

CURRENT STATUS AND OPTIONS FOR BIOTECHNOLOGIES IN FOOD PROCESSING AND IN FOOD SAFETY IN DEVELOPING COUNTRIES

SUMMARY

Food processing converts relatively bulky, perishable and typically inedible raw materials into more useful, shelf-stable and palatable foods or potable beverages. It also contributes to food security by minimizing waste and loss in the food chain and by increasing food availability and marketability. Food is also processed to improve its quality and safety. Biotechnology makes use of microbial inoculants to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of foods through fermentation which is also widely applied to produce microbial cultures, enzymes, flavours, fragrances, food additives and a range of other high value-added products. Fermentation processing in most developing countries is more art than science and, in low income economies, it often makes use of a rudimentary technological base with poor process control resulting in low yields and products of variable quality. Spontaneous fermentations and those which use “appropriate” starter cultures produced largely through backslopping (a process which uses samples of a previous batch of a fermented product as inoculants) are widely applied at the household and village levels in developing countries. With increasing research and development (R&D), a number of pre-cultured single or mixed strains of micro-organisms, called “defined starter cultures”, have been developed and are being used by small manufacturers in their fermentation processing operations. Defined starter cultures are also imported by a number of developing countries for use in processing operations.

Traditional methods of genetic improvement such as classical mutagenesis and conjugation can be applied to improve the quality of microbial cultures. Hybridization is also used for the improvement of yeast strains. Molecular biology techniques are widely employed in R&D for strain improvement. While these techniques are common in developed countries,

they are only now beginning to be applied in developing countries for the improvement and development of starter cultures. For example, random amplified polymorphic DNA (RAPD) techniques have been applied in Thailand in the molecular typing of bacterial strains for the production of fermented pork sausage (nham), leading to the development of three different defined starter cultures, which are currently used for the commercial production of products with different flavour characteristics. Genetically modified (GM) microbial cultures are used in the production of enzymes and various food processing ingredients. Rennet, which is widely used throughout the world as a starter in cheese production, is produced using GM bacteria. Thailand currently uses GM *Escherichia coli* as an inoculant in lysine production. Many industrially important enzymes such as alpha-amylase, gluco-amylase, lipase and pectinase, as well as bio-based fine chemicals such as lactic acid, amino acids, antibiotics, nucleic acid and polysaccharides, are produced in China using GM starter cultures.

Food safety is defined as the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use, and food safety along the food chain includes the good agricultural practices that establish basic principles for farming (including aquaculture), soil and water management, crop and animal production, post-harvest handling and treatment, good manufacturing practices for storage, processing and distribution to the consumer. Biotechnology is widely employed as a tool in diagnostics to monitor food safety, prevent and diagnose food-borne illnesses and verify the origins of foods. The techniques applied in the assurance of food safety focus on the detection and monitoring of hazards. Biotechnological developments have led to the widespread availability of methods of identification that are more rapid and less costly than those based on conventional techniques. Polymerase chain reaction (PCR) and enzyme-linked immunoabsorbent assay (ELISA) methods are now applied in the detection of major food-borne pathogens.

Genome sequence information coupled with the support of advanced molecular techniques have enabled scientists to establish strategies to protect consumers from pathogens and provided industry with tools for developing strategies to design healthy and safe food by optimizing the effect of probiotic bacteria, the design of starter culture bacteria and functional properties for use in food processing. These advances have, in turn, led to more precise diagnostic tools and the ability to quickly develop efficient, specific and sensitive detection kits for new microbial strains. Kits are now also available for the detection of mycotoxins which are major hazards associated with pulses and grains, the raw material inputs for a number of traditional fermented foods in many developing regions. The identification of food ingredients and the origins of foods through traceability studies have also been enhanced by molecular methods.

5.1 INTRODUCTION

Food processing makes use of various unit operations and technologies to convert relatively bulky, perishable and typically inedible raw materials into more useful shelf-stable and palatable foods or potable beverages. Processing contributes to food security by minimizing waste and losses in the food chain and by increasing food availability and marketability. Food is also processed in order to improve its quality and safety. Food safety is a scientific discipline that provides “assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use”¹.

Biotechnology as applied to food processing in most developing countries makes use of microbial inoculants to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of foods. The process whereby micro-organisms and their enzymes bring about these desirable changes in food materials is known as fermentation. Fermentation processing is also widely applied in the production of microbial cultures, enzymes, flavours, fragrances, food additives and a range of other high value-added products. These high value products are increasingly produced in more technologically advanced developing countries for use in their food and non-food processing applications. Many of these high value products are also imported by developing countries for use in their food processing applications.

This Chapter describes the prospects and potential of applying biotechnology in food processing operations and to address safety issues in food systems with the objective of addressing food security and responding to changing consumer trends in developing countries. It is important to note that food safety evaluation or risk assessment is not covered here, the Chapter instead focusing on the context of biotechnologies as applied to food safety.

Technologies applied in the processing of food must assure the quality and safety of the final product. Safe food is food in which physical, chemical or microbiological hazards are present at a level that does not present a public health risk. Safe food can therefore be consumed with the assurance that there are no serious health implications for the consumer. Recent food scares such as mad cow disease and the melamine contamination of food products have increased consumer concern for food safety. As incomes rise, consumers are increasingly willing to pay a premium for quality, safety and convenience.

A range of technologies are applied at different levels and scales of operation in food processing across the developing world. Conventional or “low-input” food processing technologies include drying, fermentation, salting, and various forms of cooking including roasting, frying, smoking, steaming and oven baking. Low income economies are likely to employ these as predominant technologies for the processing of staple foods. Many of

¹ Recommended International Code of Practice - General Principles of Food Hygiene (Codex Alimentarius Commission, 2009)

these technologies use a simple, often rudimentary, technological base. Medium levels of processing technologies such as canning, oven drying, spray drying, freeze drying, freezing, pasteurization, vacuum packing, osmotic dehydration and sugar crystallization are widely applied in middle and upper middle income economies. Higher-level, more capital-intensive food processing technologies such as high-temperature short-time pasteurization and high-pressure low-temperature food processing are widely employed in middle and upper middle income economies. Functional additives and ingredients produced using fermentation processes are generally incorporated into food processing operations that make use of higher-level technologies.

Traditional methods of food safety monitoring such as the detection of pathogenic bacteria are generally based on the use of culture media. These are the techniques of choice in low and lower middle income economies which lack the resources, infrastructure and technical capacity to utilize modern biotechnologies. Conventional bacterial detection methods are time consuming multi-step procedures. At least two to three days are required for the initial isolation of an organism, followed by several days for additional confirmatory testing. Biotechnology based methods can provide accurate results within a relatively short time frame. Biotechnological developments have resulted in the widespread availability of low-cost rapid methods of identification compared with the significant cost/time requirements of conventional techniques. Lower middle income economies apply both traditional and more sophisticated methods for monitoring the microbiological quality of foods and their conformity with international standards.

A number of case studies are described in the text to demonstrate the utility of biotechnology-based applications in food processing and food safety. These case studies provide the basis for the development of strategic interventions designed to upgrade food processing and food safety in developing countries through the application of biotechnology.

This paper is divided into two main Sections – “Stocktaking: Learning from the Past” and “Looking Forward: Preparing for the Future”. In the first Section, Part 5.2 provides a brief definition of biotechnologies; Part 5.3 gives an overview of the current status of the application of biotechnologies in developing countries; Part 5.4 provides an analysis of the successes/failures of the application of biotechnologies in developing countries and underlying causative factors; and some case studies of applications in developing countries are described in Part 5.5. In the second Section, Part 5.6 deals with a key issue in the sector where application of biotechnologies might be useful; Part 5.7 proposes options for developing countries to make informed decisions about the application of appropriate biotechnologies; and Part 5.8 presents priorities for action for the international community.

A. STOCKTAKING: LEARNING FROM THE PAST

5.2 BIOTECHNOLOGY: DEFINITION AND SCOPE

For the purpose of this Chapter, biotechnology is defined in accordance with the Convention on Biological Diversity (CBD), i.e. “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use”.

Biotechnology in the food processing sector uses micro-organisms for the preservation of food and for the production of a range of value-added products such as enzymes, flavour compounds, vitamins, microbial cultures and food ingredients. Biotechnology applications in the food processing sector, therefore, target the selection and manipulation of micro-organisms with the objective of improving process control, product quality, safety, consistency and yield, while increasing process efficiency.

Biotechnological processes applicable to the improvement of microbial cultures for use in food processing applications include traditional methods of genetic improvement (“traditional biotechnology”) such as classical mutagenesis and conjugation. These methods generally focus on improving the quality of micro-organisms and the yields of metabolites. Hybridization is also used for the improvement of yeasts involved in baking, brewing and in beverage production. *Saccharomyces cerevisiae* strains have, for example, been researched for improved fermentation, processing and biopreservation abilities, and for capacities to increase the wholesomeness and sensory quality of wine (Pretorius and Bauer, 2002). Methods employed in genetic R&D of wine yeasts are described in detail in Pretorius (2000) and some are summarized in Table 1.

