Food Processing Biotechnology

The food chain can be viewed as a continuum from the planted seed to the processing, distribution and marketing of products, to the consumer's table. The food processing industry serves as the vital link between the farmer and the supermarket. Except for fruits and vegetables that are often consumed raw, most agricultural products undergo some kind of processing after leaving the farm gate. Biotechnology can obviously be used to improve the safety and nutritional quality of the food supply at every link in the chain. Previous examples have focused on how it can be used to improve the production end of the food chain. Any genetic improvement in plants and animals that serve as raw materials for processed foods will impact the processing of that product; therefore, processing parameters are an essential element of any strain improvement strategy. However, the following discussion will focus on how biotechnology can be used to improve the processing of food — the utilization end of the food chain — from the time the raw product leaves the farm gate until it is consumed.

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A stroll through a modern supermarket vividly illustrates how processed foods have changed in the last 10 years. With increased consumer awareness and concern about food quality, safety, nutrition, and convenience, the food processing industry has responded by formulating and marketing products that meet consumer demands and expectations. It is common to see expanded refrigerated and frozen food sections, a wide variety of fresh fruits and vegetables, extensive delicatessen sections featuring partially processed foods, and a vast array of microwavable products. New product development often requires the utilization of new processing, preservation, packaging and distribution systems, and these new systems may create the potential for new microbiological, safety, quality or nutritional concerns. For example, although modified atmosphere or controlled atmosphere packaging (MAP/CAP) and vacuum packaging can be used to improve quality and extend shelf-life of minimally processed refrigerated products, it also creates an environment that could permit the growth of deadly pathogens. Restricted use of nitrate in bacon or sulfite in potato processing creates similar microbiological safety concerns. Microwave ovens have revolutionized home food preparation, yet uneven heating could create microenvironments that allow survival of pathogenic organisms. To meet consumer demands and at the same time ensure food quality and safety will require application of innovative and effective technologies, including biotechnology-

What is Biotechnology?

Biotechnology has been defined as a collection of technologies that use living systems (plants, animals, or microorganisms), or compounds derived from living organisms, for the production of industrial goods and services (Office of Technology Assessment, 1981). Biotechnology is not new to the food processing industry as humans have been exploiting living systems for the production, processing and presevation of food for centuries. Mutation and selection techniques have been used to improve strains of bacteria and yeast used to produce fermented foods, such as cheese, sausage, bread and wine. Many ingredients used in processed foods including vitamins, stabilizers, enzymes, flavor enhancers and preservatives are currently produced by bacteria. What distinguishes the more traditional "old" biotechnology from the "new" biotechnology is the emergence within the last 20 years of genetic engineering that allows the exchange of genetic information between related and unrelated organisms. Other molecular biology techniques, including hybridoma technology, DNA probe technology, enzyme and protein engineering, bioengineering and fermentation technology, and plant and mammalian cell culture, are also included under the umbrella of biotechnology.

Applications of Biotechnology to Food Processing

Genetic improvement of food fermentation microorganisms Bacteria, yeasts and molds have been used for the production of fermented foods for thousands of years. Classical strain improvement methods involving mutation and selection are imprecise and uncontrollable, it is impossible to screen for all mutations that might occur, and the screening process is laborious and time consuming. In addition, one is limited to the genetic information already present in the organism. Genetic engineering provides a mechanism for overcoming many of these limitations as it allows for the selection and transfer of single, well-defined traits from virtually any living organism in a precise, controllable and predictable manner. Table 1 illustrates how genetic engineering can be used to improve microorganisms used in food fermentations. Examples include the impact of genetic improvements on the processing, nutritional value, microbiological safety and shelf-life of fermented foods.

Table 1 Genetic improvement of food-grade microorganisms

Type of Fermentation	Nature of Improvement	Implications
DAIRY		
Cheese	Bacteriophage (virus) resistance	Eliminate economic losses due to destruction of culture by viruses
	Accelerated ripening	Decreased storage costs
Yogurt	Higher levels of betagalactosidase	More digestible product for lactose-intolerant individuals
MEAT		
Sausage	Bacteriocin production	Inhibition of pathogens and spoilage organisms
CEREAL		
Beer	Alpha-amylase production	Used for production of “lite” or low calorie beer
Bread	Higher levels of maltose permease and maltase	More consistent and improved leavening

(Taken from Harlander, 1989)

It is important to note that the first genetically engineered food-grade microorganism was approved for use by the British Ministry of Agriculture, Fisheries and Food on March 1,1990. The manufacturer, Gistbrocades, was granted permission to manufacture and supply the particular strain of yeast, *Saccharomyces cerevisiae* 352 Ng, used in the baking industry. The strain was genetically engineered to produce elevated levels of two enzymes involved in starch utilization, maltose permease and maltase. Consistent fermentations result in doughs containing widely different sugar concentrations thus ensuring product quality.

