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Biotechnology in Food Production and Processing

Dietrich Knorr and Anthony J. Sinskey

The use of biotechnology in the manufacture of food and beverages has been practiced for more than 8000 years with vinegar, alcoholic beverages, sourdough, and cheese production being the

tissue culture systems, and bioengineering offer great potential for application to several areas of food production and processing. A key question related to this issue is, what are the constraints

Summary. The food processing industry is the oldest and largest industry using biotechnological processes. Further development of food products and processes based on biotechnology depends upon the improvement of existing processes, such as fermentation, immobilized biocatalyst technology, and production of additives and processing aids, as well as the development of new opportunities for food biotechnology. Improvements are needed in the characterization, safety, and quality control of food materials, in processing methods, in waste conversion and utilization processes, and in currently used food microorganism and tissue culture systems. Also needed are fundamental studies of the structure-function relationship of food materials and of the cell physiology and biochemistry of raw materials.

most prominent examples (1). Biotechnological processes are now being used to produce other fermented products, food and feed additives, and processing aids (Table 1). In fact, the food processing industry, which has annual sales of \$300 billion in the United States and about £30 billion in Great Britain, is the oldest and largest user of biotechnological processes (2).

An important issue today is the impact that modern biology will have on the food industry. Recent advances in molecular biology, fermentation science,

hindering applications of biotechnology to the food industry? In this article, we attempt to address these points and to review the current role of biotechnology in the production and processing of food.

Biotechnology in Food Production

Biotechnology can significantly influence the food supply, including the production and preservation of raw materials and the alteration of their nutritional and functional properties. In addition,

development of production aids, processing aids, and direct additives such as enzymes, flavors, polysaccharides, pigments, and antioxidants can improve the overall utilization of raw materials.

Raw materials. Plant products derived from fewer than 30 plant species provide more than 90 percent of the human diet. Eight cereal crops supply more than half the world's calories (4). Animal products contribute over 56 million tons of edible protein and over 1 billion megacalories of energy annually (5). In addition, the increasing importance of marine food products and single cell proteins (SCP) as raw materials has been stressed (6).

Currently, the role of biotechnology in raw material production is directed toward (i) increasing productivity through improved efficiency of nutrient use and conversion, (ii) increasing productivity through improved plant resistance, and (iii) identifying new food sources with desirable properties.

Feed efficiency and productivity of animals has been increased substantially (7). Furthermore, SCP, derived from the dried cells of microorganisms for use as protein sources in human food and animal feeds, are cultivated on a large scale by using both photosynthetic and non-photosynthetic microorganisms (8). Extensive work is under way to increase the ability of plants to fix atmospheric nitrogen for their metabolic use (9), and cultured plants and plant cells are being considered for food production (10).

Additional efforts include the genetic

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Table 1. World production, market value, and end use of selected products of the biotechnology-based food industry (3).

Products	Production (metric tons per year)		Market size (millions U.S. \$)			Primary end use
	1974	1981	1974	1981	1990 (estimated)	
Amino acids		455×10^3 *	290	1.9×10^3 † 1.8×10^3 *	2.2×10^3 ‡	Feed additive, food enrichment and flavoring agent, feed preservative
Citric acid	265×10^3	300×10^3 †				Food additive, processing aid
Enzymes		65×10^3	132	310 to 400	1.5×10^3	Processing aid
Vitamins				668† 1.1×10^3		Feed and food additive, food enrichment agent
Baker's yeast	0.97×10^6	1.75×10^6 †	380			Food additive, enrichment agent
Beer	75×10^6	87×10^6 †		27×10^3 §*	44×10^3 §	Beverage
Cheese	11×10^6	12×10^6				Food
Fermented foods				3.5×10^3	6×10^3	Food
Miso		5.72×10^3				Food
Soy sauce		1.18×10^6 *				Food

*Data for 1982. †Data for 1979. ‡Fermented chemicals. §Alcoholic beverages. ||Japan only.

improvement of animal breeds; improvements in the reproductive efficiency of livestock; the use of vaccines and monoclonal antibodies in the diagnosis, prevention, and control of animal diseases; and the improvement of crop species through the regulation of endogenous genes, the transfer of DNA from one species to another (for example, fusion of cells, transfer of subcellular organelles, vector-mediated DNA transfer), the improvement of plant resistance factors (for example, plant and microbial produced pesticides), and the improvement of photosynthetic efficiency (7, 9, 11). In addition, the use of the genetic diversity in plants, new plant and animal food sources, and improved food production technologies (such as aquaculture, hydroponics, continuous tissue culture, and solid-state fermentation) are being sought continuously (9, 10, 12).

