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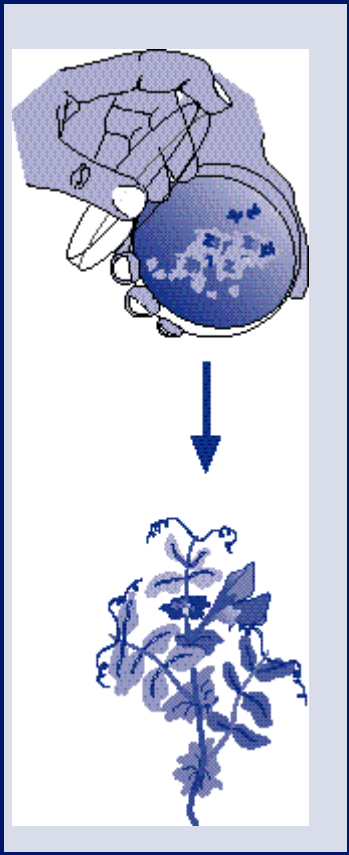

FOOD BIOTECHNOLOGY

AN INTRODUCTION

ILSI
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FOOD BIOTECHNOLOGY
AN INTRODUCTION

by Dean Madden

ILSI Europe

ILSI EUROPE CONCISE MONOGRAPHS

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FOREWORD

The professor to his cook: You are a little opinionated and I have some trouble making you understand that the phenomena which take place in your laboratory [kitchen] are nothing other than the eternal laws of nature, and that certain things which you do without thinking, and only because you have seen others do them, derive nonetheless from the highest scientific principles.

Jean Anthelme Brillat-Savarin
The Physiology of Taste, 1825

The sentiments expressed by Brillat-Savarin over a century ago are even more relevant today as the technology associated with food production, processing and preparation becomes increasingly complex. Traditional biotechnology — brewing, baking and other fermentation processes — has been associated with food for thousands of years. Now modern biotechnology, and genetic modification in particular, is beginning to make an impact. Given food's deep cultural importance, it is hardly surprising that this has created anxieties.

This monograph, which aims to address those concerns and to describe the opportunities presented by modern biotechnology, is in five sections. In the first two parts the technology of genetic modification is explained. Numerous examples of its use in food production are described in the third section. Several case studies then illustrate how enzymes are used in food processing, showing how many modern practices originated from ancient traditions. Finally, some of the social, economic and safety implications of genetic modification are examined.

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WHAT IS BIOTECHNOLOGY?

The term "biotechnology" was first coined by a Hungarian, Karl Ereky, towards the end of World War I. Ereky used the word to refer to intensive agricultural methods. Since that time biotechnology has been variously defined, but it has nearly always been associated with food production and processing. In particular biotechnology has usually encompassed the traditional manufacture of bread, wine, cheese and other fermented foods. On these grounds, biotechnology can trace its roots back several thousand years to the ancient Sumerians, who brewed beer with naturally occurring yeasts.

Ancient fermentations were not always successful. The microbes that fell into the wine maker's vat could yield the finest vintage or transform the entire product to vinegar. In the 1800s Louis Pasteur laid the foundations of microbiology and identified microorganisms — bacteria, fungi, algae and protozoa — as the cause of both desirable and undesirable changes in food. The application of Pasteur's research led to safer, more reliable food processing and preservation and helped ensure the consistent high quality of, for example, fine wines and cheeses.

Pasteur asserted that fermentation processes were inextricably linked to the activities of living microbes. Towards the end of the last century it was discovered that cell-free extracts from yeast could also bring about chemical changes without the intervention of the microbes from which they were derived. The active components of such extracts were named enzymes (enzyme means "in yeast"). Enzymes are proteins, made by all living things, that catalyse specific chemical reactions. Without realizing it, the makers of cheese had always used a mixture of natural enzymes — rennet — to transform milk into solid curds and liquid whey.

During the 1940s, large-scale fermentation equipment was developed which led to the efficient industrial production by microorganisms of pure enzymes and additives and other valuable compounds (such as vitamins) for use in food.

Just as different breweries have their own carefully maintained proprietary strains of yeast, enzyme manufacturers culture specially selected strains of their chosen microorganisms. Over many years great improvements have been made to the efficiency of production and the safety, quality and range of microbial products available. However, much still depends on chance occurrence followed by systematic isolation of organisms with desirable characteristics.

With the advent during the 1970s of the ability to make precise changes to genetic material, biotechnology was transformed. The performance of organisms can now be "fine tuned", and biotechnology has now almost became a synonym for "genetic modification". In 1980, an influential British report (the "Spinks Report") attempted to encapsulate nearly half a century of European and United States thought, defining biotechnology as "the application of biological organisms, systems or processes to the manufacturing and service industries". This broad definition suits our purposes, as it includes the production of food by living organisms, its subsequent processing with the assistance of microbes or enzymes and the assurance of food quality and safety using the tools of molecular biology.

METHODS USED IN BIOTECHNOLOGY

Genetics and genetic modification

Chromosomes, genes and DNA

Genes, passed from one generation to the next, determine all inherited characteristics. Genes are made from DNA (deoxyribonucleic acid), most of which is packaged, in fungal (including yeast), plant and animal cells, into chromosomes within the nucleus of the cell (Figure 1). Some genes are also found outside the nucleus: in the mitochondria (which release energy for cellular activities) and within the chloroplasts (sites of photosynthesis) of plant cells. In bacteria, most genes occur on a single circular chromosome, although small rings of DNA called plasmids may also be present.

The double helix of DNA can be likened to a twisted rope ladder. The two intertwined helices are chains made from sugar and phosphate molecules linked together alternately. Attached to each sugar molecule is a "base". There are four different bases: adenine (A), thymine (T), cytosine (C) and guanine (G). Weak bonds between the bases join the two strands of the double helix together like the rungs of a ladder. A always pairs with T, and C always pairs with G. This "base pairing" mechanism ensures identical replication of DNA strands during cell division (Figure 2).

A particular gene (a stretch of DNA with a particular sequence) determines the structure of all or part of a specific protein (Figure 3). The sequences of bases in the DNA specify the amino acid residues that are needed to make proteins. Three bases in a row specify each amino acid, and the sequence that specifies each — the genetic code — is the same in all living organisms (see Figure 2).

Also encoded within the DNAare instructions to regulate protein production. Although all cells of an organism will contain the same DNA, only certain proteins will be made at any one time or in any particular type of cell; that is, only certain genes will be expressed.

DNA has an identical structure in all living things, and because the genetic code is universal, the possibility is raised that genes can be transferred between completely different species. The process of transferring, removing or altering genetic information by the modification of DNA is commonly called genetic modification (or genetic engineering).

Why alter nature?

