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Applications Biotechnologies in Food Processing and in Food Safety in Developing Countries

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ABSTRACT

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 caliber technologies in order to characterize the 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.

Keywords: Biotechnologies, Food Processing, Food Safety, Developing Countries

I. INTRODUCTION

For the purpose of this paper, biotechnology is defined in accordance with the Convention on Biological Diversity, 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 makes use of 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).

Recombinant gene technology, the best-known modern biotechnology, is widely employed in research and development 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. Genetically modified (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 in order 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 will be explored and discussed in subsequent sections.

II. CURRENT STATUS OF THE APPLICATION OF TRADITIONAL AND NEW BIOTECHNOLOGIES IN FOOD PROCESSING IN DEVELOPING COUNTRIES

2.1 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 makes use of microbial inoculants for enhancing properties such as the taste, aroma, shelf-life, safety, texture and nutritional value of foods. Microbes associated with the raw food material and the processing environment serve as inoculants in spontaneous fermentations, while inoculants containing high concentrations of live micro-organisms, referred to as starter cultures, are used to initiate and accelerate the rate of fermentation processes in non-spontaneous or controlled fermentation processes. Microbial starter cultures vary widely in quality and purity.

2.1.1 Spontaneous inoculation of fermentation processes

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

2.1.2 "Appropriate" starter cultures as inoculants of fermentation processes

"Appropriate" starter cultures are widely applied as inoculants across the fermented food sector, from the household to industrial level in low-income and lower-middle-income economies. These starter cultures are generally produced using a backslopping process which makes use of samples of a previous batch of a fermented product as inoculants (Holzapfel, 2002). Appropriate starter cultures are widely applied in the production of fermented fish sauces and fermented vegetables in Asia and in cereal or grain fermentations in African and Latin American countries. The inoculation belt (Holzapfel, 2002) used in traditional fermentations in West Africa serves as a carrier of undefined fermenting micro-organisms, and is one example of an appropriate starter culture. It generally consists of a woven fibre or mat or a piece of wood or woven sponge, saturated with "high"

2.1.3 Defined starter cultures as inoculants of fermentation processes

Few defined starter cultures have been developed for use as inoculants in commercial fermentation processes in developing countries. Nevertheless, the past ten years have witnessed the development and application of laboratory-selected and pre-cultured starter cultures in food fermentations in a few developing countries. These developments have taken place primarily in Asian countries. "Defined starter cultures" consist of single or mixed strains of micro-organisms (Holzapfel 2002). They may incorporate adjunct culture preparations that serve a food-safety and preservative function. Adjunct cultures do not necessarily produce fermentation acids or modify texture or flavour, but are included in the defined

culture owing to their ability to inhibit pathogenic or spoilage organisms. Their inhibitory activity is due to the production of one or several substances such as hydrogen peroxide, organic acids, diacetyl and bacteriocins (Hutkins, 2006).

2.1.4 Defined starter cultures developed using the diagnostic tools of advanced biotechnologies

The use of DNA-based diagnostic techniques for strain differentiation can allow for the tailoring of starter cultures to yield products with specific flavours and/or textures. Random amplified polymorphic DNA (RAPD) techniques have been applied in, for example, Thailand, in the molecular typing of bacterial strains and correlating the findings of these studies to flavour development during the production of the fermented pork sausage, nham. 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).

2.1.5 GM starter cultures

To date, no commercial GM micro-organisms that would be consumed as living organisms exist. Products of industrial GM producer organisms are, however, widely used in food processing and no major safety concerns have been raised against them. Rennet which is widely used as a starter in cheese production across the globe is produced using GM bacteria. These are discussed in more specific detail in Section 2.2. Thailand currently makes use of GM Escherichia coli as an inoculant in lysine production. Many industrially important enzymes such as α-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 recombinant micro-organisms include Cuba, Brazil, India, and Argentina.

2.2 Food additives and processing aids

Enzymes, amino acids, vitamins, organic acids, polyunsaturated fatty acids and certain complex carbohydrates and flavouring agents used in food formulations are currently produced using GM micro-organisms. Examples of some of these products are listed in Table 5.