TABLE 1

SOME METHODS EMPLOYED IN GENETIC R&D OF WINE YEASTS

Method	Comments
Hybridization	Cannot generally be used directly, but the method is not entirely obsolete. Has been used to study the genetic control of flocculation, sugar uptake and flavour production. Cross-breeding and hybridization of spore-derived clones of <i>S. cerevisiae</i> have also been accomplished.
Mutation and selection	For example, to induce autotrophic and de-repressed mutants for efficient sugar fermentation and ethanol tolerance.
Rare mating	Mixing of non-mating strains at high cell density (ca. 10^8 cells/ml) results in a few true hybrids with fused nuclei. Cytoduction (introduction of cytoplasmic elements without nuclear fusion) can also be used to impart killer activity (using karyogamy [nuclear fusion] deficient mutants).
Spheroplast fusion	Spheroplasts from yeast strains of one species, the same genus, or different genera can be fused to produce intraspecific, interspecific or intergeneric fusants, respectively. The possibility exists to introduce novel characteristics into wine yeast strains which are incapable of mating.
Single-chromosome transfer	Transfer of whole chromosomes from wine yeast strains (using the karyogamy mutation) into genetically defined strains of <i>S. cerevisiae</i> .
Transformation	Introduction of genes from other yeasts and other organisms.

Recombinant gene technology is widely employed in R&D for strain improvement. The availability of genetic manipulation tools and the opportunities that exist to improve the microbial cultures associated with food fermentations are tempered by concerns over regulatory issues and consumer perceptions. GM microbial cultures are, however, used in the production of enzymes and various food processing ingredients such as monosodium glutamate, polyunsaturated fatty acids and amino acids.

Biotechnology is also widely employed as a tool in diagnostics to monitor food safety, prevent and diagnose food-borne illnesses and verify the origins of foods. Techniques applied in the assurance of food safety focus on the detection and monitoring of hazards whether biological, chemical or physical. These applications are explored and discussed later.

5.3 CURRENT STATUS OF THE APPLICATION OF BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

5.3.1 Methods of microbial inoculation in food fermentations

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

Starter culture development and improvement is the subject of much research both in developed and developing countries. While considerable work on GM starter culture development is ongoing at the laboratory level in developed countries, relatively few GM micro-organisms have been permitted in the food and beverage industry globally. In 1990, the United Kingdom became the first country to permit the use of a live genetically modified organism (GMO) in food. It was a baker's yeast, engineered to improve the rate at which bread dough rises by increasing the efficiency with which maltose is broken down. This modification was done using genes from yeast and placing them under a strong constitutive promoter. The United Kingdom has also approved a GM brewer's yeast for beer production. By introducing a gene encoding gluco-amylase from yeast, better utilization of carbohydrate present in conventional feedstock can be obtained, resulting in increased yields of alcohol and the ability to produce a full strength, low-carbohydrate beer. More recently, two GM yeast strains were authorized for use in the North American wine industry (Bauer *et al.*, 2007).

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

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

Spontaneous inoculation of fermentation processes

In many developing countries, fermented foods are produced primarily at the household and village levels using spontaneous methods of inoculation. Spontaneous fermentations are largely uncontrolled. A natural selection process, however, evolves in many of them which eventually results in the predominance of a particular type or group of microorganisms in the fermentation medium. A majority of African food fermentation processes use spontaneous inoculation (Table 2). Major limitations of spontaneous fermentation processes include their inefficiency, low yields of product and variable product quality. While spontaneous fermentations generally enhance the safety of foods owing to a reduction of pH and through detoxification, in some cases there are safety concerns relating to the bacterial pathogens associated with the raw material or to unhygienic practices during processing.

“Appropriate” starter cultures as inoculants of fermentation processes

“Appropriate” starter cultures are widely applied as inoculants across the fermented food sector, from the household to industrial levels in low income and lower middle income economies. These starter cultures are generally produced using a backslopping process which uses samples of a previous batch of fermented product as inoculants (Holzapfel, 2002). Appropriate starter cultures are widely applied in the production of fermented fish sauces and fermented vegetables in Asia (Table 3) and in cereal or grain fermentations in African and Latin American countries (Tables 2 and 4).

The inoculation belt (Holzapfel, 2002) used in traditional fermentations in West Africa serves as a carrier of undefined fermenting micro-organisms, and is one example of an appropriate starter culture. It generally consists of a woven fibre, mat, piece of wood or woven sponge saturated with high quality product of a previously fermented batch. It is immersed into a new batch to serve as an inoculant. The inoculation belt is used in the production of the African indigenous fermented porridges uji and mawe, as well as in the production of the Ghanaian beer, pito (Table 2).

TABLE 2

AFRICAN FERMENTED FOODS AND INFORMATION ABOUT THEIR FERMENTATION PROCESSES

Raw material	Local product name	Region/country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development*
A. Fermented starchy staples						
Cassava	Gari	West and Central Africa	Solid state	<i>Corynebacterium mannihot</i> , <i>Geotrichum</i> species, <i>Lactobacillus plantarum</i> , <i>Lactobacillus buchneri</i> , <i>Leuconostoc</i> species, <i>Streptococcus</i> species.	Natural/chance	1, 2, 5, 7, 8
	Fufu	West Africa	Submerged	<i>Bacillus</i> species, <i>Lactobacillus</i> species such as <i>Lactobacillus plantarum</i> ; <i>Leuconostoc mesenteroides</i> ; <i>Lactobacillus cellobiosus</i> ; <i>Lactobacillus brevis</i> ; <i>Lactobacillus coprophilus</i> ; <i>Lactobacillus lactis</i> ; <i>Leuconostoc lactis</i> and <i>Lactobacillus bulgaricus</i> , <i>Klebsiella</i> species, <i>Leuconostoc</i> species, <i>Corynebacterium</i> species and a yeast of the <i>Candida</i> species.	Natural/chance	1, 2, 5, 6
	Lafun, Konkote	West Africa	Submerged	<i>Bacillus</i> species, <i>Klebsiella</i> species, <i>Candida</i> species, <i>Aspergillus</i> species; <i>Leuconostoc mesenteroides</i> , <i>Corynebacterium manihot</i> , <i>Lactobacillus plantarum</i> , <i>Micrococcus luteus</i> and <i>Geotrichum candidum</i>	Spontaneous	1, 2, 5, 6
	Chikwangue	Central Africa / Zaire	Solid state	<i>Corynebacterium</i> , <i>Bacillus</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> , <i>Pseudomonas</i> , <i>Acinetobacter</i> and <i>Moraxella</i>	Spontaneous	1, 2, 7
	Cingwada	East and Central Africa	Solid state	<i>Corynebacterium</i> , <i>Bacillus</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> ,	Spontaneous	1, 2





Raw material	Local product name	Region/country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development*
B. Gruels and beverages						
Maize	Ogi	West Africa, Nigeria	Submerged	<i>Lactobacillus plantarum</i> , <i>Corynebacterium specie</i> , <i>Aerobacter</i> , yeasts <i>Candida mycoderma</i> , <i>Saccharomyces cerevisiae</i> and <i>Rhodotorula</i> and moulds <i>Cephalosporium</i> , <i>Fusarium</i> , <i>Aspergillus</i> and <i>Penicillium</i>	Appropriate starters produced by backslopping	1, 2, 3, 4, 5, 7
Sorghum	Abreh	Sudan	Solid state and Submerged	<i>Lactobacillus plantarum</i>	Appropriate starters produced by backslopping	1, 2
Millet	Uji	East Africa, Kenya	Submerged	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus plantarum</i>	Appropriate starters produced by backslopping/ inoculation belt	1, 2
Maize	Kenkey, Koko, Akasa	West Africa, Ghana	Solid state	<i>Enterobacter cloacae</i> , <i>Acinetobacter sp.</i> , <i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida mycoderma</i>	Spontaneous	1, 2
C. Alcoholic beverages						
Palm	Palm wine, Emu	West Africa	Submerged	<i>Saccharomyces cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i>	Spontaneous	1, 2, 7
Various types of African cereal grains (maize, sorghum, millet)	Busa	East Africa, Kenya	Submerged	<i>Saccharomyces cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i> .	Spontaneous	1, 2, 7
	Mbege	Tanzania	Submerged	<i>Saccharomyces cerevisiae</i> , <i>Schizosaccharomyces pombe</i> , <i>Lactobacillus plantarum</i> , <i>L. mesenteroides</i> .	Spontaneous	1, 2
	Burukutu	West Africa	Submerged	<i>Saccharomyces cerevisiae</i> , <i>S. chavelieri</i> , <i>Candida sp.</i> and, <i>Leuconostoc meseteroides</i> . <i>Acetobacter sp.</i>	Spontaneous	1, 2
	Pito	West Africa	Submerged	<i>Geotrichum candidum</i> , <i>Lactobacillus sp.</i> and <i>Candida sp.</i>	Natural/chance Inoculation belt	1, 2





Raw material	Local product name	Region/country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development*
D. Acid leavened bread/pancakes						
Various types of African cereal grains	Kisra	Sudan	Submerged		Appropriate starters produced by backslopping	
	Enjera, Tef, Injera	Ethiopia	Submerged		Appropriate starters produced by backslopping	
E. Legumes and condiments						
Locus bean, soybean	Iru, Dawadawa, Etchum, Kal Soumbara, Chu	West Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> and <i>Staphylococcus saprophyticus</i>	Spontaneous	1, 2, 3, 6, 7
African oil bean	Ugba			<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> and <i>Staphylococcus saprophyticus</i>	Spontaneous	
Melon seeds, castor oil seeds, pumpkin bean, sesame	Ogiri, Ogili	West, East and Central Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> , <i>Staphylococcus saprophyticus</i> , <i>Lactobacillus plantarum</i>	Spontaneous	1, 2
Cotton seed	Owoh	West Africa		<i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> , <i>Staphylococcus saprophyticus</i>	Spontaneous	1
F. Animal products						
Goat milk	Ayib	East and Central Africa		<i>Canida</i> spp., <i>Saccharomyces</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp.,	Spontaneous	1, 2
Cow milk	Leben, Lben	North, East Central Africa		<i>Candida</i> spp., <i>Saccharomyces</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp.,	Spontaneous	1, 2, 3

Source: compiled from Odunfa and Oyewole (1997)

* Personal assessment of data, literature, Internet search and other information by O.B. Oyewole as at March 2009. Key to the codes is 1 = micro-organisms involved known; 2 = roles of individual micro-organisms known; 3 = genetic improvement carried on organisms; 4 = starter cultures available for the fermentation; 5 = varieties of raw materials that are best suited for the product known; 6 = improved technology available and adopted; 7: pilot plant production; 8 = industrial plant production.