In nature, proteins are often made in minute quantities and are therefore difficult or impossible to extract and purify. These proteins include enzymes and a variety of pharmacologically active compounds such as insulin for diabetics, interferons for cancer therapy and vaccines to help prevent diseases. Often large quantities of such valuable proteins are needed. Biotechnologists can achieve this by transferring the relevant genes into microbes that can easily be cultivated in large numbers. The same technology, applied to the production of food, could bring significant benefits.

Improved varieties for agriculture. Animal and plant breeders have for centuries selected livestock and plants with desirable characteristics. Breeding from chosen stock is a very slow process that can be set back by the chance recombination of genes in the offspring. A breeder may select a preferred trait, only to find that it is accompanied by an equally undesirable one, which then has to be painstakingly bred out.

Traditional methods of selecting the best plants or animals from which to breed have been greatly aided by modern genetic techniques. Furthermore, it is now

FIGURE 1.

The essential structure of bacterial, animal and plant cells

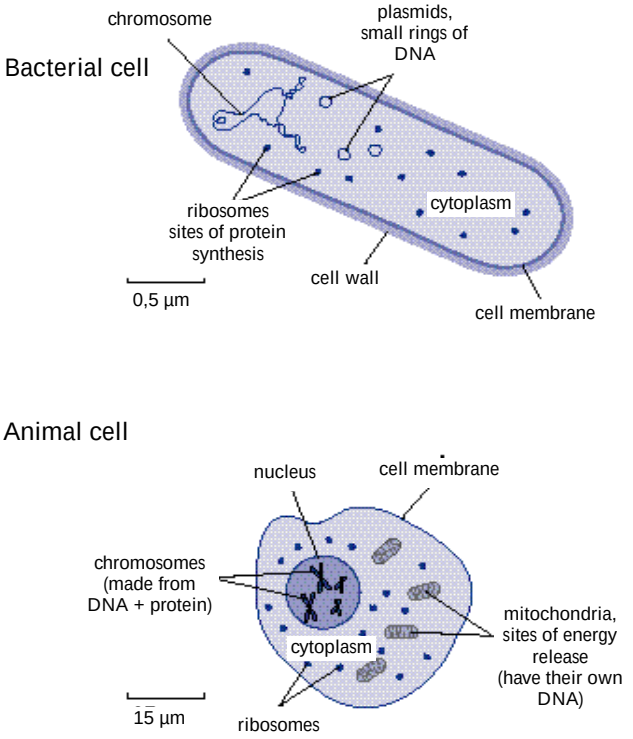
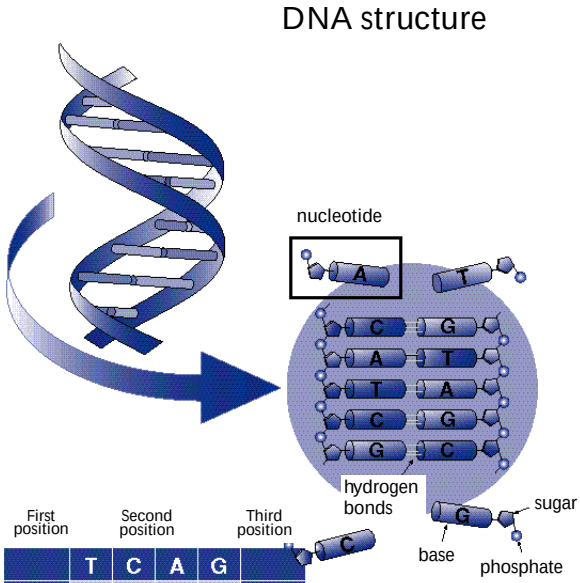


FIGURE 2.

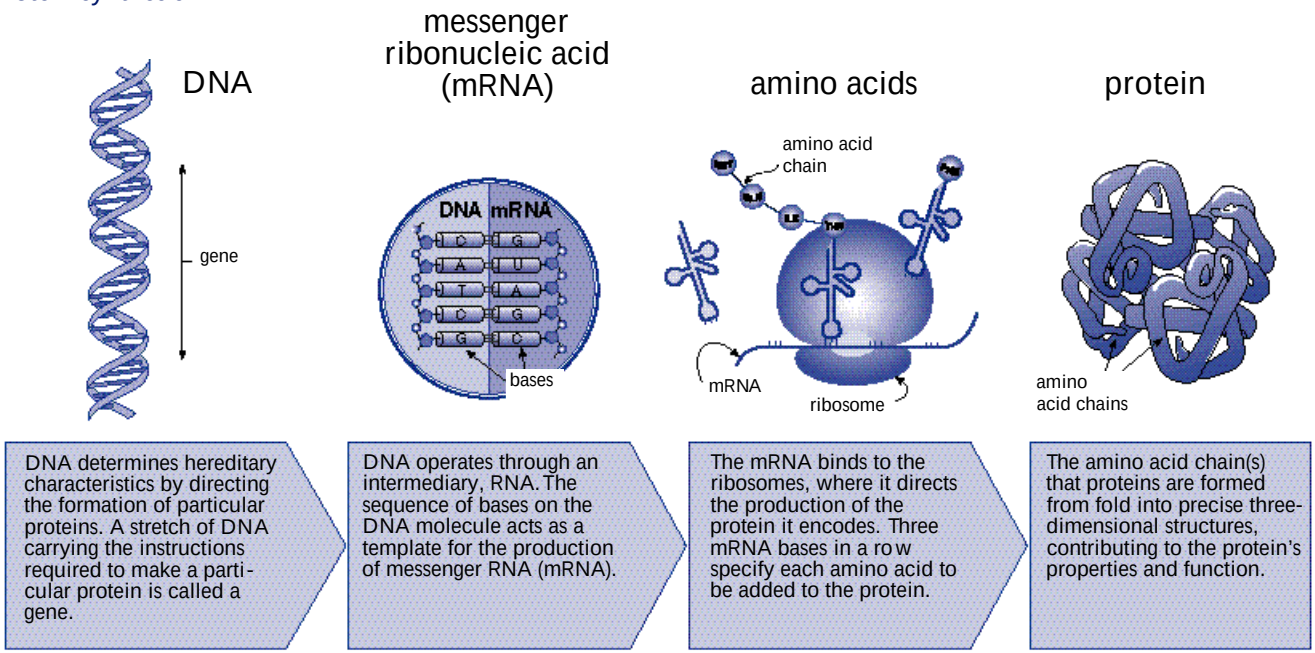
The structure of DNA, the hereditary material. Three bases in a row code for amino acids that are the "building blocks" of proteins.