Raw material modification and improvement can be applied to convert raw materials, to increase stress resistance, and to improve their functionality and nutritional quality.

In the processing of raw materials for food, polymeric carbohydrates may be removed, included in the product as dietary fiber, or converted to other products, such as sugars. The ability to convert these polysaccharides and to impart specific structural changes can result in improvements in the functionality of carbohydrates in foods and in increases in product yields (12). In vitro selection has been applied to improve the salt and cold tolerance and the herbicide and drought resistance of crop plants. Work on the improvement of such functional properties as color, flavor, and texture of raw materials is being conducted, as is work on increasing the essential nutrients and

reducing the undesirable constituents in raw materials (13).

Raw material preservation by biological processes is critical to agriculture and the food processing industry. The production of silage, the fermentation of cocoa and coffee beans, the "fermentation" (oxidation) of tea leaves, and the conversion of raw material into SCP or feedstock chemicals (1, 14) suggest the diversity and magnitude of microbial fermentation processes applied for raw material preservation and quality improvement (1, 14).

Additives and production/processing aids. Additives and production aids used in raw material production include such materials as vaccines and growth regulators in animal production and microbial insecticides and herbicides in plant production, and they all are subject to intensive investigation (11, 15). Historically, such food additives as fatty acids and other organic acids, vanilla (for flavor), and vitamins B₂, B₁₂, C, and D have been produced through biotechnological processes. Intensive work is now being carried out on the production of plant metabolites by tissue culture, including flavors, pigments, vitamins, enzymes, antioxidants, antimicrobials, and lipids and on the microbial production of flavors, pigments, vitamins, amino acids, antioxidants, biosurfactants, and polysaccharides (16). For example, the plant metabolite shikonin, a bright red naphthaquinone compound used as a dye and as an antibacterial and anti-inflammatory agent, is currently produced on an industrial scale from cultured *Lithospermum erythrorhizon* cells (17). Blue-green algae that produce tocopherols have been isolated, and a potential vitamin E precursor has been found in vari-

ous genera of bacteria and yeasts. This suggests the potential for the development of a one-step fermentation process to fulfill the demand for vitamin E (18).

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a low-calorie dipeptide sweetener that has recently been approved as a food additive. Precursors such as aspartic acid and phenylalanine are produced by fermentation processes, and the microbial production of the dipeptide aspartyl-phenylalanine has been performed at the laboratory level by recombinant DNA processes. Worldwide demands for L-phenylalanine are projected to increase from 50 metric tons in 1981 to 7900 metric tons in 1990 (19).

Improved processes for the production of other amino acids, such as L-lysine and L-threonine, have been developed recently (20).

Polysaccharides commonly derived from algae or botanical sources and used as functional agents are now being produced commercially through microbiological processes. The search for new microbial polysaccharides is an area of active research. Recent advances toward understanding the specific steps involved in the biosynthesis of specific polysaccharides offer promise for the control and manipulation of the structure and form of the final polysaccharide product (21). There are a number of food-related applications of polysaccharides; they include the microencapsulation of flavors, immobilization of enzymes, entrapment of whole cells, and aiding of flocculation in food process waste management (22).

Yeasts have been used traditionally in the production of alcoholic beverages, and attention recently has been given to

the genetic manipulation of *Saccharomyces* yeast cells to increase the efficiency of the brewing process and to prepare low-calorie, or so-called light, beers. Currently, glucoamylase enzymes from microbial sources (for example, *Aspergillus niger* and *Aspergillus awamori*) are used in the production of many light beers. These enzymes are fairly thermostable and are not destroyed by normal beer pasteurization at 60° to 62°C, so the beers become sweeter upon storage owing to the release of glucose units from dextrans by the glucoamylase. Thus, the production of a thermosensitive glucoamylase by a brewer's yeast could be of significant value (23).

Enzymes are used extensively in food production and processing. The ones most widely applied are amylases, glucose oxidases, proteases, pectic enzymes, and lipases. Excellent reviews on the production and utilization of food enzymes are available (24). Immobilized enzymes and immobilized whole cells have received significant attention as valuable biocatalysts for the food processing industry (25). The advantages of the application of immobilized systems include continuous operation, reuse of the biocatalyst, ease of process control, improved biocatalyst stability, and reduced waste disposal problems (26).

