa?

III. MUSHROOM BIOTECHNOLOGY

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Mushroom Cultivation: The Nigerian Experience

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Abstract

The cultivation of edible mushrooms is still in its infancy in Nigeria. Although many eat mushrooms, they collect them in their wild state, a practice fraught with the danger of collecting poisonous species along with edible ones.

However, the Nigerian traditional farming sysems indicate the practice of rudimentary mushroom science over the years. Edible species, generally accpeted by the people that could be cultivated commercially have been identified. Growth habits of Pleurotus tuberregium, a very widely used mushroom, have been studied. The production of its edible and medicinal sclerotia in the laboratory on wastes is reported. Its potential role in bioconversion strategy is discussed.

INTRODUCTION

There are many edible mushrooms in the continent but most Africans over the years depend on collecting these species from the wild. This practice is fraught with the dangers of mistaking poisonous for edible ones. As a result there are many reported cases of death due to mushroom poisoning.

Generally, mushroom cultivation had initial problems of development due to lack of basic information on the growth requirements of these fungi. Today the science of mushroom production has been fully developed for many species in the developed countries in Europe, North America and Asia. While these countries are now deriving great economic benefits (via mushroom exports) and enhanced dietary meals (through mushroom supplements in meals), Africa, particularly the West African sub-region, is far behind in this regard.

However, a look at the Nigerian traditional farming systems reveals that there has been a place for mushroom production in the system. African indegenous technology for mushroom production is very rudimentary.

For the continent to take a leap from its present under development of its mushroom potentials, an indigenous mushroom production technology has to be developed since such technologies will be cheaper and easier to sustain and maintain.

Some traditional technologies in mushroom cultivation in Nigeria

The aversion held against mushrooms has witheld the lical population from becoming involved in commercial mushroom cultivation.

Traditionally, there are different ways by which people have consistently maintained an interest in mushrooms. In some parts of Nigeria the local population do the following to produce mushrooms:

Site preservation

This involves taking proper note of the site of occurrence of a mushroom in the growing season (mostly the rainy season). Such sites are regularly visited for mushroom harvesting. The area is protected either by fencing or ring weeding so that it is isolated from other parts of the farm or forest. This practice is commonly associated with *Termitomyces* spp., commonly refered to in the Nigerian tradition as the "king of mushrooms". Such a harvest is highly favoured and is eaten by all members of the extended family system. Some *Coprinus* spp. are similarly treated.

These sites produce mushrooms from season to season, but obviously has limitations to yield, which cannot be improved and depends entirely on the variable natural environmental conditions of a particular season.

Log preservation

As in site preservation, logs known to grow particular edible mushrooms are cut and placed under shade and watered regularly. Mushrooms from these logs are harvested from one season to the other. This method appears to be the nearest to the modern cultivation of mushrooms. *Auricularia* sp., *Schizophyllum* sp. and some *Pleurotus* spp. have been produced by this method.

Soil burial of sclerotia

Pleurotus tuberregium (Fr) Sing produces sporophores and sclerotia when in a suitable environment. Both the sclerotia and the fruits are edible. The sclerotia, which are normally harvested from the bark of decaying or buried wood are used for many purposes, including medicinal uses.

They are buried in soils under shade or a cool place. After five to ten days the sclerotia start producing fruiting bodies in flushes, which are harvested and eaten by the local people. This appears to be the only real traditional method of cultivation, this method but

it also has problems of soil pathogens of the sclerotia as these are easily attacked by soil pathogens and pests, such as nematodes and rodents.

Traditional mushroom preservation

Unconsumed mushrooms are usually preserved by one or other of the following traditional methods:

- Sun drying, involves the direct use of solar energy. The mushrooms are laid out on a mat in an open place that allows maximum exposure to direct sunlight and heat. When the mushrooms are completely dry, they are stored away, used as the need arises, or sold in the market.
- Smoking, whereby the mushrooms are placed in a wire mesh cage, usually suspended a few feet above the traditional cooking tripod, and are left there as long as necessary until the mushrooms are dried. The regular heat/smoke from the coal or wood fire gradually dries the mushrooms. Smoked mushrooms are claimed to taste better than sun dried ones.

Common Nigerian species

In Nigeria, many species of mushrooms are popular and acceptable. These are collected from the forests or grassland areas in their seasons. They are cooked and used in soup preparations, sun dried or smoked for preservation. Mushroom gathering is quite popular among the village youth who make a bit of money from such activity.

A number of edible species (table 1) have been described and identified in Nigeria (Oso, 1975, 1977; Zoberi, 1975; Okhuoya and Okogbo, 1991).

Pleurotus tuberregium and *P. squarobulus* are found usually on wood and on dead or living hosts. The fruit bodies of both species and the sclerotia of *P. tuberregium* are used for soup and medicinal purposes (Zoberi, 1972).

P. tuberregium is a tuberous species common to both tropical and subtropical regions of the world (Okhuoya and Okogbo, 1991). Its sporophores are eaten fresh, or dried for future use. In the dry season, the fungus forms sclerotia which are regarded as a delicacy. Generally the sclerotia are spherical to oval, dark brown on the outside and whitish on the inside and may be subterranean in the host. The sclerotium is very expensive because of its many culinary uses. In soup preparation, it serves as a substitute for "egusi seeds" *(Citrullus vulgaris)*, which is very popular in Nigeria. This mushroom appears to be the most popular fungus which has been attempted for cultivation in the traditional way and most studies have concentrated on how best to cultivate it.

Cultivation of selected species

The most commonly cultivated species are *Agaricus bisporus* (temperate species), *Agaricus bitorquis* (tropical species), *Lentinus edode, Volvariella volvacea* and *Pleurotus* spp.

No one method is suitable for the cultivation of all edible commercial species. Each specie has its own recipe of production. Generally however, mushroom cultivation falls into the following stages:

a. bed preparation

b. spawning (mushroom seed planting)

c. casing

d. picking or harvesting

e. packaging

Each of these stages requires careful handling for best results. *Agaricus* mushrooms are by far the most highly cultivated mushrooms in the world (Chang, 1993). The production of *A. bisporus* stood at 37.8 per cent of world total production in 1989/90 (Chang and Piles, 1991) (see table 2).

Most of the tropical cultivated species are:

Agaricus bitorquis

This mushroom is a temperature-tolerant fungus quite suitable for the African continent, especially the West African sub-region. It grows at a higher temperature, has better shelf life, is resistant to viruses, tolerates higher concentrations of carbon dioxide and produces larger and firmer fruit bodies than A. *bisporus,* the temperate species (Dhar, 1992). It is grown in the hot summer months in Europe to reduce energy costs and grows well on wheat straw. It is yet to be cultivated commercially. Growth trials are still to be carried out in my laboratory.

Auricularia auricula

This fungus is quite common in the wild espcially during the rainy season and on logs under shade. The technology of cultivation is now available, developed mostly in Asian countries. Work is however underway in our laboratory on the indegenous spp.

Volvariella volvacea

This mushroom is a tropical and subtropical species. It is called paddy straw or warm mushroom in Asian countries where its cultivation is very popular. It is still collected from the wild in Nigeria. Another species has been identified in the wild, namely *V. esculenta* Massee.

These mushrooms grow easily on rice, sorghum and wheat straws. Other substrates, such as oil palm fruit fibre and cotton wastes, have been used successfully to cultivate them (Chang, 1993; Zoberi, 1972, 1973; Oso, 1975, 1977).

The major problem with the cultivation of these mushrooms is their poor shelf life. They degenerate very fast if not used within two days after harvest. However it is a very easy mushroom to cultivate, with a short cropping period of 14 days.

Pleurotus tuberregium

This fungus grows naturally in the wild on logs, leaf litter on forest floors and buried wood. It produces both sporophores and tuberous sclerotia on the host. The sclerotia and the sporophores are edible and very well accepted by the local populace.

In Nigeria the sclerotia are edible and used also for medicinal purposes. The situation is different in Ghana, where the sclerotia are only used for medicinal purposes and fattening of malnourished babies (Leslie, 1993, personal communication). The cultivation of this mushroom commerically has become very important because of its varied application in the sub-region.

In the wild, it infects dry wood where it produces the sclerotium, usually buried within the wood tissues, but also found between the wood and the bark (Okhuoya and Okogbo, 1991).

The fungus grows with relative ease in the laboratory (25 – 32^{0}C) and is noted for its rapid growth and causing of extensive wood decay.