TABLE 3

EXAMPLES OF TECHNOLOGIES USED IN INDIGENOUS FERMENTED FOOD PRODUCTION SYSTEMS IN ASIA

Substrate material	Indigenous fermented food	Country	Type of technology		Inoculum	Bioreactor production of starter		Nature of starter		Food safety techniques used in quality control and quality assurance
			Defined starter culture	Natural fermentation		Solid	Liquid	Dry powder	Liquid	
Soybean	Soy Sauce	China	Koji		<i>Aspergillus oryzae</i>					ELISA for detection of toxigenic fungi and mycotoxins
		Japan	Koji and Moromi		<i>Aspergillus</i> sp., <i>Saccharomyces rouxii</i>					ELISA and/or GC-MS to detect and/or monitor carcinogens 3-MCPD
		Thailand	Koji		<i>Aspergillus flavus</i> var <i>columnaris</i>	✓		✓		
Pork	Nham	Thailand, Vietnam, Lao and Cambodia	Defined Strains		Lactic acid bacteria <i>Staphylococcus xylosum</i>		✓	✓	✓	Selective cultural medium for pathogen detection (<i>Salmonella</i> , <i>Staphylococcus</i>)

TABLE 4

EXAMPLES OF FERMENTED FOODS PRODUCED IN LATIN AMERICA

Substrate	Local product name	Country	Micro-organisms associated	Uses
Maize	Abati	Paraguay, Argentina		Alcoholic beverage
Maize	Acupe	Venezuela		Beverage
Maize	Agua-agria	Mexico		Beverage
Rice	Arroz quemado	Ecuador	<i>Bacillus</i> spp., <i>Aspergillus</i> spp., <i>Actinomycete</i> spp.*	Porridge
Maize	Atole	Mexico	Lactic acid bacteria	Porridge
Black maize	Atole agrio	Mexico		Porridge
Maize, manihot or fruits	Cachiri	Brazil		Beverage





Substrate	Local product name	Country	Micro-organisms associated	Uses
Maize or rice	Champuz	Colombia, Peru		Beverage
Maize, yuca, cassava, sweet potatoes, quinoa or ripe plantains	Chicha	Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru	<i>Saccharomyces</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp., <i>Acetobacter</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp.	Alcoholic beverage
“Pulque” syrup, chili and toasted maize leaves	Charagua	Mexico		Alcoholic beverage
Maize	Fubá	Brazil		Porridge
Maize	Jamin-bang	Brazil		Bread
Maize	Napú	Peru		Beverage
Maize juice and “pulque” or brown sugar	Ostoche	Mexico		Alcoholic beverage
Cassava**	Pão de Queijo	Brazil	<i>Lactobacillus cellobiosus</i> , <i>Streptococcus lactis</i> , <i>Corynebacterium</i> spp.	Bread
Maize	Pozol	Mexico	<i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp., <i>Candida</i> spp., <i>Enterobacteriaceae</i> , <i>Bacillus cereus</i> , <i>Paracolobactrum aerogenoides</i> , <i>Agrobacterium azotophilum</i> , <i>Alkaligenes pozolis</i> , <i>Escherichia coli</i> var. <i>napolitana</i> , <i>Pseudomonas mexicana</i> , <i>Klebsiella pneumoniae</i> , <i>Saccharomyces</i> spp. and moulds	Non-alcoholic acidic beverage
Aguamiel (<i>Agave atrovirens</i> and <i>A. americana</i>)	Pulque	Mexico	<i>Saccharomyces carbajali</i> , <i>Lactobacillus plantarum</i> , <i>Leuconostoc</i> spp.	Alcoholic beverage
Maize juice, toasted maize and pirú fruits	Quebranta huesos	Mexico		Alcoholic beverage
Maize and red chili	Sendechó	Mexico		Alcoholic beverage
Maize	Sora	Peru		Alcoholic beverage
Maize, pineapple, apple or orange	Tepache	Mexico	<i>Bacillus subtilis</i> , <i>B. graveolus</i> and the yeasts <i>Torulopsis inconspicua</i> , <i>Saccharomyces cerevisiae</i> and <i>Candida queretana</i>	Alcoholic beverage
Germinated maize ground and cooked with fragments of plants	Tesgüino	Mexico	<i>Lactobacillus</i> spp., <i>Streptococcus</i> spp., <i>Leuconostoc</i> spp., <i>Pediococcus</i> spp., <i>Saccharomyces</i> spp., <i>Candida</i> spp., <i>Cryptococcus</i> spp., <i>Hansenula</i> spp., <i>Brettanomyces</i> spp., <i>Pichia</i> spp., <i>Geotrichum</i> spp. and <i>Penicillium</i> spp.	Alcoholic beverage
Maize	Tocos	Peru		Dessert
Barley	Zambumbia	Mexico		Alcoholic beverage
Maize beer and zarzaparrilla bark	Zarzaparrilla bark wine	Mexico		Alcoholic beverage

Source: Information adapted and modified from FAO (1998 and 1999); * from Van Veen and Steinkraus (1970), ** from Ray and Sivakumar (2009)

Iku, also referred to as iru, is another example of an appropriate starter culture produced by backslopping. This starter culture is produced from concentrated fermented dawadawa (a fermented legume product) mixed with ground unfermented legumes, vegetables such as pepper, and cereals, such as ground maize. It is stored in a dried form and is used as an inoculant in dawadawa fermentations in West Africa (Holzapfel, 2002).

A range of appropriate starter cultures either in a granular form or in the form of a pressed cake are used across Asian countries as fermentation inoculants. These traditional mould starters are generally referred to by various names such as marcha or murcha in India, ragi in Indonesia and Malaysia, bubod in the Philippines, nuruk in Korea, koji in Japan, and Loog-pang in Thailand. They generally consist of a mixture of moulds grown under non-sterile conditions.

Defined starter cultures as inoculants of fermentation processes

Few defined starter cultures have been developed for use as inoculants in commercial fermentation processes in developing countries. Nevertheless, the past ten years have witnessed the development and application of laboratory selected and pre-cultured starter cultures in food fermentations in a few developing countries, primarily in Asia (Table 3). Defined starter cultures consist of single or mixed strains of micro-organisms (Holzapfel 2002). They may incorporate adjunct culture preparations that serve a food safety and preservative function. Adjunct cultures do not necessarily produce fermentation acids or modify texture or flavour but are included in the defined culture owing to their ability to inhibit pathogenic or spoilage organisms. Their inhibitory activity is due to the production of one or several substances such as hydrogen peroxide, organic acids, diacetyl and bacteriocins (Hutkins, 2006).

Defined starter cultures are mainly produced by pure culture maintenance and propagation under aseptic conditions. They are generally marketed in a liquid or powdered form or as a pressed cake. Loog-pang, a defined culture marketed in Thailand in the form of a pressed rice cake, consists of *Saccharomyces cerevisiae*, *Aspergillus oryzae* or *Rhizopus* sp. and *Mucor*. Loog-pang has a shelf-life of 2–3 days at ambient temperature and 5–7 days under refrigerated conditions. Ragi cultures are commercially produced by the Malaysian Agricultural Research and Development Institute by mixing a culture inoculum which generally consists of *Rhizopus oligosporus* with moistened sterile rice flour, and incubating it at ambient temperature for four days. This starter has a shelf-life of two weeks under refrigerated conditions (Merican and Quee-Lan, 2004). It is widely used as an inoculant in the production of traditional Malaysian fermented foods. Ragi-type starter cultures for the production of a range of fermented Indonesian products such as oncom, tape and tempeh are currently marketed via the Internet.

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

alcoholic beverages. Many of these cultures are tailored to produce specific textures and flavours. In response to growing consumer interest in attaining wellness through diet, many yoghurt cultures also include probiotic strains. Probiotics are currently produced in India for use as food additives, dietary supplements and in animal feed. Methodologies used in the development and tailoring of these starters are largely proprietary to the suppliers of these starters. Monosodium glutamate and lactic acid, both of which are used as ingredients in the food industry, are produced in less advanced developing countries using defined starter cultures.

The use of DNA-based diagnostic techniques for strain differentiation can allow for the tailoring of starter cultures to yield products with specific flavours and/or textures. For example, random amplified polymorphic DNA (RAPD) techniques have been applied in Thailand for the molecular typing of bacterial strains and correlating the findings to flavour development during the production of the fermented pork sausage, nham (see Case Study 5.5.2). The results of these analyses led to the development of three different defined starter cultures which are currently used for the commercial production of products having different flavour characteristics (Valyasevi and Rolle, 2002).

GM starter cultures

To date, no commercial GM micro-organisms exist that would be consumed as living organisms. Products of industrial GM producer organisms are, however, widely used in food processing and no major safety concerns have been raised against them. Rennet which is widely used as a starter in cheese production across the globe is produced using GM bacteria. These are discussed in more detail below. Thailand currently uses GM *Escherichia coli* as an inoculant in lysine production. Many industrially important enzymes such as alpha-amylase, gluco-amylase, lipase and pectinase and bio-based fine chemicals such as lactic acid, amino acids, antibiotics, nucleic acid and polysaccharides, are produced in China using GM starter cultures. Other developing countries which currently produce enzymes using GM micro-organisms include Argentina, Brazil, Cuba and India.