Immobilized biocatalyst technology also can be applied successfully to the production of secondary plant metabolites (27). In addition, the production of enzymes with enhanced stability to temperature and other processing conditions is receiving much attention (28).

The significant impact that biotechnology can have on the production of a food ingredient is exemplified by the development of high-fructose corn syrup (HFCS) technology. The production of HFCS involves the application of two amylases and glucose isomerase to effect the liquification and subsequent saccharification of cornstarch to yield an approximately equimolecular mixture of fructose and glucose. Because fructose is sweeter than glucose, HFCS is about as sweet as a sucrose syrup of the same solids content, and it has found wide use in processed foods. About 2.5 million metric tons of HFCS (dry basis) were produced in 1981, compared to about 72,000 metric tons in 1976. Over a 10-year period (1970 to 1980), HFCS increased its share of U.S. per capita consumption of nutritive sweeteners from almost nonexistence to 16.4 percent, while sucrose usage decreased from 84.1 percent to 68.0 percent (2, 25). The production of HFCS through the use of

enzyme technology is one of the greatest commercial successes of immobilized biocatalyst technology (29).

Production methods. With the availability of high-performance bioreactors for large-scale fermentation processes (30), current emphasis is on computer process control, although there is still a need to improve bioreactor performance by overcoming the limitations of heat and mass transfer (31). This is especially important with new biocatalysts and the scale-up of such processes as animal and plant cell culture systems (32). The engineering problems are especially challenging when non-Newtonian systems are involved (33).

Extensive work on immobilization techniques, reactor design, and cell membrane permeabilization will help to overcome the current problems in continuous animal and plant cell cultures; these problems include shear sensitivity, slow growth rates, and the intracellular storage of metabolites (34).

Biotechnology in Food Processing

Biotechnology in food processing can significantly affect food product composition, quality, and functionality by providing tools for product modification, preservation, and stabilization, as well as for safety, characterization, and quality control. In addition, processing methods, especially separation and fermentation processes and waste treatment and utilization can contribute to the improvement of food products.

Product modification. Significant advances have been made in the modification of food components, such as proteins, polysaccharides, fats, and oils. Protein modifications, for example, include limited enzymatic hydrolysis to alter food functionality; the reverse process, the so-called plastein reaction, has been proposed as a method to create proteinlike materials to develop new food products. Modification of properties of proteins by combining information on crystal structure and protein chemistry with artificial gene synthesis is also being explored (3, 35).

Meat tenderization with papain is one example of the large-scale application of enzymatic hydrolysis to modify product functionality. Other potential processes are the enzymatic reduction of limonoid bitterness in citrus products to improve flavor (36) and the modification of the fatty acid composition of triglycerides by lipases. One example is the enzymatic modification of olive oil and stearic acid

to a fat similar to cocoa butter, particularly the formation of 1-palmitoyl-2-oleyl-3-stearoyl-*rac*-glycerol, the major triglyceride of cocoa butter, which has been obtained on reacting oleic anhydride with 1-palmitoyl-3-stearoyl-*rac*-glycerol in the presence of lipase. Furthermore, the development of a two-stage microbial process for producing glycerides having cocoa butter characteristics requires mention (37).

Product preservation. Historically, there has been extensive use of microbial metabolism for food preservation and stabilization, especially for dairy, meat, fish, fruit, and vegetable products (38). The efficiency of microorganisms used in the food fermentation industries potentially can be enhanced by genetic manipulation of starter cultures. However, additional fundamental knowledge of the genetics, biochemistry, and molecular biology of organisms used as starter cultures is required (39).

Product safety, characterization, and quality control. Besides the use of classical methods to ensure the quality and safety of food and to identify food components (40), three recent developments are relevant to product safety, characterization, and quality control: (i) the potential application of monoclonal antibodies to determine optimal crop harvesting and product freshness (41), (ii) the use of biosensors and DNA hybridization techniques for quality control (42), and (iii) the potential of tissue culture and genetic methods for nutrient and toxicity assessment (43). In addition, the regulatory and safety aspects of biotechnology and their impact on the nutritional quality of the resulting food products are being examined (44).