Cultivation technology

A. Sporophore production

i. Substrate trials

Several media/substrates have been tested for the growth of mushrooms. These are clay soil, loam soil, sandy soil, sawdust, oil palm fibre wastes, poultry and cattle manure. These substrates were inoculated with sclerotial pieces and left at room temperature (25 – 30^{0}C) for growth.

ii. Effect of removing the bark of sclerotium on growth

Traditionally, the bark of the sclerotium is pealed before being used for soup preparation and these peelings are usually discarded.

The effect of such bark removal on the ability to initiate growth was investigated by burying the peeled sclerotia in the substrate and then observed for sporophore production. The peelings were also tried for growth on the substrate.

iii. Use of farm wastes

Different farm wastes (table 3) were evaluated. These were cassava peelings (*Manihot* sp.), corn straw (*Zea* sp.), oil palm fibre, rice straw (*Oryza* sp.), yam peelings *(Dioscorea* sp.) wild grass straw *(Pennisetum sp).*

These substrates were separately bulked and treated with 5 per cent bleach V/V with a moisture content maintained at 70 per cent.Each substrate was inoculated with sclerotial pieces.

Spawn trials

Oil palm fruit fibre was found, from preliminary experiments, to support extensive mycelial growth of the fungus and was tested as a spawn material for the fungus. This was done by stuffing polyethylene bags with oil palm fruit fibre treated with 5 per cent bleach (V/V) and were then inoculated with sclerotial pieces (25g per bag). After 20 days of inoculation, extensive and compact mycelium (mushroom "seed") had developed on the fibre.

These bags were then opened and the mushroom seed divided into 15g portions and used to inoculate the different substrates. Fifteen days after "seeding" they were then cased with garden top soil. Fresh mushrooms start to appear 20 days after casing. The yields of fresh mushrooms under sclerotial inoculation and oil palm spawn inoculation of different substrates were compared, as shown in table 4.

Weeds and pests associated with cultivation of P. tuberregium

All fungal contaminants and other pests (table 5) associated with the different substrates and mushrooms were recorded. Fungal contaminants growing directly on the sporophores and causing damage or disfigurement were isolated and their pathogenicity tests carried out using Koch's postulates.

B. Cultivation of edible sclerotia

Laboratory induction of edible selerotia

The sclerotia of *P. tuberregium* are formed in the dry season in the host, usually dead wood. Most studies of mushrooms in general, and *P. tuberregium* in particular, have concentrated on factors influencing fruit body production. The induction of edible and medicinal sclerotia by this fungus does not seem to have been studied.

Since these sclerotia are widely used, a study was initiated to investigate their production in the field and establish means for inducing them to form in the laboratory.

Field studies

Field studies were carried out in February and April (when sclerotia of *P. tuberregium* formed in the previous dry season are common) on farms in the savannah area of Edo State of Nigeria. The study of these sclerotia in their natural habitat help to establish their mode of formation. Various trees were examined for fungal rhizomorphs, sporophores and sclerotia of the fungus. Signs of rotting on both dead (or fallen) and living trees were examined and trees affected by the fungus were identified.

Laboratory sclerotia induction

Sclerotia induction of the fungus was tested on various drill dusts in the laboratory. Fungus-free logs (without rhizomorphs) from the following trees were obtained: *Daniella oliveri* (Rolfe) Hutch and Dalz; *Blaeis guineensis* Jacq; *Ceiba petandra* (L.) Gaert;: *Triplochiton scleroxylon* (K.) Schum. Drill dusts from the logs were obtained with an electric drill (half inch diameter) by drilling from the bark towards the core (six

inches deep). The drill dusts were prepared in separate bowls (15cm x 6cm x 5.8cm) which were uncovered to provide four different culture media. Each bowl contained 50g of drill dust. A fifth treatment consisted of a mixture of all drill dusts in equal proportions. There were three replicates of each treatment. Each medium was seeded with a log sclerotium piece. The bowls were incubated at room temperature, 29 + 2^0C, and kept moist by regular watering.

Thirty days after inoculation, when the mycelium of the fungus was found to completely "run" through the various substrates, they were cased with garden soil to a depth of 2 cm. The bowls were left at room temperature and watered regularly to keep them moist.

Log inoculation

Logs of *D. oliveri, E. guineensis* and *T. scleroxylon* were obtained in the forestry department of the Ministry of Agriculture, Benin City. They were cut into sizes of four feet and holes were drilled at six inch intervals from top to bottom and at six inches from each other, using an electric drill (half inch diameter). These holes were inoculated in two ways:

- Use of mycelia spawn, using oil palm fruit fibre spawn, and
- Use of 5g sclerotial piece per hole.

There were three logs of each tree type for each type of inoculation. These logs were left in the open under shade. They were observed for both fruiting bodies and sclerotia production.

Cultivation of sclerotia on wastes

The following wastes were tested for the cultivation of sclerotia of *P. tuberregium*: melon husks, coconut fibre, oil palm fibre, milled cassava wastes, rice husks, corn cob and sawdusts.

These substrates were separately bulked and treated with 5 per cent bleach V/V. Five hundred grams of each of these substrates were separately loaded onto 15 plastic trays, 60 x 60 x 15cm. Twelve of these trays for each substrate were inocluated with 20g of fresh sclerotia at different equidistant points on the tray. Three trays of each substrate were left uninoculated with sclerotia as described, which served as controls. All the trays were uncovered and placed on a laboratory bench at room temperature with natural light/dark cycles. They were all watered every 48 hours with 150 mls sterile water and observd for growth daily.

Results and discussion

Sporophore production

Substrate trials: Loam soil gave the highest yield of mushrooms (table 6) among the different substrates tried. Casing using soil appears necessary in the cultivation of this mushroom, especially if cultivation is to be done through mycelial spawn. Debarked

sclerotia as well as the peelings produced mushrooms independently, showing that the ability to produce fruits is not restricted to any particular part of the sclerotium.

Use of farm wastes

Higher yields were produced on substrates inoculated with sclerotia than those inoculated with spawn except oil palm fibre waste and corn straw substrate. Sclerotia grow directly into sporophores and mycelium. This is not the case with spawn, which has to develop extensive mycelium before fruiting and the more the mycelium developed the greater the yield (Gray 1970).

Higher yields were recorded on substrates cased than those without casing (table 4). Casing has been principally associated with aiding the change from the vegetative phase (mycelium) to a reproduction phase (fruiting) (Lambert 1938).

All the substrates bore different fungal contaminants (table 5.). Of these pests only *Sclerotium rolfsii* caused mushroom stipe rot while the others caused crop failure or disfigurement.

The stipe rot appeared as a yellowish brown rot on the stipe, which prevented the formation of a cap. This disease occured only on substrates that were cased, suggesting that the fungus originated from the casing garden soil. To avoid this, casing soil could be screened for this fungus before use with a baiting technique described by Ikedieugwu and Osude (1977).

Edible sclerotia production

Sclerotia of *P. tuberregium* were produced in the laboratory on drilled dusts of *D. oliveri* and *E. guineensis.*

The observation that sclerotia were formed on drill dusts of *E. guineensis* was surprising, since *P. tuberregium* is not known to form sclerotia on *E. guineensis* in the wild.

Although the reason for their formation is not understood, it may be related to the physical properties of the drill dusts used, since the nature and consistency of substrates have been associated with fruit formation in *P. ostreatus* (Okhuoya and Harvey 1984). Log inoculation technique for sclerotia production was successful only on *D. oliveri* and *E. guineensis.* Both the holes inoculated with spawn as well as sclerotia pieces produced sclerotia after about three months.

These sclerotia were always formed a few centimetres away from the holes, buried between the wood bark and the hard wood.

The failure of *T. sclerozyl* on wood to produce sclerotia could be due to the lack of thick wood bark, which is not common with this plant. The formation of sclerotia appears to be between the bark of the wood and the degraded neighbouring wood tissues.

Cultivation on wastes

The results categorised the substrates into substrates that produced only sporophores, namely melon husks, coconut fibre, oil palm fibre, rice husks, while others, namely

sawdusts, corn cob and milled casava wastes, produced sclerotia. Sclerotia production appeared to be on substrates that did not support extensive mycelial growth of the fungus, while sporophore production was on substrates with much mycelial development. Although the substrates were not analyzed for nutrient, extensive mycelial growth is usually an indication of high nutrient status (Okhuoya and Isikhuemen, 1993; Okhuoya and Okogbo, 1990, 1991; Okhuoya and Ajerio, 1988; Hawker, 1959).