5.3.2 Food additives and processing aids

Biotechnologies are currently used for the production of food additives and food processing aids such as enzymes, flavouring agents, organic acids, amino acids and sweeteners.

Enzymes

Enzymes occur in all living organisms and catalyze biochemical reactions that are necessary to support life (Olempska-Beer *et al.*, 2006). They are commonly used in food processing and in the production of food ingredients. The use of recombinant DNA technology

has made it possible to manufacture novel enzymes that are tailored to specific food processing conditions. Alpha-amylases with increased heat stability have, for example, been engineered for use in the production of high-fructose corn syrups. These improvements were accomplished by introducing changes in the alpha-amylase amino acid sequences through DNA sequence modifications of the alpha-amylase genes (Olempska-Beer *et al.*, 2006). Bovine chymosin used in cheese manufacture was the first recombinant enzyme approved for use in food by the US Food and Drug Administration (Flamm, 1991). The phospholipase A1 gene from *Fusarium venenatum* is expressed in GM *Aspergillus oryzae* to produce the phospholipase A1 enzyme used in the dairy industry for cheese manufacture to improve process efficiencies and cheese yields.

Considerable progress has been made in recent times toward the improvement of microbial strains used in the production of enzymes. Microbial host strains developed for enzyme production have been engineered to increase enzyme yields. Certain fungal producing strains have also been modified to reduce or eliminate their potential for producing toxic metabolites (Olempska-Beer *et al.*, 2006). Food processing enzymes in the United States derived from GM micro-organisms are listed in Table 5.

TABLE 5

FOOD PROCESSING ENZYMES DERIVED FROM GM MICRO-ORGANISMS

Source micro-organism	Enzyme
<i>Aspergillus niger</i>	Phytase Chymosin Lipase
<i>Aspergillus oryzae</i>	Esterase-lipase Aspartic proteinase Glucose oxidase Laccase Lipase
<i>Bacillus licheniformis</i>	alpha-amylase Pullulanase
<i>Bacillus subtilis</i>	alpha-acetolactate decarboxylase alpha-amylase Maltogenic amylase Pullulanase
<i>Escherichia coli</i> K-12	Chymosin
<i>Fusarium venenatum</i>	Xylanase
<i>Kluyveromyces marxianus</i> var. <i>lactis</i>	Chymosin
<i>Pseudomonas fluorescens</i>	alpha-amylase
<i>Trichoderma reesei</i>	Pectin lyase

Source: Olempska-Beer *et al.* (2006)

Enzymes used in food processing have historically been considered non-toxic. Some characteristics arising from their chemical nature and source, such as allergenicity, activity-related toxicity, residual microbiological activity and chemical toxicity are, however, of concern. These attributes of concern must be addressed in light of the growing complexity and sophistication of the methodologies used in the production of food-grade enzymes. Safety evaluation of all food enzymes including those produced by GM micro-organisms is essential if consumer safety is to be assured (Spok, 2006). Enzymes produced using GM micro-organisms wherein the enzyme is not part of the final food product have specifically been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety evaluations have been conducted using the general specifications and considerations for enzyme preparations used in food processing (JECFA, 2006). Preparations of asparaginase enzymes have also been evaluated by JECFA (2008).

Flavours, amino acids and sweeteners

Volatile organic chemicals such as flavours and aromas are the sensory principles of many consumer products and govern their acceptance and market success (Berger, 2009). Flavours produced using micro-organisms currently compete with those from traditional agricultural sources. According to Berger (2009), more than 100 commercial aroma chemicals are derived using biotechnology either through the screening for overproducers, the elucidation of metabolic pathways and precursors or through the application of conventional bioengineering. Recombinant DNA technologies have also enhanced efficiency in the production of non-nutritive sweeteners such as aspartame and thaumatin. Market development has been particularly dynamic for the flavour enhancer glutamate (Leuchtenberger, Huthmacher and Drauz, 2005) which is produced by fermentation of sugar sources such as molasses, sucrose or glucose using high-performance strains of *Corynebacterium glutamicum* and *Escherichia coli*. Amino acids produced through biotechnological processes are also of great interest as building blocks for active ingredients used in a variety of industrial processes.

5.3.3 Current status of the application of traditional and new biotechnologies in food safety and quality improvement in developing countries

Food safety issues and concerns in food fermentation processing

Microbial activity plays a central role in food fermentation processes, resulting in desirable properties such as improvements in shelf-life and quality attributes such as texture and flavour. Pathogenic organisms are, however, of prime concern in fermented foods. Anti-nutritional factors such as phytates, tannins, protein inhibitors, lectins, saponins,

oligosaccharides and cyanogenic glucosides are naturally occurring components of raw materials commonly used in food fermentations in developing countries. Contamination of the fermentation process can pose a major health risk in the final fermented product. Methodologies for identifying and monitoring the presence of chemical (pesticide residues, heavy metals, trace elements) and biochemical (aflatoxins) hazards in fermented foods are therefore a critical need. Furthermore, with growing consumer interest in the credence attributes of the products that they consume, and the premium currently being placed on quality linked to geographical origin, the traceability of foods with selected properties is of increasing importance.

Advances in microbial genetics

In recent times, genetic characterization of micro-organisms has advanced at a rapid pace with exponential growth in the collection of genome sequence information, high-throughput analysis of expressed products, i.e. transcripts and proteins, and the application of bioinformatics which allows high-throughput comparative genomic approaches that provide insights for further functional studies. Genome sequence information coupled with the support of highly advanced molecular techniques have allowed scientists to establish mechanisms of various host-defensive pathogen counter-defensive strategies and have provided industry with tools for developing strategies to design healthy and safe food by optimizing the effect of probiotic bacteria, the design of starter culture bacteria and functional properties for use in food processing. Characterization of the genomes of lactic acid probiotics has, for example, shed light on the interaction of pathogens with lactic acid bacteria (de Vos, 2001). Nucleotide sequences of the genomes of many important food microbes have recently become available. *Saccharomyces cerevisiae* was the first food microbe for which a complete genome sequence was characterized (Goffeau *et al.*, 1996). This was followed by genome sequencing of the related yeast, *Kluyveromyces lactis* (Bolotin-Fukuhara *et al.*, 2000) as well as filamentous fungi which are major enzyme producers and have significant applications in the food processing industry.

Genome nucleotide sequences of many Gram-positive bacteria species have also been completed. The *Bacillus subtilis* genome was the first to be completed followed by that of *Lactococcus lactis*. Genome sequences of food-borne pathogens such as *Campylobacter jejuni* (Parkhill *et al.*, 2000), verocytotoxigenic *Escherichia coli* O157:H7 (Hayashi *et al.*, 2001) and *Staphylococcus aureus* (Kuroda *et al.*, 2001) have also been completed. Genome sequences of microbes that are of importance in food processing such as *Lactobacillus plantarum* (Zhang *et al.*, 2009) are likewise available. The genome of *Clostridium botulinum*, responsible for food-borne botulism, was also recently sequenced (Sebahia *et al.*, 2007).

Detection of pathogens

The rapid detection of pathogens and other microbial contaminants in food is critical for assessing the safety of food products. Traditional methods to detect food-borne bacteria often rely on time-consuming growth in culture media, followed by isolation, biochemical identification and, sometimes, serology. Recent technological advances have improved the efficiency, specificity and sensitivity of detecting micro-organisms. Detection technologies employ PCR, where short fragments of DNA (probes) or primers are hybridized to a specific sequence or template which is subsequently amplified enzymatically by the Taq polymerase enzyme using a thermocycler (Barrett, Fang and Swaminathan, 1997). In theory, a single copy of DNA can be amplified a million-fold in less than two hours with the use of PCR techniques; hence, the potential of PCR to eliminate or greatly reduce the need for cultural enrichment. The genetic characterization of genome sequence information has further facilitated the identification of virulence nucleotide sequences for use as molecular markers in pathogen detection. Multiplex real-time PCR methods are now available to identify the *E. coli* O157:H7 serogroup (Yoshitomi, Jinneman and Weagant, 2003). PCR-based identification methods are also available for *Vibrio cholerae* (Koch, Payne and Cebula, 1995) and for major food-related microbes such as *Campylobacter jejuni*, *C. coli*, *Yersinia enterocolitica*, *Hepatitis A virus*, *Salmonella* and *Staphylococcus aureus* (FDA, 2003).

Sophisticated culture media such as chromogenic or fluorogenic media are not readily used in low income economies but are relatively widespread in lower middle income and upper middle income economies. The use of immunoassays such as ELISA is also very limited in low income economies but is more widespread in the form of diagnostic kits in lower middle and upper middle income economies. DNA methods, which require elaborate infrastructure and high technical competence, find minimal application in lower income and some lower middle income economies. Biotechnologies applied in food safety assays in developing countries are summarized in Table 6.

There are movements toward implementing safety control programmes such as the application of Hazard Analysis and Critical Control Point (HACCP) in food fermentations in many developing countries. A HACCP plan for the production of the Thai fermented meat product nham is summarized in Table 7. The application of HACCP necessitates the deployment of good agricultural practices (GAPs), good manufacturing practices (GMPs), good hygienic practices (GHPs) and the monitoring of critical control points for potential microbial and chemical contamination during bioprocessing (FAO, 2006). Rigorous adherence to sanitary practices in the processing environment necessitates rapid, dynamic, sensitive, specific as well as versatile and cost-effective assay methods. The molecular approach of biotechnology entails near-time or real-time bacterial detection and offers levels of sensitivity and specificity unchallenged by traditional/conventional methods.