Processing methods. Mechanical unit operations used for product purification and recovery include sedimentation, centrifugation, and filtration, along with dialysis, flotation, and ultrafiltration (45). Biomass separation is commonly aided by bioflocculation or by the use of synthetic polyelectrolytes. Recently, natural polyelectrolytes such as chitin and chitosan have been investigated as substitutes for synthetic polyelectrolytes (45, 46). Application of aqueous two-phase (liquid-liquid) systems for the extractive purification of enzymes (47) and the supercritical extraction (48) of food ingredients are becoming increasingly important. In supercritical extraction, carbon dioxide is favored as the dense gas because it is nontoxic, nonexplosive, cheap, readily available, and easily removed from extracted products (49). Supercritical extraction is currently used

on an industrial scale for decaffeinating coffee and tea. Scale-up of high-performance liquid chromatography (HPLC) separation processes is also being explored (50).

Nonlipolytic enzymes have been used to enhance the extractability of oil from seeds (51), and pectolytic enzymes are applied to increase yields in the processing of liquid fruit and vegetable products (52). In addition, cofermentation processes have been suggested to aid the separation and purification of secondary metabolites (53).

Treatment and utilization of process waste. Because of the large volumes involved in the production and processing of food, generated wastes create disposal and pollution problems. In addition, there is a substantial loss of essential nutrients. For example, 20 million metric tons of whey, the fluid that results from the separation of curd when converting milk into cheese, accumulate annually in the United States (54). Whey contains more than half of the nutrients of the milk used in cheese production, including 1 percent protein and 5 percent lactose. Approximately 50 percent of the total whey solids is disposed of in various industrial and municipal waste-treatment operations (55).

Biomass recovery, especially isolation of valuable protein by-products, has been carried out in the food processing industry for an extended period of time. The isolation of protein concentrates from potato processing wastes, for example, has been used on an industrial scale for several decades, and the product's potential for food application has been investigated extensively (56). During the past decade, ultrafiltration has become useful in food processes, especially for the recovery of whey protein from cheese, cottage cheese, or industrial casein processing wastes (57). By-product recovery has also been explored for application to the processing of meat, cereal, dairy, fruits and vegetables, and fish and shellfish, as well as fermentation operations (58).

The multifunctional potential of food processing wastes for by-product recovery and conversion can be illustrated by the case of chitin—poly- β (1,4) *N*-acetyl-D-glucosamine—which is a waste product of the shellfish industry and one of the most abundant polysaccharides in the world. It has been shown to have numerous potential food applications, such as being used as a possible dietary fiber, a functional ingredient, and an immobilizer of enzymes. Chitosan (partially deacetylated chitin) has been effective

in aiding the separation of colloidal and dispersed particles from food process wastes and has the potential for being used for the microencapsulation of flavor and for the entrapment of whole cells (59). Chitin bioconversion to SCP has also been reported, and numerous additional applications of chitin and chitosan are being investigated (60).

Bioconversion of food processing wastes includes the use of substrates such as starch or whey. The so-called Symba process utilizes a symbiotic culture of two yeasts, *Endomycopsis fibuliger* and *Candida utilis*, to convert potato starch into SCP. A *Kluyveromyces fragilis* and *Candida intermedia* symbiotic culture, which is characterized by an exclusively oxidative lactose metabolism, is being used for production of protein-enriched whey (61). Other examples of process waste bioconversions are the application of molasses and corn steep liquor as substrates in many fermentation processes and the production of vinegar (from "waste" wine) (1). Furthermore, the anaerobic digestion of food wastes to provide methane for fuel use is now being used on a commercial scale (62).

Research Needs

As the above discussion indicates, there have been numerous achievements in the field of food biotechnology and there exist many more potential opportunities. One critical factor, however, is the formulation of achievable objectives to aid the rational improvement of food production and processing technologies and to reach desired goals for product quality. What is needed is a rational program for the food sector that has a structure based on identification of essential research needs. Also needed is increased investment in food research and development, which currently constitutes only about 0.3 percent of industry-based business in the United States, as well as long-term commitments to research projects in food biotechnology.

At a workshop sponsored by the Institute of Food Technologists, scientists from industry, government, and academia made the following statement concerning the research needs in this important area (63):

Biotechnology directed toward the general area of food can bring significant economic benefits at both the macro and micro levels. The U.S. national (macroeconomic) interests can be served by more reliable supplies of critical food and food ingredients; by the

development of methods of production which do not minimize the production capability of the growth environment; and by more efficient use of capital employed in food processing.