The yield of sclerotia was highest on sawdust, 113 + 6.2g/tray, while the lowest yield was found on milled cassava wastes, 4.5 + 3.0g/tray (table 7). This correlates with the biological efficency (BE) of the fungus on each substrate.

The higher the BE, the higher the yield. The observation that the fungus formed some sclerotia successfully on a carbohydrate waste (milled cassava waste), indicates its potential in the bioconversion of abundant carbohydrate wastes in the enviroment.

Conclusion and suggestions

1. Edible mushrooms abound in the wild in Nigeria and the continent of Africa and are yet to be commercially cultivated.
2. *Pleurotus tuberregium* is an important mushroom as its sporophores (fruiting bodies) and the tuberous sclerotia are edible. The medicinal potential of the sclerotia needs further investigation.
3. The induction of these edible sclerotia in the laboratory using wastes, makes it a potential tool for biotechnological application.
4. Mushroom cultivation should be encouraged by the respective governments through adequate funding of research.
5. Many indigenous species need to be developed through collaborative research/information exchange in a global network on mushroom technology.
6. A Mushroom Growers Association should be established on national and sub-regional levels to promote the science of mushroom biology and mushroom products.
7. Organisation of workshops on mushroom industry and economics.
8. International organizations such as UNIDO, FAO and other agencies should help in the delibrate training of mushroom scientists in institutions within and outside the country that run mushroom science courses, especially at the post graduate level.

Table 1: Common edible mushrooms collected from the wild in Nigeria

Fungi	Habitat
Agaricus spp.	Forest and savannah
Armillaria mellea (Vahlsex Fr) Kummer	Forest
Auricularia auricular Fr	Forest
Boletus spp.	Forest and savannah
Clitocybe nebularis	Forest
Collybia butyraceae Fr	Forest and savannah
Coprinus africanus Fr	Forest and savannah
Coprinus setulosus Fr	Forest and savannah
Coprinus picaceus (Fr) Gray	Forest and Savannah
Lactarius spp.	Savannah
Lepiota sp.	Forest and savannah
Mycena sp.	Forest and savannah
Pleurotus spp.	Forest and savannah
Pleurotus squarrobulus Fr	Forest and savannah
Pleurotus tuberregium (Fr) Kummer	Forest and savannah
Rusula spp.	Savannah
Schizophyllum commune Fr	Forest
Termitomyces spp.	Forest and savannah
Tricholoma sp.	Forest
Volvoriella esculenta (Fr) Sing	Forest
Volvoriella volvacea (Fr) Sing	Forest
Lycoperdon pyriforne Fr	Forest and savannah

Table 2: World production of *Agaricus bisporus* (thousand tonnes fresh weight)

Country	1986	1989/90
USA	285	302
P.R. China	185	170
France	165	200
Netherlands	115	140
United Kingdom	95	118
Italy	75	100
Canada	51	51
Spain	45	50
Germany	38	43
Taiwan, Prov. of China	35	30
Rep. of Korea	18	12
Belgium	16	20
Ireland	16	23
Australia	14	20
South Africa	11	11
Poland	4	9
Others	59	125
Total	1,227	1,424

Data from Chang and Miles, 1991

Table 3: Common wastes as potential substrates for mushroom cultivation in Nigeria

Wastes	Sources
Farm wastes:	
Rice straw	Rice farms
Wheat straw	Wheat farms
Corn straw/cob	Corn farms
Cassava peelings	Cassava mills
Yam peelings	Farms, homes, yam flour mills
Plantain/banana pseudo stems	Farms
Cocoa pods	Farms
Coconut fruit fibre	Farms
Industrial Wastes:	
Wheat husks	Flour mills, breweries
Cotton wastes	Textile mills
Sawdust	Saw mills
Oil palm fruit fibre	Oil mills

Table 4: Average yield of fresh mushrooms per tray[a]

	Sclerotial inoculation		Oil palm fruit fibre spawning	
Substrate	**Uncased**	**Cased**	**Uncased**	**Cased**
Cassava peeling (*Manihot* sp.)	12.85 ± 2.20	23.45 ± 1.70	0	6.20 ± 0.05
Corn straw (*Zea* sp.)	6.50 ± 0.30	19.74 ± 1.05	8.10 ± 3.10	64.30 ± 2.05
Oil palm fruit fibre	0[b]	123.40 ± 3.20	0	130.20 ± 2.86
Rice straw (*Oryza* sp.)	13.26 ± 3.40	39.29 ± 2.80	6.01 ± 0.02	26.08 ± 1.56
Yam peelings (*Dioscorea* sp.)	10.25 ± 1.04	15.74 ± 0.50	0	10.10 ± 0.50
Wild grass straw (*Pennisetum* sp.)	18.58 ± 1.05	32.33 ± 1.70	0	13.10 ± 0.07
River sand	16.54 ± 2.12[b]	—[c]	0	—[c]

(a) Yield is in grams ± standard deviation.

(b) Sclerotum failed to produce mushrooms on the rich oil palm fruit fibre owing to the extensive mycelia developed, but it formed (germinated) mushrooms directly when inoculated onto river sand.

(c) Not determined.

Data from Okhuoya and Okogbo, 1991.

Table 5: Common fungal weeds and pests associated with the mushroom

Fungal species/Pests	Effect[a]	State of mushroom[b]
Aspergillus flavus Link	COS	n.a.
Aspergillus niger van Tiegh	SG;DM	IS
Aspergillus tamarii Kita	COS	n.a.
Botryodiplodia theobrome Sacc.	COS	n.a.
Coprinnuss comatus Gray	COS	n.a.
Sclerotium rolfsii Sacc.	Stipe rot	IS
Penicillium sp.	SG;DM	IS
Physarum polycephalum Schw.	COS	n.a.
Rhizopus stolonifer Lind	COS	n.a.
Schizophyllum commune Fr	COS	n.a.
Xylaria hypoxylon Gray	COS	n.a.
Insects	DM	IS;MS
Nematodes	SNF	Sporophore primordia

(a) COS = contaminant on substrate;
SG = stunted growth;
DM = disfigurement of mushroom;
SNF = sporophore not formed.

(b) Before attack;
n.a. = not applicable;
IS = immature sporophore
MS = mature sporophore.

Data from Okhuoya and Okogbo, 1991.

Table 6: Sporophone production of *Pleurotus tuberregium* under different growth substrates

Substrate	Average time of emergence of sporophore ± S.D. (days)	Average number of sporophores produced per contained ± S.D. (g fresh weight)[a]
Clay soil	12 ± 2	80.5 ± 2.7
Loam soil	20 ± 2	110 ± 3.5
Sandy soil	12 ± 1	56.2 ± 1.8
Sawdust	0	0[b]
Oil palm fibre wastes	30 ± 1	101.0 ± 2.9
Poultry manure	0	0[b]
Cattle manure	40 ± 2	20.3 ± 1.3

(a) Yield after two flushes of sporophore.
(b) Sporophores produced only after fortification with oats.

Data from Okhuoya and Etugo, 1993

Table 7: Yield of sclerotia on selected substrates

Substrate	Average sclerotia yield/tray (g) ± SE	Biological efficiency (%) ± SE
Sawdust	11.30 ± 6.20	24.66 ± 1.24
Cassava waste	45.20 ± 3.0	9.04 ± 0.60
Corn cob	72.47 ± 3.40	14.49 ± 68
Melon husks	0	0(F)
Coconut fibre	0	0(F)
Oil palm fibre	0	0(F)
Rice husks	0	0(F)

S.E. = Standard error
(F) = Fruiting (sporophores formed)

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Mushroom Biotechnology in Uganda

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Abstract

Mushroom cultivation technology was introduced in Uganda in 1991. Pleurotus spp. Nos. 13,14 and 20 were first cultivated on rice straw, a technology obtained from Egypt. By the end of 1991 various materials other than rice straw had been evaluated as substrates for mushroom cultivation. Cotton seed hulls, which are otherwise an environmental hazard, were found to be the best substrate for cultivation of the Pleurotus spp. Wheat, barley and rice straws were also found to give high yields and were recommended to rural farmers who have no access to cotton seed wastes.

Bean and soyabean crop residues were found to give reasonably good yields and were recommended as the next best choice. Maize cobs, lawn grass, dry banana leaves, sugarcane trash (dry leaves) and wood shavings yielded the least, but could still be used economically by farmers who have nothing better, especially if supplemented with soya flour, maize, rice or wheat bran. Other potential substrates are mentioned.