Healthy microbes Over 70 percent of the world's population lose the ability to ferment lactose due to the gradual loss of lactase, an enzyme present in the brush border of the intestine that hydrolyzes lactose into glucose and galactose. Several investigators have demonstrated that lactase-deficient individuals digest lactose from yogurt much more efficiently than lactose from other dairy foods (Savaiavo and Levitt, 1987). Genetic engineering could be used to enhance the level of microbial betagalactosidase produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* the two organisms used in the manufacture of yogurt, making the product more easily digested by lactoseintolerant individuals. Because elderly individuals frequently experience difficulty in digesting certain food products, it may be possible to use yogurt culture as delivery systems for other digestive enzymes for certain target populations.

Probiotics Numerous strains of bacteria are capable of implanting and competing in the gastrointestinal tract of humans and animals. These organisms are often classified as "probiotic" as their function is to aid the host in some beneficial manner. Construction of strains capable of competitively inhibiting potentially pathogenic gut organisms could have several applications in agriculture and food. For example, nonpathogenic strains of *Salmonella* or other gut organisms could be engineered to produce broad spectrum bacteriocins. These strains could be supplied in poultry feed and water for biological control of pathogenic strains of *Salmonella* or other gut pathogens. The same concept could be applied to other animal species and to humans, as well. *Lactobacillus acidophilus,* a food-grade microorganism used in the production of acidophilus milk, is capable of surviving passage through the stomach, and under certain circumstances is able to colonize the GI tract. It may be possible to engineer bacteriocin-producing strains with enhanced colonization capability could be used to modulate the ecology of the gut. Consumption of products containing these engineered strains could be recommended for individuals who have completed antibiotic therapy, for travelers who might be exposed to food-borne pathogens, or for immunocompromised individuals who are susceptible to endemic diarrhea or yeast infections.

Microbially derived ingredients Microorganisms produce a host of metabolites currently used as ingredients in processed food products (Neidleman, 1990). These include acidulants (acetic, lactic, benzoic, propionic), flavors (diacetyl, pyrazines, lactones, esters), flavor enhancers (MSG), pigments (monascin, astazanthan), stabilizers and thickeners (xanthan gum, dextrans), nutritive additives (vitamins, amino acids), sweeteners (aspartame), enzymes (proteases, lipases, cellulases, pectinases) and preservatives (nisin). These ingredients add functionality, enhance nutritional quality, extend shelf-life, improve convenience and ensure safety.

Many of the ingredients listed above are produced by organisms that have a long history of safe use in foods. However, there are many microbes in nature that produce interesting compounds that could be used in processed foods. For example, many bacteria produce extracellular biopolymers that could be used as stabilizing agents, viscosifiers, surfactants, flavor encapsulating agents, noncaloric gelling agents, and as a source of soluble fiber in the diet. There is tremendous interest in transferring the gene(s) that code for production of these biopolymers into food-grade microorganisms.

Enzymes Enzymes are used extensively by the food industry as processing aids to control texture, appearance, flavor development, and nutritive value of processed foods. For example, various proteases are used to tenderize meat, pectinases are used to decloud fruit juices, amylases are used to degrade starches, caffeinases are used to decaffeinate coffee, and oxidases are used to remove off flavors (Neidleman, 1986).

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An historic event for food biotechnology was the recent affirmation (March 23, 1990) by the Food and Drug Administration (FDA) of "generally regarded as safe" (GRAS) status for the first recombinant enzyme to be used directly in food. Recombinant chymosin or rennet is an enzyme that is used to accelerate curd formation during cheese manufacture. Recombinant rennet is produced by a genetically engineered strain of *Escherichia coli* and is purified from the fermentation broth. It is interesting to note that the plasmid vector codes for an antibiotic resistance marker and that the producing strain, although not pathogenic in nature, is a gut organism that did not enjoy a long history of safe use in food prior to this application, yet achieved FDA approval. Approval of this enzyme is a significant milestone for the food industry as it establishes a critical regulatory precedent and serves as a model for other biotechnologically-derived enzymes and ingredients. It is also interesting to note that the recombinant product contains more active enzyme per unit protein and is microbiologically safer than the traditional counterpart which is extracted from the forestomach of calves.

Enzyme engineering Most enzymes function optimally at physiologic temperature and pH; this is not the conditions encountered in food processing operations that frequently involve high temperature and low pH. Genetic engineering techniques (site-specific mutagenesis) have been used to specifically alter the primary amino acid sequence of enzymes to improve their functionality in food systems. Some examples of how enzyme engineering could be used to improve enzymes used in food processing are provided in Table 2.

Table 2

Suggestions for improved enzymatic activity through enzyme engineering

Enzyme	Application	Useful Improvement
alpha-amylase	Starch liquefaction	Acid-tolerant and thermostable
amyloglucosidase	High fructose corn syrup production	Immobilized with higher productivity
esterases, lipases, proteases, etc.	Flavor development	Improved substrate specificity
glucose isomerase	High fructose corn syrup production	Increased thermo-stability
limoninase	Debittering of fruit juices	More complete limonin degradation
protease	Beer chill proofing	Improved substrate specificity
pullulanase	High fructose corn syrup production	Increased thermo-stability

(Taken from Neidleman, 1986)

More Efficient Utilization of Raw Materials

Environmental concerns and economic issues necessitate better utilization of raw materials and reduction of waste generated by the food processing industry. In the past, food processing waste streams were discharged into the environment or buried in landfills. However, the bioburden is great and soil microorganisms are not capable of degrading compounds at a fast enough rate. Whey from cheese manufacture, cellulosic waste from vegetable processing, shells from nut processing, and starch from potato processing are but a few examples of the kinds of waste streams generated by the food industry. More innovative methods for converting these materials to valueadded products must be developed. In other countries, food processing waste streams are used as feedstock for subsequent fermentation

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processes used to produce food and nonfood products including pharmaceuticals and specialty chemicals. As our supply of petroleum-based chemicals are depleted, biotechnology-based methods will be needed to more efficiently utilize waste streams, surplus commodities and other renewable agriculture resources.