In addition, faster innovation, particularly in agricultural raw material development, can occur, thereby maintaining a competitive international position. Also, lower energy consumption in food processing can be expected, as well as the provision of an added value usage for agricultural commodities currently in surplus.

At the microeconomic level of individual food sectors benefits will come from more effective production, improved ability to meet the consumers' demands for natural foods and food ingredients, less waste, improved processing characteristics, consistent quality, and a greater nutritional value.

The following programs reflect research needs at the various steps in the path that leads from agricultural production to the consumer:

1) *Application of biotechnology to the structural-functional relationship of food material.* This program aims to improve the utilization of biomaterials by applying modern biotechnological principles to control the functional performance of foodstuffs. In addition, biotechnology will contribute analytical tools and processing procedures that will aid in the implementation of this new knowledge.

2) *Cell physiology and biochemistry of agricultural raw materials.* The potential exists to lower the cost of agricultural raw materials, both plant and animal, by application of biotechnological techniques. Potential targets for improvement are (i) solids content, sensory properties (color, flavor, texture), environmental adaptation, secondary metabolites (vitamins), and postharvest storability in crops and (ii) feed efficiency, palatability, fat/protein ratios, fertility, and maturation time of juveniles in animals.

To realize these benefits, a vast increase is necessary in our understanding (at the molecular level) of the cellular physiology, including biosynthetic and regulatory pathways, of the appropriate animal and plant species.

3) *Improvement of enzymatic processing.* Enzyme processes can reduce the high cost of traditional food processes and also permit development of totally novel foods and food ingredients. To expand the range of possible processes and to improve on the economics of current enzyme-based processes, increased basic knowledge is needed on enzyme isolation and characterization, the mechanisms of enzyme action, and enzyme incorporation into food processes. Specific needs are to understand the mechanisms of enzyme inactivation; to

utilize enzymes for biosynthetic processes and redox reactions relevant to foods, including the low-cost production and recycling of cofactors; and to develop new process procedures using immobilized whole cells. Fundamental studies are needed on the control of mass transfer in food systems, maintenance of catalytic activity, and prevention of contamination. Also needed are computer modeling and understanding of the mechanisms of action of food processing enzymes in sufficient detail to permit systematic protein engineering to improve enzymes.

4) *Improvement of food-grade microorganisms.* Microorganisms—bacteria, yeasts, and fungi—are all used extensively in various aspects of food processing. To improve the economics (yield and productivity) and new product characteristics achievable with these organisms, major advances are needed in our understanding of their biochemistry and genetics. Specific research needs are (i) to establish recombinant DNA technologies and a fundamental understanding of microorganisms useful in food fermentation and preservation processes; (ii) to quantitatively describe the microbial ecology and biochemistry of mixed-culture and solid-state fermentations important in foodstuffs; (iii) to isolate, select, and genetically manipulate organisms capable of synthesizing food additives—such as biopolymers, colorants, natural flavorings, and preservatives—by fermentation and cell culture; and (iv) to develop economically viable bioprocesses as sources of raw materials for the food processing industry.

5) *Methods development.* To improve the production costs, nutritional value, and cost in use of some of the major agricultural crops, particularly cereals, further fundamental advances in cell culture methods and recombinant DNA technologies are necessary. Specific research needs are (i) vector development and transformation procedures for cereal crops, (ii) improved regulation and expression of foreign genes, and (iii) techniques to regenerate and propagate crops that cannot now be so handled. To reduce the time and cost of developing new crop species, rapid screening methods are required to identify the desired genotype at the cell culture stage.

6) *Food safety.* There is an urgent need to improve and to accelerate techniques of food safety assessment. Biotechnology can contribute to food safety by increasing the sensitivity and specificity of such assays and by developing faster and more meaningful methodolo-

gies based on DNA hybridization, sequencing, and monoclonal antibody techniques.

The most critical research needs, in addition to basic studies on the structure-function relationship of food materials, are fundamental studies in the cell physiology and biochemistry of agricultural raw materials and improvement of food-grade microorganisms.

Conclusions

Biotechnology applied to food production and processing clearly encompasses a very large and diverse field. The utilization of the capabilities of biological systems is rapidly expanding into a variety of food applications, and consequently many new food sources, processes, and products are being developed. In addition, the identification of critical research needs will help to enhance food production and processing.

We have attempted to highlight biotechnology in food production and processing in broad terms, and consequently we recognize that we have only touched on many of the exciting involvements of biotechnology in providing, securing, and improving the world's food supply.

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