Various methods of substrate treatment, inoculation and pinning are described. Use of spent compost as manure helps recycle the crop residues and hence avoid soil exhaustion through crop removal.

INTRODUCTION

Mushroom biotechnology was introduced in Uganda in 1991. The idea was conceived by the Ugandan Minister of Agriculture as a project that would be suitable for women farmers to enhance their income as well as improve the nutritional status of the Ugandan people. In other words, mushrooms are a nutrition food that fetches a high price on the market and yet their cultivation can be done at home rather than in outside fields. The technology developed in Uganda was therefore aimed at the women small-scale farmers. The technology therefore had to be simple and cheap.

Preliminary research work

From Egypt Pleurotus strains No. 13, 14 and 20 were obtained. In Egypt, these *Pleurotus* strains were being cultivated on rice straw, which is abundant there. In Uganda, rice straw is available in only a few places, so a farmer would need to travel long distances to get it. Therefore there was a need to identify as many organic substrates as possible that would be available to farmers anywhere in the country.

Experiments were then set up to evaluate various organic materials as substrates for *Pleurotus* mushroom cultivation. By the end of 1991 various materials had been identified as suitable for cultivation of *Pleurotus*. Tables 1 and 2 show the materials tried and the yields obtained from each.

Table 1: Mean yield of oyster mushrooms on different organic materials on the first two flushes (Gm)

Treatment Rep.	1	2	3	4	5	6	7	8	Totals
1	137	350	350	240	155	214	173	1224	1,743
2	123	425	144	340	186	172	120	128	1,638
3	104	300	280	274	391	160	106	200	1,815
4	373	360	254	286	280	66	89	250	1,958
5	404	205	146	188	306	110	78	210	1,647
6	250	300	206	200	330	200	62	200	1,748
7	604	270	238	134	302	136	160	100	1,944
8	140	330	350	300	258	177	143	130	1,828
9	160	385	300	360	289	138	59	83	1,774
10	363	380	370	190	330	132	118	62	1,945
Total	2,658	3,305	2,638	2,512	2,827	1,505	1,108	1,487	18,040
Mean	265.8	330.5	263.8	251.2	282.7	150.5	110.8	148.7	

CV = 38.87 per cent

LSD = 78.33

Treatment:

1. Rice straw
2. Barley straw
3. Wheat straw
4. Bean trash
5. Soyabean trash
6. Maize cobs
7. Dry banana leaves
8. Paspalum lawn grass

After this experiment, cotton seed hulls from a soap factory, sugarcane trash from the sugar plantation and wood shavings from lumber yards were tried and gave the following yields:

Table 2: Yields of *Pleurotus* per flush on cotton seed hulls, sugarcane and wood shavings (mean for three flushes)

Material	1	2	3	Total	Mean
Cotton seed hulls	700	1,020	950	2,670	890
Sugarcane trash	300	350	320	970	323
Wood shavings	180	98	112	390	130

From these trials, cotton seed hulls, which are otherwise an environmental hazard, were found to be the best substrate for the cultivation of *Pleurotus* spp. Wheat, barley and rice straws were also found to give high yields and were recommended for rural farmers who have no easy access to the cotton seed processing industries. Bean and soyabean crop residues were found to give reasonably good yields and were recommended as the next choice, and especially for areas where other materials mentioned above are not available. Maize cobs, paspalum lawn grass, dry banana leaves, sugarcane trash (dry leaves) and wood shavings yielded the least, but could still be used economically by farmers in areas where they are readily available and where they have no better choice. These materials could be supplemented with soyaflour, maize bran, rice bran or wheat bran, but farmers have not taken up that technology confidently enough to try it.

Bagasse from the sugar factory and malt refuse from the breweries are potential substrates for *Pleurotus* cultivation and which are abundantly available in some areas, but these have not yet been tried at the research station. They have therefore not yet been recommended to farmers as their treatment and yields are not known. Yet these accumulating materials are an environmental hazard in the areas around the relevant industries. For example,. bagasse is a potential fire hazard, apart from occupying great expanses of land and may also harm the soil. Too much of anything is always bad. If used in *Pleurotus* cultivation it would be turned into a useful material.

Malt from breweries is mainly thrown into Lake Victoria, a fresh water lake that supplies most of the fish eaten in Uganda, Kenya and Tanzania. I am not sure what effect this has on the lake, but it is certainly not the right place for it. It could be used in bioconversion to produce mushrooms or maybe turned into fertilizer.

Treatment of substrate for cultivation of *Pleurotus*

Organic substrates used in the cultivation of *Pleurotus* are mainly ligno-cellulosic materials which the fungus is capable of breaking down by means of enzymes. To facilitate the breakdown of these materials, and also to ensure that only the required fungus, *Pleurotus*, is utilising them, it is necessary to pre-treat them before utilisation by the mushrooms.

The treatment method copied from Egypt was by boiling in water for two hours. This helps to kill other microorganisms and remove some unwanted sugars and phenolic compounds as well as soften the materials for easier breakdown. After boiling, the water has to be drained out to 70 per cent moisture content before inoculation. This could be very inconvenient, especially during the rainy season, as the materials have to be hung up outdoors on a raised rack. Sometimes the farmers are not patient enough to wait till the moisture content is correct so they inoculate wet substrate, which ends up suffocating the mycelia of *Pleurotus*. Another method has been tried and found more suitable for such farmers, i.e. steaming. In this method the moistened substrates are packed in raffia bags of 100 kg capacity then lowered into a drum with little water. When the water is boiled the steam goes through the substrate in the bag since it has no outlet. This continues for six hours. When the substrate is removed it only needs to cool to 30^0C or 25^0C before inoculation. This results in the right moisture content.

Inoculation

This is the addition of mushroom mycelia into the substrate where it will grow and finally produce fruiting bodies. In Uganda, inoculation is done by putting the substrate and spawn in a plastic bag, layer by layer. 100g of spawn is used to inoculate 4 to 5 kgs of substrate. The bag with the inoculated substrate is then sealed and incubated at about 22 to 25^0C, which is the normal ambient temperature in Uganda for most of the year. Incubation, or spawn-run period, lasts for three to four weeks, after which the mycelia will have completely colonised the substrate, which is then ready for fructification.

Pinning

The first stage of fructification is known as pinning. This is achieved when the physical conditions of temperature, relative humidity, light and aeration are changed from those of the spawn-run period, i.e. from 25 to 20^0C from RH 60 to 90 per cent from darkness to light and from semi-aerobic to good ventilation (aeration).

This can be achieved by:

1. Opening windows that were previously closed to allow in air and light.
2. Maintaining porous clay pots full of water in the cropping shed to increase humidity and reduce temperature.
3. Making slits or holes in the bag, or even removing the bag completely to allow outside conditions into the mycelia. Making holes gives the best yield.

The altered conditions stimulates change from the vegetative to the reproductive phase. Four to seven days after this change pinheads appear on the mycelia. These develop and form edible fruiting bodies within four days. After harvesting another flush of mushrooms should occur within ten days. In Uganda, at least six flushes can be obtained under good conditions, but the quality of the mushrooms deteriorates after the third flush. When it is no longer economical to harvest, the spent compost is thrown away into the garden where it is recycled as manure in crop production. This helps to return the crop residues to the soil. Thus soil exhaustion by crop removal is avoided. In an experiment which was not repeated, spent mushroom compost from soyabean trash was found to be

comparable to coffee husks as manure in tomato production. However, this requires further investigation.

New introductions

In 1993 cultures of other species of mushrooms were introduced to Uganda from Italy, Mauritius, Poland, and Thailand. These are currently being multiplied before field trials on various composts can be carried out. The species include *Agaricus bisporous, Agaricus bitorquis, Lentinus edodes, Volvariella volvacea, Auricularia auricular, Flamulana velutipes, Pholiota nameko, Pleurotus ostreatus, Pleurotus pulmonarius, Pleurotus sarjo caju, Pleurotus colombinus* and *Pleurotus cornucoepia.* It is hoped that each agro-ecological zone in Uganda can find among these a suitable strain. There is therefore a need to set up a genebank to ensure availability and preservation of these strains.