Food Safety

Although it is generally agreed that the U.S. enjoys the safest food supply in the world, emerging pathogens, not previously associated with food, have been responsible for recent outbreaks of foodborne illness. Within the last five years, the dairy industry has had to cope with the emergence of the pathogenic organism, *Listeria monocytogenes,* which is capable of causing spontaneous abortion in pregnant women, and meningitis in infants, the elderly, and immunocompromised individuals. A strain of *Salmonella enteritidis* has been isolated from intact eggs and entire flocks of poultry appear to be endemically infected with the organism. There is also increasing consumer concern about microbial toxins, aflatoxin, chemical residues (herbicides, pesticides, fertilizers and fungicides), antibiotics, and animal drug residues in raw and processed foods. Rapid and sensitive methods based on the development of DNA probes and poly and monoclonal antibodies could revolutionize quality control and quality assurance in the food industry. Theoretically, these tests should be capable of detecting a single organism or toxin molecule, thus dramatically increasing the sensitivity over current methods. In addition, test results are available in hours or days rather than days or weeks.

Biosensors

The highly specific action of enzymes and microbial cells can be exploited as analytical tools to measure the concentration of specific components in complex mixtures. Enzymes, antibodies or whole cells can be immobilized onto solid surfaces, and the specific reactions they mediate can be detected electrochemically, photometrically, thermometrically or mechanically (Wagner and Schmid, 1990). In food systems, biosensors can be used to measure low molecular weight, single compounds such as glucose, organic acids, amino acids, alcohols or food additives; complex compounds such as microorganisms and biological or chemical contaminants; and complex quality parameters such as freshness, shelf-life prediction, flavor, maturity or thermal stress. Some examples of biosensors currently under development are listed in Table 3.

Table 3 Commercially available biosensors for food analysis

Analyte	Biocomponent	Application
Glucose	Whole bacterial cells Glucose oxidase enzyme	Molasses production Brewing, various fermentations, fruit juice and soft drink manufacture, banana maturation
Lactose	P-galactosidase enzyme	Raw milk
Sucrose	Invertase enzyme	Instant cocoa manufacture
Lactate	Lactate dehydrogenase enzyme	Dairy products, yogurt, whey
Ethanol	Alcohol dehydrogenase enzyme	Alcoholic beverages, wine, beer, cider, fermentations
Peptides	Amino peptidase enzyme	Casein hydrolysis
Amino acids	Amino acid dehydrogenase enzyme	Many foods
Glutamate	L-glutamate oxidase enzyme	Soy sauce manufacture
Aspartame	L-aspartase enzyme or alcohol oxidase enzyme	Level of sweetener in many foods including soft drinks
Ascorbic acid	Ascorbate oxidase enzyme	Fruit juices
Sulfite	Sulfite oxidase enzyme	Dry fruit, wine, vinegar, juices, potato flakes
Penicillin	Antibody-enzyme conjugate	Milk
PHB ester	phydroxybenxoate hydroxylase enzyme	Fruit juices and drinks

Taken from Wagner and Schmid, 1990).

Conclusions

Biotechnology could have a dramatic impact on the entire agriculture and food sector. It has the potential to reduce the need for agricultural chemicals; improve the productivity, efficiency, and profitability of food production and processing; open new markets for improved or unique processed food products; and, improve the nutritional quality, safety, cost, and convenience of consumer food products. Any improvement of the food supply at any point in the food chain will ultimately impact the utilization end of the system and the ultimate beneficiary of the improvement — the consumer.

References

Harlander, S.K. (1989) Food biotechnology: Yesterday, today, and tomorrow, *Food Technology* 43:196.

Neidleman, S. (1986) Enzymology and food processing. In: *Biotechnology and Food Processing.* Harlander, S. & Labuza, T. (Eds.)Noyes Publications, Park Ridge, N.J.

Neidleman, S. (1990) The microbial production of biochemicals. *In: A Revolution in Biotechnology.* Marx, J.L. (Ed.). Cambridge University Press, New York, N.Y.

Office of Technology Assessment. (1981) *Impacts of Applied Genetics: Microorganisms, Plants and Animals.* U.S. Government PrintingOffice, Washington, D.C.

Savaiavo, D. and Levitt, M. (1987) Milk intolerance and microbecontaining dairy *loads, Journal of Dairy Science* 70:397.

Wagner, S. and Schmid, R.D. (1990) Biosensors for food analysis, *Food Biotechnology* 4:215.