2.2.1 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 α-amylase amino acid sequences through DNA sequence modifications of the α-amylase genes (Olempska-Beer et al. 2006). Bovine chymosin used in cheese manufacture was the first recombinant enzyme approved for used 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 by deleting native genes encoding extracellular proteases. Certain fungal producing strains have also been modified to reduce or eliminate their potential for producing toxic metabolites (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, however, 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 (FAO, 2006). Preparations of asparaginase enzymes have also been evaluated by JECFA (Olempska-Beer, 2008).

2.2.2 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 the 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.

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

2.3.1 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.

2.3.2 Advances in microbial genetics

In recent times, the 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 the Lactococcus lactis genome. 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 also available. The genome of Clostridium botulinum, responsible for food poisoning, was also recently completed (Sanger Institute, 2009).

2.3.3 Detection of pathogens

The rapid detection of pathogens and other microbial contaminants in food is critical to assess 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 the polymerase chain reaction (PCR) assay. Short fragments of DNA (probes) or primers are hybridized to a specific sequence or template, which is subsequently enzymatically amplified 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 2 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 Weagan, 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, Staphylococcus aureus (Bacteriological Analytical Manual, 2003).

Sophisticated cultural 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 enzyme-linked immunosorbent assay (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 7.

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 is summarized in Table 8. The application of HACCP necessitates the deployment of good agricultural practices, good manufacturing practices (GMPs) and good hygienic practices (GHPs) and the monitoring of critical control points for potential microbial and chemical contamination during bioprocessing (FAO, 1995). 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 sensitivity and specificity unchallenged by traditional/conventional methods.

2.3.4 Mycotoxin detection

The problem of mycotoxin contamination in food including fermented foods is a global concern. Mycotoxin contamination is particularly prevalent in developing countries in tropical areas such as in 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 in use, sensitive and easy to use would greatly facilitate the detection and quantization 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).

2.3.5 Detection and identification of foods and food ingredients

The DNA-based identification code system is reliant 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 in the identification of 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. The DNA barcode has been used for the identification of many products for export in countries such as Thailand, China, Brazil, Cuba and Argentina, The DNA barcode of microsatellite markers has also been successfully used in differentiating and identifying fermented products such as premium wines, cheeses and sausages on the basis of their origins. Basmati rice varieties and olive cultivars used in olive oil production (Sefc et al., 2000) have also been differentiated.

III. 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.

3.1 Socio-economics of the consumer base

The consumer base of traditionally fermented staple foods in most developing countries is largely poor and disadvantaged. Price, rather than food safety and quality, is therefore a major preoccupation of this group when purchasing food. Fermented foods provide that target group with an affordable source of food, and make a substantial contribution to their food and nutritional security. These foods are

generally produced under relatively poor hygienic conditions at the household and village level. Fermentation processing is practised largely as an art in such contexts.

Interventions designed to upgrade processes used in the production of these traditionally fermented staples have been largely carried out through donor-funded projects and have focused primarily on reducing the drudgery associated with the fermentation processes. Improvements have also targeted the upgradation 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. 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 4.1 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 4.2 highlights how consumer demand for safe food led to research and development into starter culture development designed to improve the safety of nham in the marketing chain.

3.2 Changing consumer demand trends

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

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

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

3.3 The enabling environment for starter culture development

A considerable amount of research in developing countries has focused on the identification of starter micro-organisms associated with the fermentation of these staple foods. The greatest strides in starter culture development have, however, been realized in countries that have prioritized the development of technical skills, the infrastructural support base and funding support for research into the upgradation of fermentation processes. Linkages between research institutions and the manufacturing sector have also been critical to the successful introduction of starter cultures.

Collaborative initiatives among research institutions have also had a major positive impact on biotechnological developments in developing countries. Collaboration among African institutions and their counterparts in the North has greatly facilitated improvements in biotechnological research and capacity development in the area of food biotechnology on the continent. One major success story in this regard has been collaborative projects involving Burkina Faso, Ghana and institutions in the Netherlands. This programme facilitated the typing and screening of microbial cultures associated with fermented African foods as a basis for starter culture development. Results of this work (Mengu, 2009) led to improvements in the production of gari, a fermented cassava product and dawadawa, a fermented legume product.

3.4 Proactive industrial strategies

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

3.5 Export opportunities for fermented products

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

IV. CONCLUSION

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 caliber technologies in order to characterize the 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.

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