Mushroom culturing and spawn production

Culturing and spawn production are specialized technologies that require sophisticated equipment. Lack of this has greatly inhibited the expansion of spawn supply to farmers and hence mushroom cultivation in Uganda. Mushroom culturing means obtaining mushroom mycelia by germination of spores. Spores are contained in a mushroom fruiting body in the gills. Spores can be obtained individually by spore print and germinated to obtain different strains or hybrids, or they can be germinated using tissue culture to obtain offspring similar to the mother. All this must be done under sterile conditions. The media mostly used is potato dextrose agar/PDA (solid) or potato-dextrose liquid media. In Uganda, only PDA is being used. No breeding has yet been carried out in Uganda, only tissue culture is done. Mother cultures are used to make mother spawn by inoculating pieces of culture into sterilised wheat or sorghum grain in bottles. This mother spawn is then used to inoculate other bottles of sterilised grain to make spawn that is supplied to farmers.

PDA media and grain media are sterilised in an autoclave at 121^0C (15 psi) for 20 minutes and two hours respectively. This requires electric power, which is sometimes lacking.

When cultures are mature, they require refrigerated storage, which also depends on unreliable electric power. All the same, culturing and spawn production at the Kawanda Research Institute, using some improvisation, has been able to cater to over 300 farmers, mainly around the city of Kampala, but also upcountry. With the arrival of some equipment in the near future, the prospects of mushroom cultivation are very encouraging.

The Utilisation of Alien Invader *Acacia* Woods for the Production of the Mushroom *Pleurotus*

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In the 19th century, a series of Australian nitrogen fixing *Acacia* spp. were introduced into South Africa for a variety of purposes, including bark production for the tanning industry and stabilisation of sand dunes in the Cape Peninsula. The soils of the southern Cape coast are low in nitrogen and this resulted in the rapid spread of these *Acacia* into areas of indigenous vegetation, the fynbos. Economically, it is very expensive to rehabilitate these areas, with costs exceeding US$ 1,000 per hectare in many cases. The woods from many of these trees has no economic importance, being unacceptable even as a fuel source. The aim of this project is to develop added value to the cleared wood by production of edible mushrooms and so reduce the cost of clearance of these invader plants.

The *Acacia* initially produce dense thickets consisting of trees with a diameter of two to four centimetres, which as the thicket ages, can increase up to a maximum of 30 to 40 cms. Clearing can be carried out totally manually, which is labour intensive and important in areas of high unemployment, or using less labour with the aid of power tools. In order to reduce the presence of piles of dead wood, mechanical chippers are used to reduce the volume of the cleared wood. The cleared ground is usually quite bare and requires reseeding, especially if the thicket is dence and quite old. The seed bed produced by the *Acacia* thicket is very dense and will produce new growth for five years or more if not controlled. Seed germination is induced by heat and therefore controlled burning is not an alternative. Neither is clearing by bulldozers, as the seedbed is disturbed and fresh germination immediately occurs. The cleared wood is chipped using

a mechanical chipper to reduce the volume of the dead wood and avoid burning. It is this chipped wood which is the starting point of these studies.

In this present study we have used three strains of *Pleurotus* obtained from the American Type Culture Collection. All three grow quite well on 100 per cent *Acacia longifolia*, the predominant invader in the Eastern Cape area around Grahamstown, the moisture content being 60 – 70 per cent and 1 per cent ammonia being added. Growth rate was measured by colonisation rate of mycelium and by rate of utilisation of substate measured by the dry weight method. They were also shown to grow on a number of other woods obtainable in the area, namely South African oak, meranti and black wattle, the former two being hard woods obtained from sawmills and the latter, a second invader *Acacia*, originally imported from Australia to be used in the production of bark for the tanning industry.

Addition of either oak or meranti to give 25, 50 or 75 per cent *A. longifolia* mixtures showed that maximum rate of colonisation of the mixture was obtained with a 50 per cent mixture. However, the results do not show a large enough difference to warrent use of a mixture in preference to using 100 per cent *Acacia*. Preliminary studies on other *Acacia* species are equally promising.

When the three *Pleurotus* strains were compared against each other for growth rate on 100 per cent *Acacia* significant differences between strains were obtained. This suggests that further studies on a wider range of strains might identify a strain with a significantly faster growth rate on our substrate. It should also be noted that seasonal temperature variation in South Africa can be as much as 30^0C and choice of *Pleurotus* strain might depend on the climate of the area in which the mushrooms are to be produced. To this end, 22 new strains of *Pleurotus* were brought in from Taiwan Province of China and are at present under analysis for growth rate on various substrates.

Initial studies were carried out on 250g samples. We are now carrying out scale-up trials involving 1 kg and 5 kg production lots. Our productivity at present is on the low side at between 10 and 20 per cent, but this is in part due to problems of controlling humidity levels.

The original aim of this work was envisaged as resulting in small scale production of these mushrooms by farmers for the food market using waste material from the clearing of invader plants from their farms. This will have two economic benefits: first, it will make the clearing of such invaded areas and their return to farmland more economic, and secondly, employment will be created by clearing the land and in mushroom production. In South Africa, a small amount of *Pleurotus* is already on sale produced by conventional technology on maize straw. However, the market isnot large and a marketing/costing study is at present underway as part of this project. Interest has also been expressed by forestry companies as a way of disposing of their sawmill waste and negotiations are underway in this area. It should be noted that in South Africa, mushroom production from cellulose substrates is envisaged as a cash crop which will require distribution and sales rather than a supplementary food resource for the rural population.

Bioconversion of Agricultural Wastes: Mushroom Cultivation in Thailand

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Abstract

The straw mushroom (Volvariella volvacea) was the first mushroom cultivated in Thailand. Its cultivation technology was successfully developed in 1937 and extended to the rice farmers in 1938. Using rice straw as the substrate it was widely accepted and has become an integral part of the rice farming system in the central region of the country where rice straw is always abundant. With slight modification, farmers in other parts of the country can cultivate the straw mushroom, utilizing their locally available agricultural wastes, such as mungbean pods and cassava wastes. In addition to the straw mushroom, six other kinds of mushroom are being cultivated. These are the oyster mushroom (Pleurotus ostreatus), P. sajor-caju, the ear mushroom (Auricularia polytricha), shiitake (Lentinula edodes), hed khon (Lentinus squarrosulus) and button mushroom (Agaricus bisporus). With the exception of the button mushroom, the rest are cultivated on pararubber (Hevea brasiliensis) sawdust. The current annual mushroom production volume exceeds 80,000 tons with a value of over US$ 80 million. Approximately 10 per cent of the production is exported in both processed and preserved forms.

INTRODUCTION

One of the most efficient ways of making use of agricultural wastes and plant residues is to convert them first to edible mushrooms and then turn them into compost. Even though Thailand is moving towards industrialization, the most important sector in her economy is still agriculture. Being an agricultural country, she has abundant supplies of agricultural wastes and clop plant residues. Rice straw alone is produced at around 40 million tons annually. Other major crop residues are cassava wastes, sugarcane bagasse, corn cobs and stems, pararubber sawdust and pineapple leaves and stems.

These are usually thrown away or turned into compost except for those for which technologies have been developed for their use as substrates in mushroom production.

The first mushroom cultivated in Thailand was the rice straw mushroom (*Volvariella volvacea*). Its cultivation technology was sucessfully developed in 1937 and extended to rice farmers in 1938. Using rice straw as the substrate, it was widely accepted and straw mushroom cultivation has become an integral part of the farming system of rice farmers in the central region of the country ever since.

Current mushroom production

At least seven species of edible mushroom are commercially produced in Thailand. They are the straw mushroom (*Volvariella volvacea*), the oyster mushroom (*Pleurotus ostreatus*), *P. sajor-caju*, the ear mushroom (*Auricularia polytricha*), shiitake (*Lentinula edodes*), hed khon (*Lentinus squarrosulus*) and the button mushroom (*Agaricus bisporus*). With the exceptions of shiitake and the button mushroom, which are temperate species, the rest are tropical. The current production exceeds 80,000 tons with a value of over 2,000 million baht (US$ 80 million). Details are given in table 1.

Table 1: Species of edible mushroom commercially cultivated, volume and value of production in 1992

Species	Volume (tons)	Value (million baht)*
Volvariella volvacea	65,000	1,625
Pleurotus spp.	12,000	240
Auricularia polytricha	5,000	75
Lentinus squarrosulus	6,000	180
Agaricus bisporus	300	10
Lentinula edodes	150	15
Total	88,450	2,145

* 25 baht = US$ 1

Mushroom trade

Most of the mushrooms produced are domestically consumed. Usually they are consumed fresh with the exceptions of the ear mushroom and shiitake, which are prefered in the dried form. The straw mushroom and button mushroom are also canned. About 10 per cent of the mushrooms produced are exported in both canned and dried forms. The import-export statistics from 1982-1992 taken from the Department of Custom's Annual Reports are given in table 2.