The genetic code

First position	Second position: T	C	A	G	Third position
T	PHE	SER	TYR	CYS	T
	PHE	SER	TYR	CYS	C
	LEU	SER	STOP	STOP	A
	LEU	SER	STOP	TRP	G
C	LEU	PRO	HIS	ARG	T
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLN	ARG	A
	LEU	PRO	GLN	ARG	G
A	ILE	THR	ASN	SER	T
	ILE	THR	ASN	SER	C
	ILE	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	T
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLU	GLY	G

FIGURE 3.
Protein synthesis

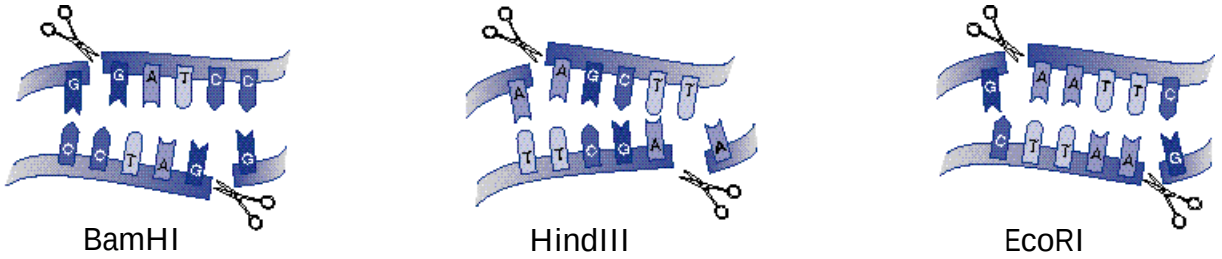


possible to make very precise changes to the genetic material. This can help improve the resistance to disease and environmental stress amongst crop plants and farm animals. It can also help boost agricultural productivity and enhance the nutritional status, storage properties and ease of processing of food products.

Food additives and processing aids. Enzymes are specialized proteins that are essential for life. They catalyse all biological processes and thus control metabolism in living organisms. Once extracted from living organisms, these proteins allow certain processes in food production to be conducted. For thousands of years enzymes such as rennet from animals and papain from plants have been used to enhance the flavour, texture and appearance of food. Because of the diversity of microorganisms, it has been possible to find a wide range of microbial enzymes that are active in the conditions encountered in food processing. With genetic modification a greater range of pure and highly specific enzymes can be produced more efficiently. These enzymes can be used to make desirable changes to food both rapidly and at relatively low temperatures, with a subsequent reduction in fuel requirements and in the environmental impact of food processing. To the consumer, the direct benefits include better flavour, texture and shelf life of food, often with a reduction in the need for processing and additives.

FIGURE 4.

Restriction enzymes "recognise" and cut DNA molecules at precise locations. The names of these enzymes are derived from those of the organisms that produce them. The sequences of bases that are recognised by three enzymes (BamHI, HindIII and EcoRI) are shown here.



How genetic modification is done

Cutting and pasting DNA. Special enzymes, obtained from bacteria, are an essential tool of the molecular biologist. In nature, these enzymes help bacteria fend off viral attack by precisely dissecting the foreign DNA of invading viruses. In this way, the proliferation of the viruses is restricted. Restriction enzymes (as they are known) recognize and cut DNA molecules at specific locations (Figure 4). Many hundreds of restriction enzymes have been isolated from different microbes and are available commercially. With restriction enzymes almost any section of DNA, and consequently any single gene, can be excised at will. The end of one DNA molecule will readily link to that of another that has been cut with the same enzyme. To join two DNA molecules permanently it is necessary to form chemical bonds along the DNA's sugar-phosphate backbone. An enzyme called DNA ligase can do this job. The function of these "cut and paste" enzymes in assembling novel DNA molecules is obvious, but the genetic engineer's tool kit would be incomplete without one or two other enzymes. To understand their role it is necessary to appreciate how proteins are made.

A genetic intermediary. The genetic information encoded in DNA lies within the nucleus of the cell. However, proteins are not made in the nucleus, but elsewhere, at special structures called ribosomes. Before a particular protein can be made, a copy of the appropriate instructions must first be transcribed from the DNAand then ferried to the ribosomes. The copied instructions are made from mRNA (messenger ribonucleic acid). This mRNA is virtually a mirror image of the sequence of bases on one DNA strand, according to the base-pairing rules. Upon arrival at the ribosomes the base sequence within the mRNA directs the construction of proteins from amino acids. A sequence of three adjacent bases in the mRNA molecule is needed to determine each amino acid in the protein (see Figure 3).

Cells that are producing a particular protein will have many identical copies of that protein's mRNA inside them. It is often easier to search for genes among the small mRNA molecules rather than along the entire length of the cell's DNA. Once a desired length of mRNA has been isolated, two additional enzymes are needed.

DNA from RNA. The enzyme reverse transcriptase assembles a single strand of complementary DNA alongside a corresponding piece of mRNA. A second enzyme (DNA polymerase) can then be used to construct a double-stranded helix using the first DNA strand as a template. DNA made in this way is called copy or complementary DNA(cDNA). A copy of a gene from a donor cell is used in genetic modification.

Gene synthesis. By the judicious use of restriction and other enzymes, molecular biologists are able to assemble DNA molecules which contain one or more genes of interest. Where a particular piece of DNA is difficult to isolate, it is sometimes possible to make it artificially using a DNA synthesizer. Under computer control, these devices string together the biochemical precursors needed to make short stretches of DNA. Of course, to programme the synthesizer it is necessary to know the sequence of bases present in the desired gene; this too can be determined automatically using a DNA sequencer. It is also possible to copy specific genes using the polymerase chain reaction (PCR). The PCR has been likened to a "genetic photocopier". From a very small amount of DNA millions of copies of a specific section of DNAcan be made quickly. The PCR lies behind many of the spectacular successes of forensic genetic fingerprinting, where criminals have been identified from the DNA in just a few drops of blood or even a couple of cells on a cigarette butt.

Plasmids. Once a suitable DNA molecule has been constructed, it must be moved into a cell in which it can be expressed and duplicated so that it passes from one cell division to the next. For microorganisms, one of the most successful methods involves the use of plasmids as a vehicle for transferring genes. Plasmids are rings of DNA that are found in some cells. They carry a limited set of genes and normally constitute only a few percent of a cell's total DNA. During the course of evolution, plasmids carrying genes that help their microbial hosts survive have been selected by nature. Some plasmids confer on their hosts the ability to degrade substances in the environment such as nutrients and antibiotics. Many traditional foods, such as yoghurt, cheese and other fermented dairy products, contain large numbers of living microbes that naturally harbour plasmids.