TABLE 6

BIOTECHNOLOGIES APPLIED IN FOOD SAFETY ASSAYS IN DEVELOPING COUNTRIES

Food production chain	Risk factor	Hazard profile	Biotechnology		Country level Development
			Traditional	New	
I. Pre-processing of incoming raw material from producers (farms)	Improper practice	Chemical <ul style="list-style-type: none"> • Pesticide residues • Unapproved chemotherapeutics 	Chromatography TLC (thin layer chromatography) GC (gas chromatography) HPLC (high-performance liquid chromatography)	Biosensors for pesticides, metals, antibiotics and micro-organisms ELISA for aflatoxins and natural plant toxins	Low income
	Presence of contaminants	<ul style="list-style-type: none"> • Heavy metals • Dioxins 	Atomic absorption spectrophotometry Chromatography	GC-MS	Some lower middle and middle income
II. Processing raw material	Improper handling (time/temperature)	Chemical <ul style="list-style-type: none"> • Undeclared additives and supplements • Economic adulteration 	TLC, GC, HPLC	Mass spectrometry (MS) methods for additives	Low, lower middle and middle income economies
	Fermentation procedures involving micro-organisms	Microbial	Growth in culture media	Chromogenic/fluorogenic indicator culture media ELISA, Antibody based biosensors PCR detection of specific genes	Middle income
	Quality parameters	<ul style="list-style-type: none"> • Consistency • Composition 	Biochemical and enzyme assays pH measurements	Biosensing of fermentation-related enzymes Monitoring of sugars, alcohol, organic and inorganic ions Surface plasmon resonance	
III. Packaging and end product analysis	Contamination from packaging material	Chemical <ul style="list-style-type: none"> • Undeclared allergens and additives 	Chromatography TLC, GC, HPLC, fast protein liquid chromatography	GC-MS	Some lower middle and middle income economies
		Microbial <ul style="list-style-type: none"> • Non-sterile conditions leading to microbial growth 	Growth in culture media	Chromogenic/fluorogenic indicator culture media ELISA PCR detection of specific genes	
		Physical <ul style="list-style-type: none"> • Pieces, fragments of materials 	Inspection and sampling	Metal detection systems	Middle income economies

Mycotoxin detection

The problem of mycotoxin contamination in food, including fermented foods, is a global concern. Mycotoxin contamination is particularly prevalent in tropical areas such as South Asia and Africa. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are two of the most widely used methods for the detection and quantification of mycotoxins in developing countries. These methods, however, are time consuming, difficult to use and require laboratory facilities. Immunoassays that are economical, sensitive and easy to use would greatly facilitate the detection and quantification of mycotoxins. A number of ELISA kits are now commercially available for the detection of aflatoxins, deoxynivalenol, fumonisins, ochratoxins and zearalenone (Schmale and Munkvold, 2009).

Detection and identification of foods and food ingredients

DNA-based identification systems rely on polymorphisms at the nucleotide level for the differentiation of living organisms at the variety and species levels. Currently, PCR-based methods are used either for the purpose of detecting single nucleotide polymorphisms (SNPs) giving rise to restriction fragment length polymorphisms (RFLPs) or for detecting small sequence length polymorphisms (SSLPs), often known as variable number tandem repeats (VNTRs). These methods facilitate the identification of unique polymorphisms of a variety of food commodities and can be used to identify their source or origin. These unique polymorphisms are often referred to as DNA barcodes (Teletchea, Maudet and Hänni, 2005). The DNA barcode is used for the identification of specific varieties in food detection and in food traceability and, for example, for the identification of many products for export in countries such as Argentina, Brazil, China, Cuba and Thailand. The DNA barcode of microsatellite markers has also been successfully used for differentiating and identifying fermented products such as premium wines, cheeses and sausages on the basis of their origins, as well as for differentiating Basmati rice varieties and olive cultivars used in olive oil production (Sefc *et al.*, 2000).

5.4 ANALYSIS OF THE REASONS FOR SUCCESSES/FAILURES OF APPLICATION OF BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

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

Socio-economics of the consumer base

The consumer base of traditionally fermented staple foods in most developing countries is largely poor and disadvantaged. Price, rather than food safety and quality, is therefore a major preoccupation of this group when purchasing food. Fermented foods provide that target group with an affordable source of food and make a substantial contribution to their food and nutritional security. These foods are generally produced under relatively poor hygienic conditions at the household and village levels. Fermentation processing is practised largely as an art in such contexts.

Interventions designed to upgrade processes used in the production of these traditionally fermented staples have been largely carried out through donor-funded projects and have focused primarily on reducing the drudgery associated with the fermentation processes. Improvements have also targeted the upgrading of hygienic conditions of fermentation processes and the introduction of simple and “appropriate” methodologies for the application of inoculants such as the use of backslopping. While the uptake of simple backslopping technologies at the household level has, in general, been very good by that target group, the uptake of defined starter cultures has been less successful owing to cost considerations. Case Study 5.5.3 on the household level production of Som Fug in Thailand highlights the poor uptake of improved starter culture technologies by household-level processors, primarily on the basis of cost.

With growing incomes and improved levels of education in urban centres across a number of developing countries, dietary habits are changing and a wider variety of foods is being consumed. Fermented foods are no longer the main staples but are still consumed as side dishes or condiments by that target group. The demand of that target group for safe food of high quality has begun to re-orient the traditional fermented food sector and led to improvements in the control of fermentation processes through the development and adoption of defined starter cultures, the implementation of GHPs and HACCP in food fermentation processing, and the development of bioreactor technologies, coupled with appropriate downstream processing to terminate fermentation processes and thus extend the shelf-life of fermented foods. The packaging of fermented products has also improved. Case Study 5.5.1 on soy sauce production in Thailand highlights an example of how starter culture development coupled with bioreactor technology has improved yields and the efficiency of fermentation processes, while Case Study 5.5.2 highlights how consumer demand for safe food led to R&D into starter culture development designed to improve the safety of nham in the marketing chain.

Changing consumer demand trends

Apart from their changing dietary patterns and their demand for safety and quality, higher income consumers demand convenience and are increasingly concerned about deriving health benefits from the foods they consume. Many of these consumers also show a preference

for shopping in supermarkets. Consumer demand for deriving wellness through food consumption has stimulated the development of industrial fermentation processes for the production of functional ingredients such as polyunsaturated fatty acids and probiotic cultures for use as food ingredients in developing countries. These functional ingredients are currently applied in the fortification of fermented foods as well as in the production of dietary supplements in countries such as India.

The growth of supermarkets in developing countries has promulgated the need for standardized products of a reasonable shelf-life that meet safety and quality criteria. Packaged fermented products such as kimchi, miso and tempeh, for example, are widely available in supermarkets across Asia. The production of traditional beer in a powdered format and in ready-to-drink containers in Zambia is a good example of product development that has taken place in response to consumer demand for convenience, both in local and export markets.

Shifting consumer preferences in South Africa away from basic commodity wine to top quality wine is yet another example of how market demand has led to research and biotechnological innovation in the wine industry. Biotechnological innovations in that country are currently focused on the improvement of *Saccharomyces cerevisiae* strains to improve wholesomeness and sensory quality of wines.

The enabling environment for starter culture development

A considerable amount of research in developing countries has focused on the identification of starter micro-organisms associated with the fermentation of staple foods. The greatest strides in starter culture development have, however, been realized in countries that have prioritized the development of technical skills, the infrastructural support base and funding support for research into the upgrading of fermentation processes. Linkages between research institutions and the manufacturing sector have also been critical to the successful introduction of starter cultures. Case Study 5.5.1 on soy sauce production exemplifies how success was achieved through such collaboration. Case Study 5.5.2 on nham production in Thailand also highlights how collaboration between the manufacturing sector and public sector research institutions resulted in the development of improved starter cultures and the uptake of these cultures by nham manufacturers to assure product safety.

Collaborative initiatives among research institutions have also had a major positive impact on biotechnological developments in developing countries. Collaboration among African institutions and their counterparts in the North has greatly facilitated improvements in biotechnological research and capacity development in the area of food biotechnology on the continent. One success story in this regard has been a series of collaborative projects on traditional African fermented foods involving research institutions in Africa and Europe (Mengu, 2009). The programme facilitated the typing and screening of microbial cultures

associated with fermented African foods as a basis for starter culture development, and results of this work led to improvements in the production of gari, a fermented cassava product, and dawadawa, a fermented legume product.

Issues related to the protection of intellectual property rights (IPR) are of growing concern with respect to starter culture development. Case Study 5.5.4, describing flavour production using alkaline-fermented beans highlights the critical importance of IPR in reference to processes applied in the production of traditional fermented foods.

Proactive industrial strategies

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

Export opportunities for fermented products

Increasing travel due to globalization has changed the eating habits of consumers across the globe. Export markets for fermented foods have grown out of the need to meet the requirements of the developing country diaspora in these markets as well as to satisfy growing international demand for niche and ethnic products. Indonesian tempe and Oriental soy sauce are well known examples of indigenous fermented foods that have been industrialized and marketed globally. The need to assure the safety and quality of these products in compliance with the requirements of importing markets has been a driving force for the upgrading of starter cultures as well as for diagnostic methodologies for verifying their quality and safety. Growing interest and trade in fermented food products is also likely to lead to the greater use of the DNA barcode for identifying the origins of specific fermented food products produced in developing countries.

5.5 CASE STUDIES OF APPLICATIONS OF BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

5.5.1 Fermented soy sauce production

This Case Study on the production of soy sauce highlights success in the application of starter culture technology and the use of improved bioreactor technology. It exemplifies the transition of a craft-based production system to a technology-based production system. Research leading to these developments was supported by an international organization, followed by funding support from the Government of Thailand and the Thai soy sauce

industry. Developments of the process were largely driven by the demand pull created by a soy sauce industry consortium in Thailand in order to meet market requirements.