Most of the imported mushrooms are dried shiitake from the Republic of Korea, The People's Republic of China and Japan, and most of the exported mushrooms are dried ear mushrooms and canned straw mushrooms. These are exported to countries in Asia,

Table 2: Import-export value of mushrooms from 1982 to 1992 (baht)*

Year	Import	Export
1982	19,978,114	6,717,721
1983	29,946,854	6,124,084
1984	38,006,216	8,272,649
1985	13,681,913	13,762,217
1986	4,814,622	7,721,847
1987	1,892,514	11,566,318
1988	9,564,291	37,324,987
1989	13,127,314	140,352,751
1990	40,040,010	252,285,982
1991	73,422,600	245,037,777
1992	70,336,624	259,232,327

* 25 baht = US$ 1

the Middle East, Europe and the USA. The larger markets by far are Europe and the USA. Other countries that also export tropical mushrooms are the People's Republic of China, Indonesia, Viet Nam and Taiwan Province of China. From table 2 it is very interesting to note that mushroom exports have increased dramatically from 1989 onwards. This is mainly due to significant increases in the export volume of preserved straw mushrooms.

Number of mushroom farms

There are two types of mushroom growers in Thailand – those who grow mushrooms as their main occupation, and those who grow mushrooms as an additional source of income. In the first category, the growers cultivate mushrooms in permanent mushroom houses. Species of mushrooms grown are *Pleurotus* spp., *L. edodes, L. squarrosulus, A. polytricha, A. bisporus* and *V. volvacea* (protected culture). Growers in the second category are mostly rice farmers who grow straw mushrooms, utilizing the remaining rice stubble left over in the fields after harvesting. They grow straw mushrooms by the open field method during the slack period (February to May) of each year. The number of growers in the first category is about 5,000. The number of non-permanent growers fluctuates from year to year, depending on the price of straw mushrooms and the availability of straw. However, in certain areas, such as Pa-chee District of Ayudhya Province in the central region, and the Chum-pae District of Khon Kaen Province in the Northeast, growers produce straw mushrooms throughout the year, employing the open field method. The number of growers of this type is very difficult to estimate. There are roughly over 10,000 non-permanent (or seasonal) mushroom growers in the country.

Size distribution of production unit

Mushroom farms are rather small, although this depends on the species of mushroom being grown and the size of the local market. The average mushroom farm that produces 60 to 100 kg of fresh *Pleurotus* mushrooms per day usually has two fruiting houses with 5,000 polypropylene fruiting bags in each house. The farm will need to produce 300 to 500 new bags daily to be able to sustain this level of production.

For the open field straw mushroom farms, the size of each operation (bed area) ranges from 47 m^2 to 118 m^2, depending on the season and the substrate used. Details of the average size of open field straw mushroom production per family per cycle of production yield are given in table 3.

Table 3: Average bed area of straw mushroom production per family per cycle (15 days)

Substrate		Area (m^2)	Yield (kg)	Income (baht)
Mungbean pod	S	82	70.9	925
	R	76.1	60.0	766
	W	77.6	74.0	1,014
Rice stubble	S	94.7	69.6	1,220
	R	86.2	55.4	1,548
	W	46.4	41.9	1,465
Rice stalk	R	117.3	75.4	1,800
	S	117.8	79.3	2,076.7
	W	107.5	51.8	1,675.8

S = Summer
R = Rainy season
W = Winter

Current production technology

Technology for producing mushrooms in Thailand could be divided into two systems, depending on the type of production unit. One system is to produce mushrooms in polypropylene bags, another is to produce mushrooms in a bed-type unit. The latter system is further sub-divided into open field culture and protected culture where mushroom beds are constructed within the house, i.e., *Agaricus*, and the protected culture of *Volvariella volvacea*.

The polypropylene bag method

This method utilizes heat resistant propylene bags as the production unit. The major substrates used in Thailand are pararubber (*Hevea brasiliensis*) sawdust supplemented with various organic and inorganic compounds such as rice bran, sugar, rice flour,

magnesium sulphate, calcium sulphate, etc., to render the final mixes suitable for the target mushrooms. Partially composted rice straw is also used. The prepared substrate is packed into 13 x 17 inch polypropylene bags and sterilized for three hours at 98^0C and left to cool. Spawns, which are usually made from sorghum grains, are then inoculated into the bags. After the bags are incubated for the required period of time, the mushroom mycelium will ramify throughout the substrate. Depending on the mushroom species, the length of time required is between 22 days for *P. sajor-caju* to 120 days for *L. edodes.* They are now ready for fruit body induction, maturation and harvesting. Mushrooms produced by this method are *Pleurotus* spp., *A. polytricha*, *L. edodes* and *L. squarrosulus* and can generally be used to produce wood-colonizing edible mushrooms.

Thai mushroom growers extensively use pararubber sawdust in producing wood-colonizing mushrooms. The sawdust is obtained from sawmills that work only on pararubber wood. The supply is abundant and continuous because certain acreages of pararubber plantations are becoming too old to be economically viable. The old trees need to be removed and seedlings planted. Pararubber wood makes good furniture and there are quite a number of factories that produce it. Pararubber sawdust is quite suitable for growing all wood-colonizing edible mushrooms and needs no composting.

The low-bed method

The low-bed method is the simplified version of the original high-bed method for growing rice staw mushrooms in the open field condition. This method requires a wooden frame of 100 x 30 x 30 cms (length by width by height) dimension. Rice straw is steeped in water for two to three hours and packed into the wooden frame until the layer is 15 cms thick. Spawn is then placed on top of this layer along the rim of the bed. The second and third layers of straw and spawn are similarly packed into the wooden frame. The top layer is then loosely covered with rice straw and the whole bed is further covered with a plastic sheet. Finally, the plastic sheet is covered with straw so that the bed surface is not directly exposed to sunlight. A group of 10 to 20 beds could also be constructed in a long row and covered together with one plastic sheet. Temperature and moisture of the bed is maintained by properly manipulating the rim of plastic sheeting, e.g. to let in air. Mushroom primordia begin to appear on the nineth day and picking is already possible on the twelfth day. The second and third (final) flushes come three days apart. Average yield per bed is 0.8 to 1 kg from 8 kgs dry rice straw.

After the harvest is completed, the partly degraded rice straw can be turned into compost. This compost has been extensively used in Thailand to improve soil texture and fertility. It contains higher total nitrogen (N) content, but lower potassium (K) content than the original rice straw. Results of the chemical analysis of rice straw and its derived compost after growing straw mushrooms are given in table 4.

In the areas where straw is sometimes not available there have been some modifications to the technique to accommodate the locally available wastes. For example, in the Northeastern part of the country where serious drought is common, farmers have to grow mungbeans instead of rice. The mushroom growers can grow straw mushrooms on mungbean pods supplemented with cattle manure and coarse rice bran. Bed dimensions have to be adjusted to a mere 100 x 30 x 15 cms, because the bed temperature rises very

Table 4: Chemical analysis of rice straw and its derived compost after growing of straw mushrooms

	Nitrogen %	Phosphorus %	Potassium %	Oxygen/ carbon %	Carbon : Nitrogen
Rice straw	0.565	0.117	3.171	45.3	80.2
Compost	1.05	0.165	1.732	38.4	36.6

Source: A.N. Yadav, 1988.

quickly. In a traditional bed size, the heat will accumulate and eventually kill off the mushroom mycelium. Similar adjustment is also needed when cassava wastes are used as the growing substrate. In contrast, in the Southern part of the country where pararubber sawdust and water hyacinths are abundant and rice straw is scarce, the mushroom growers can also grow straw mushrooms on these two substrates. Bed dimension in this case is altered to 100 x 30 x 30 cms with eight alternated layers of pre-wetted sawdust and fresh water hyacinth.

The protected culture method

This method is used for growing straw and button mushrooms. It requires accurate composting and peak-heating processes and relatively larger capital input than the other methods. Superior compost for growing straw mushrooms is usually made from cotton waste amended with a few other organic and inorganic compounds. Since cotton waste is scarce in Thailand it has to be imported from other countries just for mushroom growing.

Research programmes

Current research programmes place special emphasis on strain improvement of all economic mushrooms such as the straw mushroom, the button mushroom, *Pleurotus* spp., shiitake and tropical *Lentinus spp.* Included in the programmes are improvement of substrate mixes, spawn production, disease and pest controls and the collection and exploitation of naturally occurring mushrooms. One additional area that is very interesting is the development of a growing method for *Pleurotus*. spp. on untreated rice straw, which will lend itself to mechanization and subsequent cost reduction.