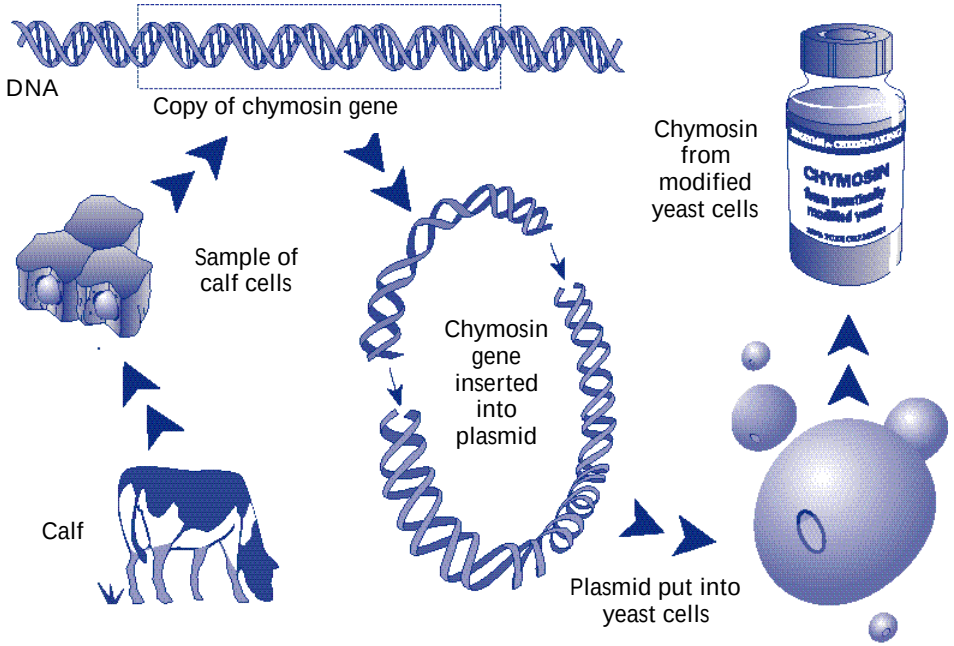
Like the DNA of chromosomes, that of plasmids can be cut with restriction enzymes and additional DNA pasted into it. The result is a ring of "recombinant" DNA that can be put into a bacterium. Specialized plasmids can be used to ferry genes from bacteria into yeast cells or even plants (Figure 5).

A limitation of plasmids is that they cannot accommodate DNAfragments longer than 15 000–20 000 base pairs. However, some harmless, specially tailored viruses can package larger DNA molecules. Such viruses have been used to transfer genes into microbes, plants and animals and even to treat human disease.

Genetically modified plants. A vector system that is used for a wide variety of plants is the plant tumour-inducing plasmid (Ti-plasmid) found in the soil bacterium Agrobacterium tumefaciens. Through its plasmid, Agrobacterium has the ability to naturally engineer plant cells so that they grow tumours that produce compounds which the bacteria need to sustain themselves. Molecular biologists use disarmed (non-tumour-inducing) versions of this plasmid to introduce foreign genes of their choice into plants. Because every cell carries a complete copy of all the plant's genes in its chromosomes, it is possible to regrow an entire plant from a single modified cell (see Cell culture, below). Specially modified Ti-plasmids have now been produced which help transfer fairly large genes into plants. Unfortunately, monocotyledons (including the important cereal crops) are resistant to Agrobacterium.

FIGURE 5.

Pure chymosin for cheese making can now be obtained from genetically modified yeast



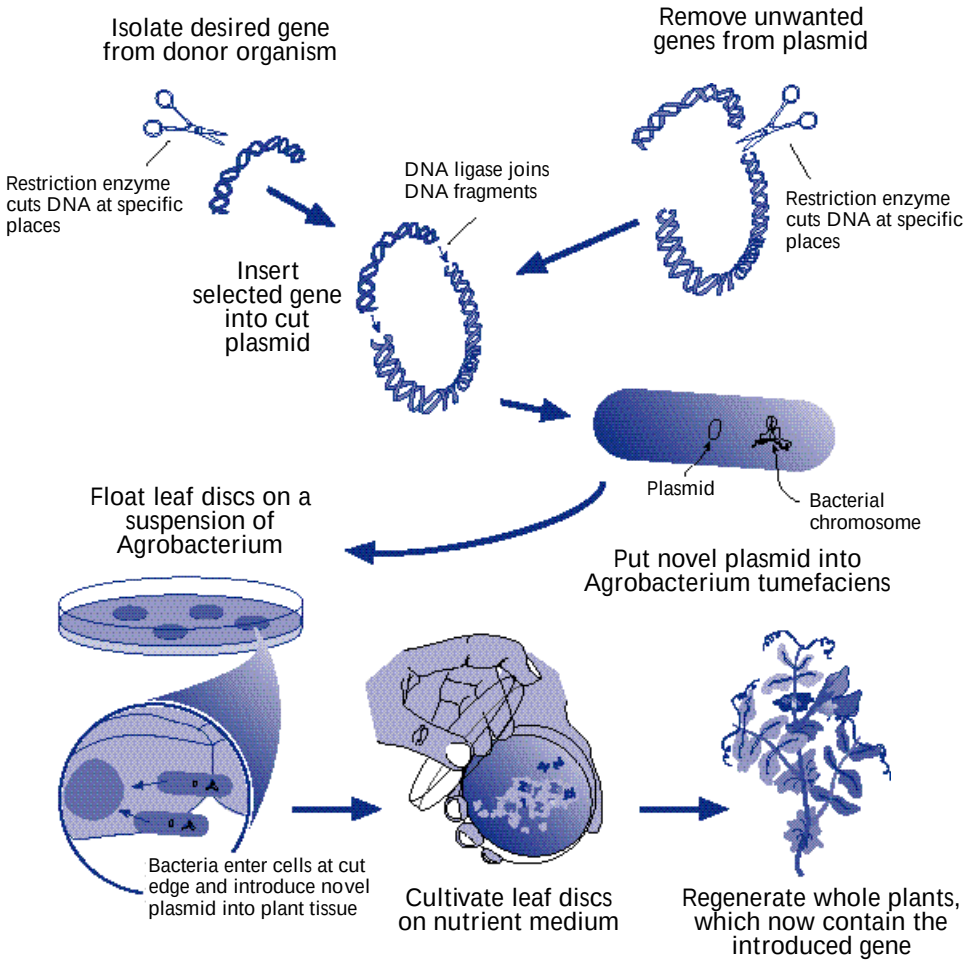
Agrobacterium has proved especially useful when working with trees, which, because they are slow growing and large, are difficult to improve by conventional breeding. Apricot, plum, apple and walnut trees have all been genetically modified with Agrobacterium (Figure 6).

Gene ballistics. A procedure called ballistic bombardment has achieved success with several crops, including rice, wheat and soya. With this method, the DNA to be introduced into the plant cells is first stuck onto minute tungsten or gold particles. The DNA-coated particles are fired at high velocity into soft plant tissue, usually callus (see Cell culture, below). This introduces functional DNA into the plant cells.