Soy sauce production involves a two-step fermentation process that makes use of koji inoculants in the initial phase followed by moromi inoculants in the second phase. The initial phase of the fermentation involves the soaking of soybeans in water for 1–2 hours, boiling for approximately 17 hours to hydrolyze the protein complex, and the addition of the koji culture *Aspergillus oryzae* for proteolysis of the soy proteins. Using this traditional method of production, the process of proteolysis takes between 40 hours and seven days depending on the method and the conditions used. The second phase of the fermentation process, referred to as a moromi fermentation, involves the addition of brine solution to the koji. *Saccharomyces rouxii*, a salt-tolerant yeast, is the predominant micro-organism in this phase of the fermentation which lasts as long as 8–12 months. Moromi fermentations are traditionally conducted in earthenware jars, which often pose a limitation to the manufacturers both in terms of expansion and in terms of production capacity (Valyasevi and Rolle, 2002). The soy sauce industry has moved up the ladder of development from an “art” to a technology-based process through the introduction of defined starter cultures and improvements in the control of the fermentation process. Physical and biological parameters of the fermentation process are controlled through the use of koji and moromi cultures and koji and moromi fermentors.

Use of the koji starter, *Aspergillus flavus* var. *columnaris*, was found to enhance product safety and uniformity. The introduction of pressure cookers as an innovation for hydrolyzing the soybeans reduced the time required for solubilization from 17 hours to 2.5 hours. Moreover, the use of starter culture technology facilitated the development of fermentation chambers with controlled temperature and humidity conditions, which resulted in shortening the duration of the fermentation process. The resulting soy sauce had a higher (6 percent) soluble protein content than that derived from boiled soybeans. These developments resulted in economic gain for the soy sauce industry and greater value added to the product in terms of quality and safety.

5.5.2 Traditional fermented pork sausage (nham)

This Case Study demonstrates how consumer demand for safe food resulted in the commercial use of defined starter cultures, with the collaboration and support of government agencies. The diagnostic role of biotechnology in starter culture development for the tailor making of cultures is also highlighted.

Nham is an indigenous fermented pork sausage produced in Southeast Asia. It is prepared from ground pork, pork rinds, garlic, cooked rice, salt, chili, sugar, pepper and sodium nitrite. It is traditionally consumed as a condiment in the uncooked state in Thailand. It is generally produced using an uncontrolled fermentation process. Fermentation of the product

occurs during transportation from the manufacturer to the point of retail. The product is generally retailed under ambient conditions. Traditionally produced nham is considered high risk by the Thai health authorities, who require a warning label on the package stating that the product “must be cooked before consumption”.

The first step in the transition to science-based technology for nham fermentations was the development of a starter culture. This starter was subsequently adopted by a nham manufacturer who also implemented HACCP in his operation to assure safety and to satisfy the compulsory standard requirements of GMP in the food processing industry imposed by the Thai Food and Drug Administration. A microbiological hazard profile was developed for nham by the manufacturer in collaboration with scientists from the Ministry of Science, who established that the prevalent pathogens in nham were *Salmonella* spp. (16 percent), *Staphylococcus aureus* (15 percent) and *Listeria monocytogenes* (12 percent) (Paukatong and Kunawasen, 2001). Nitrite, an additive used in nham production, was identified as a chemical hazard and the metal clips used for closing the package were identified as physical hazards. A HACCP plan which included four critical control points was developed for nham (Table 7).

The critical control point on nitrite was monitored by checking the pre-weighed nitrite prior to adding it to the product formulation. Scientific data from studies on starter cultures showed that a rapid increase in acidity within 36–48 hours of fermentation inhibited the growth of bacterial pathogens such as *Staphylococcus aureus* and *Salmonella* spp. (Paukatong and Kunawasen, 2001; Chokesajjawatee *et al.*, 2009). With the application of these starter cultures, the final product was sent to retailers after the fermentation reached its end-point (pH < 4.5). An innovative pH indicator which undergoes a colour change on attainment of the end-point of the fermentation process (pH < 4.5) was included in the package. With these innovations and the implementation of a HACCP plan, local health authorities waived the requirement for the warning “must cook before consumption” on the package. This authorization was seen by the public as an endorsement of product quality and safety by the health authority. Subsequently, three medium-sized manufacturers followed suit in adopting the improved technology. Recognition of the starter culture technology as a food safety measure by the health authority was, of itself, an effective public awareness campaign.

RAPD markers were used for molecular typing of approximately 100 bacterial strains at 12-hour intervals during nham fermentations. These studies resulted in the development and commercialization of three different starter formulae for use by larger manufacturers of nham. These starter cultures are marketed in a liquid form which requires refrigeration. Dried starter cultures have a shelf-life of one month at ambient temperature. Further innovations

have led to the incorporation of local yeast extracts into starter culture development, resulting in a 20 to 30 fold reduction in cost. The adoption of starter culture technology in nham fermentations has had a positive impact on the industry in terms of safety assurance to consumers and product consistency.

5.5.3 Traditional fermented fish paste – Som Fug

Som Fug is a traditional fermented minced fish cake. It is considered a healthy and highly nutritious product and is an excellent source of protein (protein content: 15.7 percent, fat: 3.2 percent and total carbohydrates: 4 percent). It is produced using a spontaneous microbial fermentation process similar to that used for producing nham and many other Southeast Asian fermented foods. This Case Study demonstrates that the uptake and use of starter culture technologies is still largely contingent on cost considerations and consumer appreciation of the nutritional value of the product.

TABLE 7

HACCP PLAN FOR THE PRODUCTION OF NHAM

Process step	Hazard	Critical limits	Monitoring procedures	Corrective actions	HACCP records
Weighing nitrite	Improper nitrite weight: if too high - chemical hazard, if too low - may result in microbiological hazard	100 ppm \leq initial nitrite level \leq 200 ppm	The quality control (QC) supervisor randomly checks the pre-weighed nitrite according to appropriate sampling frequency	Supervisor reweighs every bag of nitrite since last satisfactory check; record deviation; recalibrate the weighing balance	<ul style="list-style-type: none"> • nitrite weighing records • deviation records • balance calibration records
Stuffing	Failure to remove metal clips may contaminate product	No metal in product	Line worker to visually inspect each nham product during stuffing. Change worker every 30 minutes	Line worker notifies supervisor; separate contaminated product; segregate metal; and record deviation	<ul style="list-style-type: none"> • visual inspection log • deviation records
Labelling	Failure to provide microbiological safety information to the consumer	Label to contain information such as "safe if cooked before consumption" on each nham product	Line worker randomly checks the label on nham products	Line worker notifies supervisor; recheck nham product; label product; and record deviation	<ul style="list-style-type: none"> • visual inspection log • deviation records
Fermentation	Inadequate fermentation resulting in growth of pathogens	The pH of nham product lower than 4.6	QC worker randomly monitors pH of nham in each lot	QC worker notifies supervisor; hold lot; prolong fermentation; and record deviation	<ul style="list-style-type: none"> • monitoring pH records • holding records • deviation records

Source: Paukatong and Kunawasen (2001)

Compositionally, Som Fug consists of minced freshwater fish (mud carp, *Cirrhina microlepis*) 84 percent (by weight), garlic 8 percent, water 4 percent, salt 2 percent, boiled rice 1 percent, sucrose 0.1 percent and black pepper. It is fermented for about 2–4 days at ambient temperature. Lactic acid bacteria are the dominant microflora associated with the fermentation (Paludan-Muller, Huss and Gram, 1999). RAPD-PCR analyses determined that the garlic-fermenting lactic acid bacteria associated with Som Fug fermentations belonged to *Lactobacillus pentosus* and *Lact. plantarum* (Paludan-Muller *et al.*, 2002). Furthermore, the studies concluded that fructans from garlic are important carbon sources which catalyze the fermentation. The studies on Som Fug illustrate the high discriminatory power of biotechnology in differentiating lactic acid bacteria at the strain level. The Som Fug industry did not see the benefit of implementing starter culture technology. Although the important micro-organisms for Som Fug fermentation had been identified, there were no attempts to develop starter cultures. One major reason for the lack of development of starter culture technology was the widespread production of Som Fug at the household level. Household manufacturers do not see the benefit of starter culture technology but rather view starter cultures as a burden to the cost of production. Moreover, there is no scientific information to substantiate the nutritional value of Som Fug and hence there is very little public awareness of the nutritional value of the product.

5.5.4 Flavour production from alkaline-fermented beans

This Case Study on the indigenous fermentation of the locust bean is a classic example of how traditional fermentations can be exploited for the production of high-value products such as flavour compounds. The work, however, was undertaken by a large corporation with little involvement of local researchers. Returns on commercial successes derived from this study did not go back to the people who invented the traditional method of producing this indigenous fermented food. This Case Study, therefore, serves to highlight the critical issue of IPR in traditional production systems.

Dawadawa is produced by alkaline fermentation of the African fermented locust bean (Steinkraus, 1995). It is an important condiment in the West/Central African Savannah region (Odunfa and Oyewole, 1986). Similar fermented food products can be found throughout Africa with regional differences in the raw materials used as processing inputs or in post-processing operations. Similarly, fermented products are referred to as *kinda* in Sierra Leone, *iru* in coastal Nigeria, *soumbara* in Gambia and Burkina Faso, and *kpalugu* in parts of Ghana (Odunfa and Oyewole, 1986). Foods produced by alkaline fermentation in other parts of the world include *natto* in Japan, *thua noa* in Thailand and *kinema* in India (Tamang, 1998). These are mainly used to enhance or intensify meatiness in soups, sauces and other prepared dishes.