Conclusion

During the period of 56 years from the inception of the mushroom industry in 1938, many new developments of the production technology have taken place. Some were locally developed by researchers or growers and others were introduced. Many more so-called agricultural wastes have been added to the substrate list for mushroom cultivation. Mushrooms are now grown more for income than for food. It is observed that canneries which export processed mushrooms play a vital role in price stablization and expansion of the industry. There have been gradual changes in the production strategy and the technology used. Farms are becoming larger and the technology more cost-effective to be able to deliver products that are more competitive. It is evident that continuous improvement of the production technology is crucial to the sustainability of the mushroom industry in Thailand.

In addition to mushrooms, which is the direct product of this technology, there is huge a amount of compost by-product which is almost equally important. Activators for different types of substrate have been developed in this connection.

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CONCLUSION

The conclusions reached by the symposium are very well defined. The papers which dealt with the lactic fermentations of foods gave examples of such fermentations and delved into the possibility of actually developing and using lactic starter cultures composed of LAB strains isolated from the microflora indigenous to the particular food. The difficulties to be encountered in this respect were discretely pointed out and solutions suggested. The lactic acid bacterial species involved in lactic food fermentations and the ones selected to be used as starters have, in some instances, been identified. The importance of fermentation in general as a low-cost method of preservation has been emphasized and the role played by lactic acid bacteria in making foods safer to eat in warm climates and under unsanitary conditions of preparation have been pointed out. It was subsequently concluded that these food fermentations are very useful for Africa and that the fermentation processes known in the continent be advanced and modernized. The microorganisms involved should be isolated, identified and deposited safely in some culture collection facility. Research in this area should continue and be supported by such organizations as UNIDO and the African research workers be trained and organized into a biotechnology association. In addition, support should be given to entrepreneurial efforts to upgrade and commercialize fermented food products in Africa.

With respect to cassava biotechnology, it has been pointed out that cassava has a number of advantages as a food source in Africa. It can be grown in a wide range of soil types and climates, requires little attention, and can be stored underground for years, which makes it an excellent famine reserve. It is in fact a major food crop in Africa today and a variety of food products are made from it. The processing of cassava involves a fermentation step during which the toxic substances, linamarin, is removed and a desirable flavour developed. There is more than one technique for cassava fermentation, and various microorganisms seem to be involved in these methods, including bacteria, yeasts and moulds. But lactic acid bacteria seem to be the most dominant among the microflora of fermentation. Lactic bacterial strains which possess the characteristics required in a suitable starter culture, with even linamarase activity, have been isolated and tried as starter cultures in some instances in an attempt to modernize cassava processing technology. Two problems of cassava as a food source are its low protein content and the residual toxicity detected in the final food product, even after fermentation and heat treatment. Both these problems can be solved through biotechnology.

The culturing of non-toxigenic moulds on cassava was found to increase the protein content substantially, and the use of linamarase-processing starter cultures is envisioned to help remove the last traces of toxicity from cassava foods. It was the view of the symposium participants that all efforts to modernize the processing of cassava in Africa be supported strongly. It is therefore concluded that measures should be taken to isolate and characterize the microflora involved in cassava fermentation and preserve them in centres for culture collection. It is important, too, to develop effective starter cultures for cassava fermentation. Research scientists in the field of cassava biotechnology should be linked through networking and the exchange of relevant information be made to flow

easily between them through electronic systems. Furthermore, efforts must be made in the development of cassava bioengineering to simplify such processes as peeling of the tubers. Training of scientists must be well sustained and research on cassava coordinated.

In the field of mushroom biotechnology it was stated that many communities in Africa have known traditional mushroom collection and consumption and some even carry out a form of cultivation of these macrofungi. But modern ways of mushroom farming have only reached the continent very recently, and that only in research institutions which made use of experiences in this field in non-African countries, such as those of Asia and Europe. Simple and economically feasible techniques of mushroom cultivation are now being sought after in some African countries, especially suitable for poor farmers, in particular women farmers. Both locally collected and imported mushroom strains are being tested for production on various substrates of agricultural wastes. The indigenous mushrooms which abound in the wild are being characterized and their properties studied in order to assess their potential for commercial production by modern controlled cultivation. Their natural pests and diseases that hamper growth and minimize yields are also being studied. Some research centres are already releasing spawn to farmers on a limited scale, using imported mushroom strains. Agricultural wastes tested as substrates for mushroom cultivation are variable and include sawdust, cereal straw, water hyacinths, cotton seed hulls, etc. It was thus decided that an African Mushroom Biotechnology Council be formed and linked to the international Mushnet. In fact, immediate steps were taken to establish this body by naming the members of the council, which consisted of five persons. Moreover, the symposium recommended that electronic systems should be established to facilitate dissemination of information and interconnect researchers in the field of mushroom biotechnology. It was also recommended that five centres for mushroom studies be established across Africa for research spawn production and culture collection of mushrooms. At the same time, enterprises in mushroom technology should be encouraged.

In addition to the above three themes on food fermentation, cassava biotechnology and mushroom biotechnology, the symposium discussed and gave recommendations on the advancement of biotechnology in general in Africa. This can be summarized in the following points:

1. Networking of the African biotechnologists and information through electronic systems.
2. Developing culture collection centres for microbial strains involved in food fermentations and mushroom technology.
3. Training African biotechnologists.
4. Encouraging biotechnology aspects other than those of the three themes of the symposium above. This includes the raising of virus-resistant strains of plants and mushrooms, biological control of pests, etc.

5. Connecting and linking African scientific organizations with similar regional and international organizations.

6. Urging UNIDO and similar agencies to help fund the activities of the networks and organizations suggested or actually formed by the participants in the Dakar and Ibadan symposia.

7. Training and organizing women working in the area of traditional biotechnology and helping them modernize their enterprises.

APPENDIX

TECHNICAL SYMPOSIUM ON FOOD FERMENTATION TECHNOLOGY
Dakar (Senegal), 13 – 17 December 1993

EXECUTIVE SUMMARY

The African Agency for Biotechnology (AAB), African Regional Centre for Technology (ARCT), and Food Technology Institute (ITA) of Senegal co-sponsored UNIDO's 13 – 17 December 1993 Dakar Technical Symposium on Food Fermentation Technology. The Symposium participants initiated the creation of a professional organization of African biotechnologists whose mission incorporates developing new and enhancing existing African (1) biotechnology networks, (2) scientific databases, (3) culture collections, (4) subject matter electronic exchange networks and help desks and regional (North, South, East, West, and Central) bioinformatics nodes, and (5) technical training of biotechnologists in these and related fields.

These and other biotechnology capacity-building initiatives, adopted unanimously by the Symposium, offer an enabling framework for African scientists to reinforce and focus biotechnology more effectively in the service of Africa's scientific research, biodiversity conservation, technology transfer, and sustainable development interests. Electronic networking and bioinformatics help desks acting as clearinghouses would strengthen mutual African and international scientific and development interests. The Symposium recommendations and action items respond to UNIDO's call for "dialogues by biotechnology associations" and complement UNIDO's thematic, commercialization and biosafety aims for biotechnology in less developed countries.

This nascent African biotechnology organization will link its five core activities with efforts in the areas of: (1) lactic acid bacteria fermentation, (2) cassava processing, and (3) mushroom and microbial biomass conversion biotechnologies targeted by UNIDO. Initiatives enhancing the introduction, breeding and diffusion of viral-resistant plants and fungi, biological control of pests, and other biotechnological work in sustainable natural resources development (especially biodiversity conservation– and food-related) are identified as needed and complementary.

African network development priorities include: promoting expanded and new collaborations with the international scientific community (Microbial Research Centres and international agricultural research centres, Microbial Strain Data Network, etc.); cooperation with African multilateral organizations such as AAB and ARCT, African nongovernmental scientific organizations such as the African Academy of Science, and African expatriate and African-American science and technology capacities; enhancing the status of African women in biotechnology, including support for African women-led

biotechnology industry initiatives; and linking international environmental programs with African biotechnology developments.

The Symposium selected a Steerng Group to implement these initiatives. The representative of ARCT expressed official support. Symposium participants unanimously recommended an early dialogue with UNIDO and a group of experts, to address these capacity-enhancing steps as well as the funding needs of the proposed organization's Secretariat and networking activities.