Electroporation. DNA can also be introduced into the thin-walled tubes which develop from pollen grains by subjecting them to microsecond pulses of a strong electric field. This technique, called electroporation, causes pores to appear momentarily in the pollen tubes through which DNA from a surrounding solution can enter. Seeds that develop from ovules fertilized with such pollen carry the introduced genes. Electroporation also works with plant cells from which the cell wall has been removed by enzyme treatment. From these naked plant cells, whole plants can be regenerated by cell culture (see Cell culture, below). Electroporation has also been used to transfer DNA molecules into a broad spectrum of microorganisms.

FIGURE 6.

Agrobacterium naturally infects some types of plants and introduces novel DNA into them. Biologists use specially tailored forms of Agrobacterium to modify plants genetically.

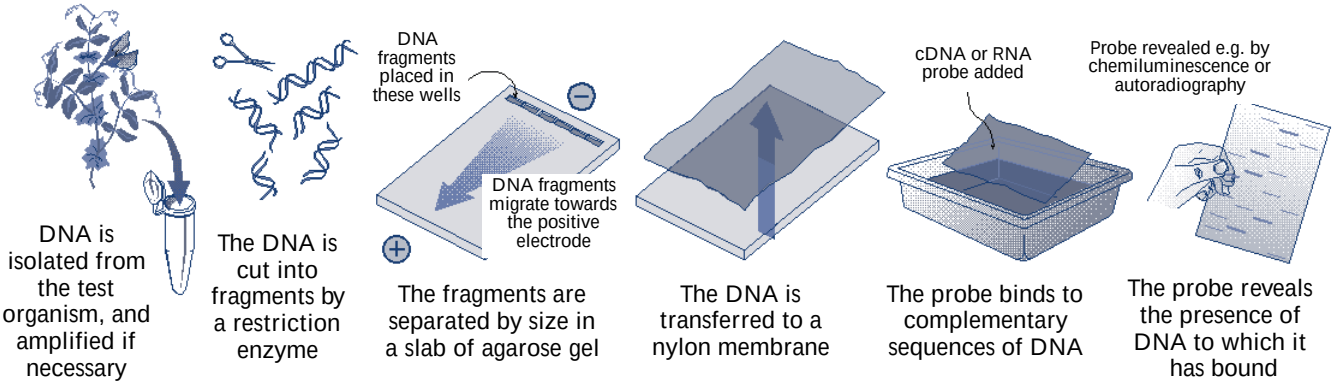


Genetically modified animals. The DNA of animals can also be modified by genetic engineering. It is necessary to introduce genes at an early stage of development if they are to be present in all of the cells of a mature animal and be passed on to its offspring.

DNA can be injected into newly fertilized egg cells through a very fine glass pipette. Only a small proportion of such injected eggs take up the new genes. The injected eggs are transferred into the uterus of a suitable foster mother. This is the only method so far

FIGURE 7.

DNA probes can be used to detect particular genes or fragments of DNA that have been isolated from organisms. This allows the presence of genetic characteristics to be confirmed rapidly, without the need to, for example, grow a plant to maturity.



that works for cows, pigs, sheep and goats. Microinjection can also be used to introduce new genes into fish eggs, but it is not suitable for the eggs of birds. However, specially modified viruses have been used to introduce, for example, disease resistance into chickens. The viruses, which are made harmless, are inserted through the shell of the egg.

Marker genes and gene probes. Whatever method is used, at best only a small proportion of treated cells take up the introduced DNA. Screening is therefore necessary to discover which cells have done so. As mentioned above, plasmids often carry genes which help the microbes that possess them break down particular antibiotics. These genes can be used as "markers" to identify those cells which have taken up plasmids, for when the cells are placed in a growth medium which contains an appropriate antibiotic, only those with plasmids will thrive.

Several different types of marker genes exist within the plant kingdom, and these are being developed as alternatives to antibiotic markers. Other methods for identifying transferred genes include the PCR. This method enables the amplification of transplanted DNA sequences, which can then be detected using "gene probes". Gene probes are small fragments of single-stranded DNA or RNA which bind to complementary sequences in the DNA that is being sought out (Figure 7). Probes can also be used to detect the DNAof microorganisms that might contaminate food. They are so sensitive and specific that it is possible to use probes to differentiate between strains of the same species and to determine whether particular microorganisms are capable of producing toxins.

Genetic switches. To ensure that the recipient cell's biochemical machinery will allow introduced genes to be expressed, sequences of DNA called control regions are required. One well-studied control region switches

on and off a gene for making β-galactosidase. This enzyme enables bacteria to metabolise lactose, but it is produced only when that sugar is present in the surrounding medium. The "genetic switch" associated with the gene encoding β-galactosidase can be placed in front of other genes, so that the addition of lactose to broth in which the engineered cells are growing triggers expression of the new genes. There are several other genetic switches, some of which can even be thrown by a simple change in temperature.

Other biotechnological methods

Cell culture

Many types of plant cells from a variety of species can be cultivated in vitro by supplying them with nutrients and growth substances under strict aseptic conditions (Figure 8). Illumination may also be required. Cultures of individual cells, grown in a fermenter, can be used in preference to whole plants for producing high-value products such as natural food colourings (for example, betanin, the red colour from beetroot) and flavourings (for example, vanilla and mint oil). Problems with this technique arise from the fact that most of these substances are produced only by mature cells that are not dividing. In culture, the cells tend to be actively growing, and so do not yield large quantities of these desirable products.

A recent development with considerable promise is the cultivation of "hairy root cultures". By infecting plant tissues with Agrobacterium rhizogenes (a relative of A. tumefaciens), the production of root-like structures can be triggered. These transformed cells can be grown indefinitely on simple solid or liquid media. It is possible that they could be used to produce natural food flavourings and colourings without having to rely on plants grown in the open, which are subject to variations in availability and quality. However, work on hairy root cultures is still at the experimental stage.