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

B. LOOKING FORWARD: PREPARING FOR THE FUTURE

5.6 EMERGING PATHOGENS: A KEY ISSUE WHERE THE APPLICATION OF BIOTECHNOLOGIES COULD BE USEFUL

The identification of infectious agents requires high-end technologies which are not usually available in developing countries. Developing countries must therefore seek assistance from countries with higher calibre technologies in order to characterize infectious agents, put in place surveillance and monitoring systems and develop strategies to contain the disease(s). Biotechnology can play a key role in facilitating the characterization of new emerging pathogens.

Traditional cultural methods for the detection and enumeration of microbial pathogens are tedious and require at least 12–18 hours for the realization of results. By that time, the food products would have been distributed to retailers or consumers. Immunoassay diagnostic kits facilitate near-real-time monitoring, sensitivity, versatility and ease of use. The emergence of multi-antibiotic resistance traits is prevalent in intensive farming in developing countries due to the abuse of antibiotics. The spread of multi-antibiotic resistant micro-organisms poses public health concerns because pathogens exhibiting such resistance would be difficult to control with the use of currently available antibiotics. The rapid detection of these pathogens with high sensitivity is one way of monitoring and containing the spread of multi-antibiotic resistant traits. A strategic approach being employed by some is the development of affinity biosensors with an antibiotic resistant nucleotide sequence as the detection probe.

5.7 IDENTIFYING OPTIONS FOR DEVELOPING COUNTRIES

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

Regulatory and policy issues

- Governments must be committed to protecting consumer health and interests, and to ensuring fair practices in the food sector.
- There has to be consensus at the highest levels of government on the importance of food safety, and the provision of adequate resources for this purpose.

- Government policy must be put in place that is based on an integrated food-chain approach, is science-based, transparent and includes the participation of all the stakeholders from farm to table.
- The importance of the regional and international dimensions of using biotechnologies in food processing and safety must be recognized.
- Priority must be accorded to promoting fermented foods in the food security agendas of countries.
- Governments must also provide an enabling environment that is supportive of the growth and development of upstream fermentation processes such as the production of high-value fermented products, such as enzymes, functional food ingredients and food additives.

International cooperation and harmonization

- The organization and implementation of regional and international fora are critical requirements for the enhancement of national organizational capability and performance and to facilitate international cooperation. Further, the setting up of administrative structures with clearly defined roles, responsibilities and accountabilities could efficiently govern processed foods and safety issues.
- Biotechnology-based standard operating procedures (SOPs) for food safety should also be documented for use in authorized laboratories.
- National food control databases for the systematic collection, analysis and reporting of food-related data (food inspection, analysis, etc.) with set regulations and standards based on sound science and in accordance with international recommendations (Codex) are key requirements.

Education policy

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

- Strategies should therefore be developed for the dissemination of knowledge about food biotechnology and particularly fermented foods. Targeted consumer education on the benefits of consuming fermented food products and on applying good practice in their production is required.
- Food biotechnology should be included in educational curricula in order to improve the knowledge base in countries on the contribution of fermented foods to food and nutritional security and to generate awareness of the growing market opportunities for fermented foods and high-value products derived from fermentation processes.

Information-sharing

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

Legislation and policy on technologies

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

Intellectual property rights

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

Communication and consumer perceptions

- Communication between various stakeholders is critical in proactively engaging with consumers. Communication must be established with the public at large on processed food and associated hazards. Communication gradually builds confidence and will be critical to advancing the application of biotechnologies in food processing and safety. The primary role of communication in this respect is to ensure that information

and opinions from all stakeholders are incorporated in the discussion and decision-making processes. The need for specific standards or related texts and the procedures followed to determine them should also be clearly outlined. The process, therefore, should be transparent.

- Public awareness and education are critical to the success of food bioprocessing and food safety in developing countries.
- Greater attention must be directed toward understanding consumer and producer (processor) perceptions on food safety and quality in developing countries.
- If foods are to be promoted as being safe and healthy, their nutritional and safety attributes must be transparently demonstrated by presenting scientific data to substantiate the nutritional and health benefits and by applying good manufacturing/hygiene practice and HACCP as safety measures to ensure that issues of consumer concern are addressed.

Technical capacities and technology transfer

- Traditional fermented foods should be viewed as valuable assets. Governments should capitalize on these assets and add value to them by supporting research, education and development, while building on and developing the indigenous knowledge base on food fermentations.
- Government agencies in developing countries should focus on the development of technical capacities to deal with emerging technical issues.
- The technical capacities of academic and research institutes should be strengthened in the fields of food biotechnology, food processing, bioprocess engineering and food safety through training and exchange programmes for researchers. Such programmes should emphasize collaboration with both developed and developing country institutions engaged in work on food biotechnology, starter culture development, bioprocess engineering and food safety.
- Training capabilities in food biotechnology and food safety should be developed within developing country institutions through the introduction of degree courses in order to broaden the in-country technical support base for food bioprocess development. Given the similarities among fermentation processes across regions, an inventory of institutions engaged in food biotechnology in developing countries would be an asset in facilitating networking among institutions. Food processors, policy-makers and equipment manufacturers should also be integrated into the networking activities.
- The development of appropriate levels of bioreactor technology with control bioprocess parameters will be necessary to improve the hygienic conditions of the fermentation processes.

- Research and infrastructural development to enable the cost-effective production of defined starter cultures in a stable format (i.e. cultures which do not require refrigeration and have prolonged shelf-lives under ambient conditions) should be prioritized.
- Infrastructure development to facilitate the transfer and adaptation of fermentation technologies developed at the laboratory level to the household and village and, where necessary, the enterprise level should be prioritized.
- Appropriate levels of equipment will also be required to facilitate the downstream processing of these products.
- Traceability systems that facilitate the differentiation and identification of food products should be prioritized in order to broaden market opportunities for these products.
- A food-chain approach to assuring food safety should be prioritized by governments.
- Food safety management systems should be strengthened by implementing systematic food safety measures such as GHP, GMP and HACCP in food fermentation operations. Diagnostic kits are important tools for monitoring and verifying the level of sanitation in processing plants.

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

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

5.8 IDENTIFYING PRIORITIES FOR ACTION FOR THE INTERNATIONAL COMMUNITY

The last decade has witnessed considerable change with respect to the applications of biotechnology in food processing and food safety. Market forces have been the major drivers of change in the food sector of developing countries. Modern biotechnological tools are likely to play a greater role in the development of efficient science-based processes for food processing and safety in order to respond to consumer demand. The production of high-value fermented products such as enzymes, functional food ingredients and food additives is likely to continue to increase in developing countries.

The international community (FAO, UN organizations, NGOs, donors and development agencies) can play a major role in assisting developing countries to maximize the benefit to be derived from food bioprocessing. The adoption of biotechnology-based methods in food processing and for food safety and quality monitoring is dependent on several factors that include capacity building in technical and regulatory areas, policy formulation, regulatory frameworks and regional networks.

Based on the analysis in this Chapter, a number of priority areas are identified for support by the international community. These are:

Capacity building and human resource development

- support basic and advanced education;
- prioritize specific areas for investment;
- develop policy options, priorities and action programmes that promote food fermentation as a means of addressing food security;
- support human resource development in a range of scientific disciplines – food biotechnology, food safety, bioengineering and enzyme technology;
- support capacity building initiatives for household-level, small- and medium-scale processors of fermented foods;
- support IPR development.

Technology transfer and support for R&D

- improve the relevance of national research to the needs of the food sector in developing countries;
- enhance competitiveness and the creation of an enabling environment that is conducive to private sector investment in research, development and innovation for the upgrading of food fermentation processes to respond to market demand;

- establish and strengthen the research and infrastructural support base for work on starter culture development, bioreactor design and for the development of diagnostic tests and equipment for monitoring food safety and traceability. This infrastructural support base would include laboratories, laboratory equipment and cell bank facilities for the proper preservation and storage of microbial culture preparations;
- develop scientific data to examine the nutritional, health and health-benefit claims associated with fermented foods;
- establish pilot processing facilities for the scaling-up and testing of technologies to facilitate their adoption.

Networking and clusters

- support the development of regulatory frameworks for food safety;
- support North-South and South-South training and exchange on food biotechnologies, bioprocess engineering and food safety;
- promote and facilitate networking among scientists, researchers, small- and medium-scale food processors and the retail sector to facilitate knowledge and information-sharing;
- support leveraging the traditional knowledge base in the upgrading of food fermentation processing operations.