STEERING COMMITTEE MEMBERS

The participants in the Dakar Technical Symposium unanimously agreed to establish a "steering group". This Steering Committee is intended to serve both as a mechanism for following up with UNIDO the recommendations and decisions of the Symposium participants and as the governing board of a Secretariat for an organization of African biotechnologists.

The following individuals were nominated and, by concensus, affirmed as members of this Steering Commiftee:

1. N.Gnange Felix, University of Abidjan Labo-Genetique (FAST), Abidjan, Côte d'lvoire
2. Papa Kandji, Institute of Food Technology (ITA), Dakar, Senegal. Dr. Kandji was nominated by Dr. Felix as his alternate member of the Steering Group and this was agreed by concensus.
3. Nabil Mohammad Magdoub, Microbial Research Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt
4. Frederica N. Nkakyekorera, Kawanda Agricultural Research Institute, Kampala, Uganda. (Dr. Nkakyekorera is also the chairperson of the African Mushroom Biotechnology Council advanced at the Dakar Symposium.)
5. Olusola B. Oyewole, Department of Food Science, University of Agriculture, Abeokuta, Nigeria

In addition, Dr. J.J. Kojo Asiedu, African Regional Centre for Technology (ARCT) offered himself to the Symposium as the Centre's representative to, and liaison with, the Steering Group. This was unanimously agreed to by the Symposium participants.

Chairperson — Although no chairperson of the Steering Group was elected by the participants in the Symposium, the members of this Steering Group concurred in Dr. Olusola B. Oyewole's role as the Group's liaison and coordinator.

FOOD FERMENTATION TECHNOLOGY INITIATIVES

The Symposium's Lactic Acid Fermentation Technology Working Group emphasized the value of a UNIDO-supported, diversified biotechnology capacity development initiative in Africa. It recommended that UNIDO consider taking measures to advance

fermentation technology capacities in three food products (cereals, kissra, risogurt), in culture collections development, and in the area of technical training. It called on UNIDO to:

1. Facilitate establishment of a comprehensive system of culture collections of important food industry micro-organisms for Africa. This could involve upgrading existing culture collection facilities such as the one located at the Ain-Shams University-based Microbial Research Centre (MIRCEN), which could serve as one of the regional culture collections for Africa and support the needs of national agricultural research systems (NARS) in Africa;

2. Sponsor workshops and training courses in state-of-art biotechnology in such areas as genetics of lactic acid bacteria and recombinant DNA technology for African scientists in the national agricultural research systems (NARS);

3. Support the research project on "Industrialization of Lactic Acid Bacteria Fermentation Technologies of Cereals and its Dissemination in Developing Countries" undertaken in the collaborative effort led by the Korea University in Seoul, and the promotion of a collaboration between African institutions, such as the Institute of Food Technology (ITA) of Senegal, and the Korea University;

4. Support small-scale entrepreneurs who produce *Kissra* (a staple sorghum bread) with provision of specific starter cultures and technical assistance from food technology and/or other appropriate divisions of such facilities as at the University of Khartoum, for the development and applications of appropriate technologies for the dissemination and propagation of cultures by end-users; and

5. Support the Institute for Food Technology (ITA) in Dakar, Senegal, for technology transfers of processing techniques developed at Korea University, Seoul, for the production of "Risogurt".

CASSAVA BIOTECHNOLOGY INITIATIVES

The Cassava Working Group confined its formal recommendations to its particular areas of interest. However, it also discussed broader concerns about enhancing African biotechnology capacities.

Among this Working Group's specific recommendations to UNIDO were the following:

1. Assist in establishing a culture bank for microbial cultures for food fermentation in Africa, with emphasis on, but not limited to cassava;

2. Support research on the development of starter cultures and the efficient transfer of such technology to end-users;

3. Assist in establishing an electronic network among Africa's national agricultural research systems (NARS); regional institutions, such as the African Regional Centre for Technology (ARCT); international institutions, such as the International Institute for Tropical Agriculture (IITA), the Centro Internacional de Agricultura Tropical (CIAT); and others, for the electronic exchange of information on cassava processing;

4. Support a clearinghouse and coordination activity regarding the many cassava fermentation and cassava biotechnology meetings regularly held in Africa and beyond. It was requested that UNIDO intercede to help facilitate coordination of such meetings;

5. Assist in improved and continued dissemination of existing cassava processing technology, such as peeling, and assist institutions involved in the development of cassava technology;

6. Strengthen bioengineering capacity in Africa through collaborative programs between relevant research institutions from Africa and beyond, such as the Korea University, Seoul;

7. Facilitate the exchange of information on training for African scientists in food biotechnology; and

8. Assist in accessing support funds, such as stipends and scholarships, for African students and research scientists for technical training on existing and new food fermentation technologies in the industrialized countries. Where appropriate facilities are available in Africa, such technical training should also be provided within African countries.

MUSHROOM BIOTECHNOLOGY INITIATIVES

The UNIDO December 1993 Dakar Technical Symposium on Food Fermentation Technology adopted its Mushroom Biotechnology Working Group's call for a comprehensive African Mushroom Science and Industry Initiative. This incorporates (1) forming an African Mushroom Biotechnology Council (AMBC) in cooperation with international collaborators and linked to MUSHNET (the international mushroom information initiative of UNIDO), (2) five regional mushroom technology centres, (3) a culture collection system, and a programme for (4) studies and technical exchanges, (5) education and training, and (6) mushroom enterprise development.

1. The AMBC is intended as a network for African biotechnologists involved in mushroom-related development, to work in cooperation with UNIDO, MUSHNET and the Chinese University of Hong Kong and international scientific organizations, such as the Microbial Strairi Data Network (MSDN). The effort is envisioned as a long-term collaboration, strengthened by electronic communications, training and technical exchanges with biotechnology resources of other African organizations, such as the food fermentation and cassava biotechnology networks, Africa-based international agricultural research centres (IARCS) and Microbial Research Centers (MIRCENs), the University of Benin, University of Zimbabwe, Ain-Shams University, and other African Universities, and African technical institutions, including the food technology institutes.

2. AMBC mushroom biotechnology centres in North, South, East, West and Central Africa are to serve as regional and inter-regional information clearinghouses and technical assistance facilities. Help desks and local information nodes are to provide access to scientific information facilities and culture collections worldwide.

3. The five centres are to be linked to an African culture collection system for indigenous mushroom and general fungal culture collections and to microbial laboratory and bioinformatics capacity development. The centres are to facilitate exchange of strains, spawn production, importation of new strains, and distribution of cultures.

4. A development studies program will foster research in such key areas as pest and virus control; new strains, culture collection development, and mushroom and general fungal conservation; and the development of manuals (for growers, spawn producers, food safety, uses of biomass wastes, preservation and packaging, and marketing). The emphasis on end-user needs.

5. Education and training include: design of public education materials for general consumers and curricular guidance materials for mushroom, general fungal and microbial biomass science in elementary, secondary and higher education; intensive training and technical exchanges for growers, food processors, and scientists; and information dissemination and training regarding intellectual property rights in research and development of mushroom, general fungal and microbial biomass uses. The centres are to facilitate technical training in culture collection, laboratory and bioinformatics development for national and regional agricultural research systems and other public and private sector interests, including African women's organizations and development programmes (both professional and grassroots level) in particular.

6. The enterprise development component emphasizes biotechnology opportunities by African women. It includes development skills transfer, a finance facility supporting venture opportunities for both food growers and processors, and the design of cost-efficient model mushroom production units (for large and small scale production).

AFRICAN MUSHROOM BIOTECHNOLOGY COUNCIL (AMBC)

Initial Members

1. Frederica N. Nkakyekorera, Kawanda Agricultural Research Institute, Kampala, Uganda. **Chair;**

2. J.J. Kojo Asiedu, African Regional Centre for Technology (ARCT), Dakar, Senegal;

3. John A. Okhuoya, Department of Botany, University of Benin, Benin City, Nigeria;

4. Moussa Souane, Head, Biotechnology Laboratory, Institute of Food Technology (ITA), Dakar, Senegal

5. Ralph Kirby, Department of Microbiology, Rhodes University, Grahamstown, South Africa;

In addition, four other Symposium participants located in Africa noted their interest in the AMBC.

These were Nabil Mohammad Magdoub, Microbial Research Centre (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt; Hamid A. Dirar, Faculty of Agriculture, University of Khartoum, Sudan; Mohammed A.M. Msabaha, M.A.R.T.I. Uyole, Ministry of Agriculture, Mbeya,Tanzania; and I. Robertson, Crop Science Department, University of Zimbabwe, Harare, Zimbabwe.

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