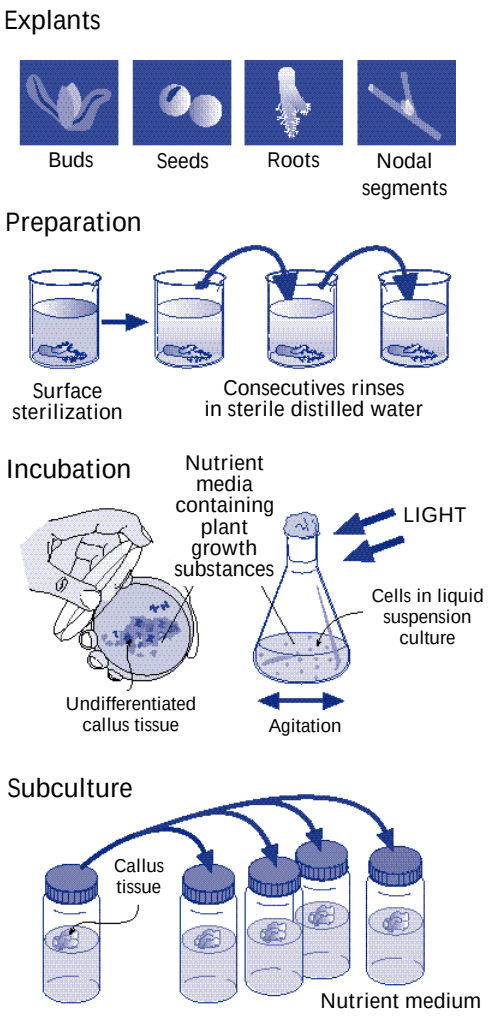
From undifferentiated cells (or callus) grown in vitro, whole plants can be regenerated by adjusting the proportions of various growth factors in the surrounding medium. This enables the propagation of many thousands of plants from just a few cells, greatly accelerating the process of plant breeding. Although plants produced by cell culture should be genetically identical, the chromosomes in callus cells frequently undergo considerable rearrangement. Potato cells are especially prone to this. Such variation is a rich source of new strains, often with desirable characteristics. However, this phenomenon can be a serious drawback when great effort has been devoted to cloning a precious new plant variety. Problems of this nature beset a major project to grow oil palms in the mid-1980s. Cell culture is also used for the conservation of those plant varieties which cannot be maintained in a normal seed bank. Genetic changes are in this case highly undesirable.

For some species, plantlets raised from cloned cells can be planted directly in farmers' fields. Although expensive, this method is especially suited to crops such as bananas, which do not have seeds. More often, plant cell culture is used to generate uniform, virus-free plants of high value (such as ornamental species) or the plants from which seeds are subsequently obtained. Cauliflower seeds, for example, are often obtained from such stock.

Cell culture can also be used to make "artificial seeds" — cultured plant embryos that are subsequently coated by a protective layer of hard gel. The cost of producing such artificial seeds currently restricts their use to high value crops. However, the process is highly productive; one estimate suggests that from a 10-litre fermenter sufficient embryos could be produced to satisfy French farmers' entire annual demand for carrot seeds.

FIGURE 8.

Plants can be cultured on sterile solid or liquid media that contain nutrients and plant growth substances ("plant hormones"). Using this method, many thousands of identical plants can be produced from a small amount of plant tissue.



The walls of plant cells are composed mostly of cellulose. A cellulase enzyme preparation can be used to remove these walls, leaving spherical protoplasts bounded by a thin membrane. The absence of a cell wall makes protoplasts especially amenable to genetic engineering by electroporation or ballistic impregnation (see page 7).

Animal cells may also be cultured in vitro, although it is not possible to grow whole animals or anything other than sheets of tissue from them. Their main use is in medical research, where, for instance, they are used to maintain viruses which cannot be cultured other than by infecting living cells.

Cell fusion

Plant cell protoplasts from different species can be fused to create complex "hybrids" with two or more nuclei. Even protoplasts derived from different genera can be joined; it does not matter that these plants are unable to interbreed. In theory, hybrid plants with characteristics from both donor cells can be regenerated from fused protoplasts. This could provide a simple route for introducing, say, the ability to fix atmospheric nitrogen from legumes into cereals. However, enthusiasm for this technique has waned since the first successful experiments during the 1970s. Protoplasts of many species, fused or not, have proved difficult or impossible to culture, let alone regenerate into entire plants.

Cell fusion of a different type, using animal cells, has been more valuable. Antibodies can be produced by hybridomas grown in vitro. A hybridoma is an antibody producing cell (which is normally difficult to culture in isolation) fused with a tumour cell (which is virtually immortal in culture). Large quantities of specific antibodies (called monoclonal antibodies because they are all identical) can be produced by cultivating hybridomas in a fermenter. These antibodies can be

used in diagnostic tests of great sensitivity. For example, aflatoxins are potentially lethal compounds that are produced by fungi which contaminate stored food. Antibodies raised against aflatoxins are now used routinely in inexpensive and simple-to-use diagnostic kits. The same antibody technology is also used to detect the hormones associated with ovulation and pregnancy. Such knowledge brings significant advantages to animal breeding programmes.

FOOD PRODUCTION

Microbial production of food, food additives and processing aids

Fermented foods

Fermented foods are foods in which desirable changes have been produced by the action of microbes or enzymes. Over 3 500 different traditional fermented foods have been catalogued. They include the bread, yoghurt and cheese that are familiar in Europe and North America. In Africa, foods made from fermented starch crops (yams, cassava, etc.) are more important, whereas in Asia products derived from fermented soya beans or fish predominate. Fermented beverages include not only the obvious alcoholic drinks, but also tea, coffee and cocoa (fermentation of the leaves or beans occurs after tea, coffee or cocoa have been harvested).

Fermentation can make the food more nutritious, tastier or easier to digest or can enhance food safety. Fermentation also helps preserve food and increase its shelf life, reducing the need for additives, refrigeration or other energy-intensive preservation methods. For several thousand years, traditional fermentation (such as brewing) has given people the opportunity to cultivate microbes on a large scale and in a safe manner.

Single-cell protein. In the 1960s, protein from microbial sources (single-cell protein or SCP) was thought to have considerable potential, particularly for Third World countries. Few of the early projects aimed to produce food for humans, aiming instead to provide nutritious, low cost animal feed.

Unfortunately, most of the SCPorganisms (yeasts, fungi, and bacteria) used petrochemical derivatives as a source

of carbon. Even when oil prices were low, the processes were only marginally economic. Consequently the oil price rises of the 1970s ended most SCP projects. One company had an efficient large-scale process using the bacterium Methylophilus methylotrophus that could utilize methanol to produce a partially purified protein for animal feed. However, the cost of soya or fishmeal for animal feed remained considerably lower than that of the bacterial protein. Consequently, production of the protein ceased in the late 1980s.

Of all the SCP projects started in the 1960s, only one has survived to become a commercial success. Fungal protein (in the form of mushrooms and yeasts) has been accepted as food for generations. One company pioneered the production of fungal protein from the mycelium of Fusarium graminearum, which is cultivated on glucose made from maize starch. The food is marketed in the United Kingdom, Ireland, Germany, Switzerland and Belgium. By 1995, annual production is expected to reach 14 000 tonnes — enough for about 28 million meals for a family of four. Unlike soya products, the fungal protein has an excellent texture, attributed to a special linear arrangement of the fungal hyphae which is similar to that of muscle fibres in meat. It has a high protein and fibre content, yet contains almost no fat, which accounts for its popularity with health-conscious consumers. The product has little taste of its own, yet absorbs other flavours readily and can be combined with a wide range of ingredients.