5.9 REFERENCES

- Barrett, T., Fang, P. & Swaminathan, B. 1997. Amplification methods for detection of food-borne pathogens. In H. Lee, S. Morse & O. Slovak, eds. *Nucleic acid amplification techniques: Application to disease diagnosis*, pp. 171–181. Boston, USA, Eaton Publishing.
- Bauer, F.F., Næs, T., Esbensen, K., Young, P.R., du Toit, M. & Vivier, M.A. 2007. Functional wine-omics. In R.J. Blair & I.S. Pretorius, eds. *Proceedings 13th Australian wine industry technical conference*, 29 July–2 August 2007, Adelaide, Australia, pp 178–183.
- Beaumont, M. 2002. Flavouring composition prepared by fermentation with *Bacillus* spp. *Int. J. Food Microbiol.*, 75: 189–196.
- Berger, R.G. 2009. Biotechnology of flavours – the next generation. *Biotechnol. Lett.*, 31: 1651–1659.
- Bolotin-Fukuhara, M., Toffano-Nioche, C., Artiguenave, F., Duchateau-Nguyen, G., Lemaire, M., Marmeisse, R., Montrocher, R., Robert, C., Termier, M., Wincker, P. & Wésolowski-Louvel, M. 2000. Genomic exploration of the hemiascomycetous yeasts: 11. *Kluyveromyces lactis*. *FEBS Lett.*, 487: 66–70.
- Chokesajjawatee, N., Pornaem, S., Zo, Y-G., Kamdee, S., Luxananil, P., Wanasen, S. & Valyasevi, R. 2009. Incidence of *Staphylococcus aureus* and associated risk factors in Nham, a Thai fermented pork product. *Food Microbiol.*, 26: 547–551.
- Codex Alimentarius Commission. 2009. *Food hygiene (basic texts)*. Fourth Edition. Secretariat of the Joint FAO/WHO Food Standards Programme, Rome, FAO. (also available at www.fao.org/docrep/012/a1552e/a1552e00.htm).
- de Vos, W.M. 2001. Advances in genomics for microbial food fermentation and safety. *Curr. Opin. Biotechnol.*, 12: 493–498.
- FAO. 1998. *Fermented fruits and vegetables: A global perspective*, by M. Battcock & S. Azam-Ali. FAO Agricultural Services Bulletin No.134. Rome (also available at www.fao.org/docrep/x0560E/x0560E00.htm).
- FAO. 1999. *Fermented cereals: A global perspective*, by N.F. Haard, S.A. Odunfa, C-H. Lee, R. Quintero-Ramirez, A. Lorence-Quiñones & C. Wachter-Radarde. FAO Agricultural Services Bulletin No. 138. Rome. (also available at www.fao.org/docrep/x2184e/x2184e00.htm).
- FAO. 2006. *FAO/WHO guidance to governments on the application of HACCP in small and/or less-developed food businesses*. FAO Food and Nutrition Paper 86. Rome. (also available at www.fao.org/docrep/009/a0799e/a0799e00.htm).
- FDA. 2003. *Bacteriological analytical manual*. US Food and Drug Administration.
- Flamm, E.L. 1991. How FDA approved chymosin: A case history. *Biotechnol.*, 9: 349–351.
- Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., Johnston, M., Louis, E.J., Mewes, H.W., Murakami, Y., Philippsen, P., Tettelin, H. & Oliver, S.G. 1996. Life with 6000 genes. *Science*, 274: 546–567.
- Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K., Han, C-G., Ohtsubo, E., Nakayama, K., Murata, T., Tanaka, M., Tobe, T., Iida, T., Takami, H., Honda, T., Sasakawa, C., Ogasawara, N., Yasunaga, T., Kuhara, S., Shiba, T., Hattori, M. & Shinagawa, H. 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Research*, 8: 11–22.
- Holzappel, W.H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.*, 75: 197–212.

- Hutkins, R.W. 2006. *Microbiology and biotechnology of fermented foods*. Oxford, Blackwell Publishing.
- JECFA. 2006. *Combined compendium of food additive specifications Volume 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications*. Joint FAO/WHO Expert Committee on Food Additives. FAO JECFA Monograph 1. Rome (also available at www.fao.org/docrep/009/a0691e/a0691e00.htm)
- JECFA. 2008. *Asparaginase from Aspergillus niger expressed in A. niger: Chemical and technical assessment (CTA)*, by Z.S. Olempska-Beer. Paper prepared for the 69th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 2008. 7 pp.
- Koch, W.H., Payne, W.L. & Cebula, T.A. 1995. Detection of enterotoxigenic *Vibrio cholerae* in foods by the polymerase chain reaction. *FDA Bacteriological Analytical Manual*, 8th Edition, pp. 28.01–28.09. Gaithersburg, AOAC International.
- Kuroda, M., Ohta, T., Uchiyama, I. et al. 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet*, 357: 1225–1240.
- Leuchtenberger, W., Huthmacher, K. & Drauz, K. 2005 Biotechnological production of amino acids and derivatives: Current status and prospects. *Appl. Microbiol. Biotechnol.*, 69: 1–8.
- Mengu, M. 2009. Overview of FP and Africa FP lessons learnt and success stories: Research on traditional African fermented foods. *Proceedings science and technology Europe-Africa project workshop*, Nairobi, Kenya, pp. 16-18. (available at www.st-eap.org/pdfs/report_st-eap_ws_mar2009.pdf).
- Merican, Z. & Quee-Lan, Y. 2004. Tapai processing in Malaysia: A technology in transition. In K.H. Steinkraus, ed. *Industrialization of indigenous fermented foods*. New York, Marcel Dekker, pp.169–190.
- Odunfa, S.A. 1981. Micro-organisms associated with fermentation of African Locust bean during iru preparation. *J. Plant Foods*, 3: 245–250.
- Odunfa, S.A. & Oyewole, O.B. 1986. Identification of Bacillus species from iru, a fermented African Locust bean product. *J. Basic Microbiol.*, 26: 101–108.
- Odunfa, S.A. & Oyewole, O.B. 1997. African fermented foods. In B.J.B. Wood, ed. *Microbiology of fermented foods*, 2nd Edition, Vol. II, pp. 713–715. London, Blackie Academic and Professional.
- Ogbadu, L.J. & Okagbue, R.N. 1988. Bacterial fermentation of soya bean for ‘daddawa’ production. *J. Appl. Bacteriol.*, 65: 353–356.
- Ogbadu, L.J., Okagbue, R.N. & Ahmad, A.A. 1990. Glutamic acid production by bacillus isolates from Nigerian fermented vegetable proteins. *World J. Microbiol. Biotechnol.*, 6: 377–382.
- Olempska-Beer, Z.S., Merker, R.I., Ditto, M.D. & DiNovi, M.J. 2006. Food-processing enzymes from recombinant microorganisms – a review. *Regul. Toxicol. Pharmacol.*, 45: 144–158.
- Paludan-Muller, C., Huss, H.H. & Gram, L. 1999. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. *Int. J. Food Microbiol.*, 46: 219–229.
- Paludan-Muller, C., Valyasevi, R., Huss, H.H. & Gram L. 2002. Genotypic and phenotypic characterization of garlic-fermenting lactic acid bacteria isolated from som-fak, a Thai low-salt fermented fish product. *J. Appl. Microbiol.*, 92: 307–314.
- Parkhill, J., Wren, B. W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A.V., Moule, S., Pallen, M.J., Pennk, C. W., Quail, M. A., Rajandream, M-A., Rutherford, K. M., van Vliet, A.H.M., Whitehead, S. & Barrell, B.G. 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature*, 403: 666–668.

- Paukatong, K.V. & Kunawasen, S.** 2001. The hazard analysis and critical control points (HACCP) generic model for the production of Thai fermented pork sausage (Nham). *Berl. Münch. Tierärztl. Wschr.*, 114: 327–330.
- Pretorius, I.S.** 2000. Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of winemaking. *Yeast*, 16: 675–729.
- Pretorius, I.S. & Bauer, F.F.** 2002. Meeting the consumer challenge through genetically customised wine yeast strains. *Trends Biotechnol.*, 20: 426–432.
- Ray, R.C. & Sivakumar, P.S.** 2009. Traditional and novel fermented foods and beverages from tropical roots and tuber crops: Review. *Int. J. Food Sci. Tech.*, 44: 1073–1087.
- Schmale III, D.G. & Munkvold, G.P.** 2009. Mycotoxins in crops: A threat to human and domestic animal health. The American Phytopathological Society Education Center.
- Sebaihia M., Peck M.W., Minton N.P. et al.** 2007. Genome sequence of a proteolytic (Group I) *Clostridium botulinum* strain Hall A and comparative analysis of the clostridial genomes. *Genome Res.*, 17:1082–1092.
- Sefc, K.M., Lopes, M.S., Mendonca, D., Rodrigues dos Santos, M., Laimer da Camara Machado, M. & da Camara Machado, A.** 2000. Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. *Mol. Ecol.*, 9: 1171–1173.
- Spok, A.** 2006. Safety regulations of food enzymes. *Food Technol. Biotechnol.*, 44: 197–209.
- Steinkraus, K.H.** 1995. Indigenous fermented foods involving an alkaline fermentation. In: K.H. Steinkraus, ed., *Handbook of indigenous fermented foods*, 2nd Edition, pp. 7–110. New York, Marcel Dekker.
- Tamang, J.P.** 1998. Role of microorganisms in traditional fermented foods. *Indian Food Ind.*, 17: 162–167.
- Teletchea, F., Maudet, C. & Hänni, C.** 2005. Food and forensic molecular identification: Update and challenges. *Trends Biotechnol.*, 23: 359–366.
- Valyasevi, R. & Rolle, R.S.** 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope of their improvement. *Int. J. Food Microbiol.*, 75: 231–239.
- VanVeen, A.J. & Steinkraus, K.H.** 1970. Nutritive value and wholesomeness of fermented foods. *J. Ag. Food Chem.*, 18: 576–578.
- Yoshitomi, K.J., Jinneman, K.C. & Weagant, S.D.** 2003. Optimization of 3'-minor groove binder-DNA probe targeting the uidA gene for rapid detection of *Escherichia coli* O157:H7 using real-time PCR. *Mol. Cell. Probes*, 17: 275–280.
- Zhang Z.Y., Liu C., Zhu Y.Z., Zhong, Y., Zhu, Y.Q., Zheng, H.J., Zhao, G.P., Wang, S.Y. & Guo, X.K.** 2009. Complete genome sequence of *Lactobacillus plantarum* JDM1. *J. Bacteriol.*, 191: 5020–5021.