As a form of SCP, algae are of interest in subtropical and tropical regions, where sunlight can be utilized as an energy source. Often the production of algal protein is associated with fish farming. Algae of the genera Chlorella and Scenedesmus have been used as food in Japan, and Spirulina is being produced commercially in several countries, including the United States, Mexico and Israel. Often the product is sold as a high-value "health food".

Improved microbial cultures. All SCP processes use naturally occurring microorganisms that have been carefully selected from wild populations. Production of the fungal protein, for example, uses a strain of Fusarium graminearum that was isolated from a soil sample obtained close to a research laboratory. Before that, many thousands of samples from around the globe had been laboriously screened to see whether they contained a fungus with a suitable nutritional profile, pattern of growth and other desirable properties. For the microbial production of a substance such as an amino acid, it is occasionally possible to find a rare natural variant or "mutant" which lacks a critical step in one of its biochemical pathways and consequently overproduces the desired material. Where such mutants are hard to find, they can sometimes be induced artificially, but such techniques are slow and rely heavily on chance.

The goals of genetic modification applied to strain improvement are essentially the same as those of traditional methods, but the techniques are more precise and far quicker. In addition, genetic modification allows the genetic "solutions" developed by nature to be transferred from one species to another, so that this species can also benefit from certain genetically determined traits, for example, disease resistance.

Genetically modified yeasts. In 1990 the United Kingdom became the first country to permit the use of a live, genetically modified organism in food. This was a special strain of baker's yeast engineered to make bread dough rise faster. Existing genes were placed under the control of stronger, constitutive promoters, which helped the yeast break down sugar maltose faster than usual.

Ordinary brewer's yeast (Saccharomyces cerevisiae) is able to utilize a variety of carbohydrates as an energy source. These include glucose, sucrose and maltose.

Although sucrose is readily available (as cane or beet sugar), glucose and the other sugars must be prepared by the enzymic breakdown of starch (see Sweetener production, below). Unlike S. cerevisiae, the closely related yeast S. diastaticus is able to grow on starch and dextrins because it makes an extracellular enzyme, glucoamylase, which catalyses the breakdown of starch. Saccharomyces diastaticus cannot be used directly for brewing because it produces a compound which gives beer a spicy flavour. Great interest has therefore focused on transferring the gene for glucoamylase from S. diastaticus into S. cerevisiae. Such a yeast would be better able to utilize the carbohydrate present in conventional feedstocks, which would increase the yield of alcohol and enable the production of a full-strength, low-carbohydrate beer without the use of extra enzymes after the beer had been brewed. A modified yeast of this sort, produced by a research foundation, recently received approval for use in beer production in the United Kingdom.

Food yeasts which have been genetically modified to metabolise a wider range of sugars also help reduce the levels of polluting waste in effluent. Sugar beet molasses is widely used as the main raw material in the production of baker's yeast. Beet molasses contains, in addition to sucrose, a small proportion of raffinose. This sugar is not fully broken down and utilized by the yeast, and the unused part (melibiose) is found in waste water from factories. The new strains of yeast utilize raffinose completely, enhancing the yield of baker's yeast and leading to a cleaner effluent.

Improved starter cultures for the dairy industry. When cheese, yoghurt and similar dairy products are made it is important that only the desired microorganisms be allowed to ferment the milk. Failure to exclude unwanted organisms can lead to poor flavours, low yields and even food poisoning. Scrupulous hygiene is required, and often the milk is heat treated to kill all or some of its microbial flora. Starter cultures of only the desired microbes are then added to the milk in sufficient volumes and in the appropriate conditions to ensure their rapid growth.

When improved starter cultures are developed, it is sometimes possible to choose between long-established, conventional techniques and the modern methods of genetic modification. However, the organisms that are produced may be identical, irrespective of the method chosen. One example where different routes led to the same result is a new yoghurt that keeps its fresh "home made" taste for several weeks without the risk of turning acidic and bitter.

Normally, the starter culture bacteria in yoghurt turn the milk's sugar, lactose, into lactic acid within days. All that is needed to produce a yoghurt that stays mild is a Lactobacillus strain that cannot metabolise lactose. Such variants already exist in nature. One possibility is therefore to search for these organisms in nature. Another conventional approach consists of treating the lactobacilli in various ways to increase their mutation rate in the hope that the desired trait will accidentally be created.

A third option involves modern genetic techniques: identifying the precise gene (or genes) responsible for lactic acid production, isolating and modifying them to inactivate lactic acid production, and then replacing them in the bacteria.

All three techniques have proved successful and led to identical mild-tasting yoghurts. In many other cases, however, only the "designer gene" approach can deliver the desired results. Although this work is still at the laboratory stage, starter cultures for yoghurt and cheese production have now been altered to provide built-in protection against other microbes that cause food poisoning. The starter cultures have been modified so

that they produce a compound which breaks down the cell walls of the potentially lethal food poisoning organism Listeria monocytogenes. Modified bacteria could also be put into other foods as a fail-safe mechanism in case the food is stored in the wrong conditions. It might also be possible to design foods that could be protected against a range of other food-poisoning organisms, such as Salmonella.

Other modified dairy starter cultures under development produce flavour compounds for better-tasting products and are able to resist attack from viruses that would otherwise ruin the production of yoghurt, cheese and similar products.

Food additives and processing aids

For many years, a wide range of food additives, supplements and processing aids have been obtained from microbial sources. These include amino acids, citric acid, vitamins, natural colourings and gums as well as enzymes. The production microorganisms have been selected from nature to ensure that, for example, they produce high yields of a good-quality product and/or are easy to grow or that the product is easy to separate and purify. Laborious screening and selection processes are now being augmented by modern genetics, allowing quicker, more precise selection and improvement of existing production strains.

Amino acids. In foods, amino acids are used to enhance flavours and to act as seasonings, nutritional additives and improvers (in flour). They are used in both human food and animal feed. Bacteria or fungi which have been specially selected to overproduce specific amino acids are grown in large fermenters. The acids are secreted into the fermentation medium and harvested. The most important commercial products are glutamic acid, which is used as monosodium glutamate, a flavour enhancer; lysine, cysteine and methionine, which are used as supplements in animal feeds that are usually deficient in these essential amino acids; and phenylalanine, which is used in animal feed and in the manufacture of the sweetener aspartame (see Sweeteners, below).

Most of the 20 amino acids needed to make proteins are produced by fermentation in hundred- or thousand-tonne quantities. In 1990, a company made a batch of tryptophane that was subsequently implicated in a rare degenerative disorder. Concern arose because the tryptophane had been produced by a genetically engineered strain of Bacillus. However, it is thought that the illness was caused by insufficient purification of the tryptophane and not by the genetic modification itself.

Gums. Several gums produced by microorganisms and plants are used widely in the food industry as thickeners, emulsifiers and fillers. Recently a process was developed to turn relatively cheap guar gum (obtained from seeds) into something akin to the more expensive locust bean gum. An enzyme (α-galactosidase) from the guar seeds is responsible for this transformation. The gene encoding the enzyme was inserted into baker's yeast, and α-galactosidase can now be produced in quantity. Production and processing of α-galactosidase by modified organisms brings several other benefits (Table 1). Bacterial polysaccharides currently occupy only a small fraction of the food ingredients market, but genetically modified cells could produce a wide range of novel gums with improved properties.

Sweeteners. Aspartame, a peptide which is 160 times sweeter than sucrose, is now used in an increasing range of foods and beverages. Aspartame's sweetness was (allegedly) discovered by accident when James Schlatter, working in the United States, licked his fingers to separate a stack of papers. He had previously spilt some aspartame on his hands while working in the

TABLE 1

A comparison of production and downstream processing requirements of α-galactosidase produced by standard and modified yeasts

	Standard yeast	Modified yeast
Production		
Yeast required	236 tonnes	10 tonnes
Waste water	2 000 tonnes	90 tonnes
Waste yeast	400 tonnes	12 tonnes
Downstream processing		
Ammonium sulphate	1 100 tonnes	25 tonnes
Potassium sulphate	25 tonnes	1 tonne
Aluminium gel	1 tonne	None
Filtration aid	133 tonnes	5 tonnes
Solid waste	540 tonnes	18 tonnes
Liquid waste	1 125 tonnes	26 tonnes
Demineralized water	3 700 m^3	50 m^3
Iced water	52 000 m^3	2 000 m^3
Energy	44 500 kW	9 000 kW
Steam	220 tonnes	50 tonnes

laboratory. Chemists at another laboratory in the United Kingdom had independently synthesized aspartame some years before but had failed to notice its sweetness.

The original manufacturing process linked the two amino acids phenylalanine and aspartic acid (both products of fermentation) by chemical means. Today a more efficient enzymatic method has been developed.

Other food additives from microbes. Citric, acetic, lactic and ascorbic acids are produced in large volumes for the food industry by microbial fermentation.

Enzymes. A wide range of microbial enzymes are used by the food industry. Some of the more important applications are shown in Table 2.

Plant biotechnology

Plant breeding techniques

Since the origins of agriculture some 10 000 years ago, farmers have been trying to improve their crops. Initially, people simply replanted some of the seeds from their best plants each year. Crosses between chosen individuals of the same species led to gradual improvements in the stock. Ancient South American civilizations relied on sweet corn (maize), the product of a cross between two dissimilar wild plants, for much of their diet. For thousands of years this species has been totally dependent on human intervention because it has no natural mechanism for dispersing its closely bound seeds. Around the turn of the century the scientific

TABLE 2

Uses of enzymes in the food industry

Enzyme	Product	Use
α-Amylase	Sweeteners	Liquefaction of starch
	Beer	Removal of starch haze
	Bread, cakes and biscuits	Flour supplementation
Amyloglucosidase	Sweeteners	Saccharification
	Low-carbohydrate beer	Saccharification
	Wine and fruit juice	Starch removal
	Bread manufacture	Improved crust colour
β-Galactosidase (lactase)	Whey syrup	Greater sweetness
	Lactose-reduced milk and dairy products	Removal of lactose for those who are lactose intolerant
	Ice cream	Prevention of "sandy" texture caused by lactose crystals
Chymosin (rennin)	Cheese	Coagulation of milk proteins
Glucose isomerase	High-fructose syrup	Conversion of glucose to fructose
Glucose oxidase	Fruit juices	Removal of oxygen
Invertase	Soft-centred sweets	Liquefaction of sucrose
		Sugar syrups
Lipases	Cheese	Flavour development
		Accelerated ripening
	Flavourings	Ester synthesis
Papain	Beer	Removal of protein
Pectinases	Wine and fruit juice	Increased yield, clarification
	Coffee	Extraction of the bean
Proteases (various)	Dairy products	Modification of milk proteins
	Caviar	Viscosity reduction of "stickwater"
	Bread, cakes and biscuits	Gluten weakening
	Meat	Tenderisation
		Removal of meat from bones

principles which govern inheritance started to be understood, and plant breeding began to be conducted on a more systematic basis.

Artificially induced mutation and selection, like that applied to microbial strains, has yielded more productive varieties of the world's major cereal crops. Genetic mapping methods similar to those used to pinpoint human genes have enabled scientists to identify precisely those plants which carry specific desirable genes. Such techniques have and will continue to lead to major improvements in yield, quality and resistance to disease. Despite these refinements to the crude hybridisation methods of former centuries, several problems remain. For instance, although plant breeders would like to introduce specific genes into crops, conventional breeding permits the recombination only of whole sets of chromosomes. Undesirable traits are therefore likely to be inherited alongside the desirable ones. Artificially induced mutation occasionally gives rise to improvements, but more often the mutations have a deleterious effect. Traditional plant breeding remains a tediously slow process, governed mainly by the time it takes plants to grow and set seed. Genetic modification, while it will likely never replace traditional methods, complements them and offers the opportunity to overcome some of their limitations.

About 80% of contemporary research in plant biotechnology is directed towards the improvement of food plants; the remaining 20% is concerned with nonfood products such as cotton, tobacco, ornamental plants and medicines. The initial emphasis in plant biotechnology has generally been directed towards the improvement of agronomic qualities. The second and third generations of genetically modified food plants will bring direct advantages to the consumer and commercial food processor.

Pest resistance

The main emphasis in this area has been the development of crops that produce a bacterial protein from Bacillus thuringiensis (Bt). Proteins from different strains of Bt act on specific pests such as beetles, moths and soil nematodes but do not affect mammals. Over many years, more than 2 000 tonnes of Bt spores or proteins derived from them have been used as biological alternatives to conventional pesticides. However, Bt pesticides are expensive to produce, and because they break down quickly frequent reapplication is necessary. Several genes encoding Bt proteins have been inserted into a variety of food plants, including cabbage, mustard, oilseed rape, maize, potato and tomato. The hope is that crops with such built-in resistance will reduce the reliance on conventional pesticides.

Herbicide tolerance

The production of genetically engineered plants that are tolerant of or even resistant to herbicides has generated considerable interest and much controversy in recent years. Critics fear that the availability of herbicide-tolerant plants will lead to an increase in the use of herbicides. The true picture is more complex.

Farmers currently apply herbicides as a routine preventative measure, usually before weed infestation has progressed too far. It is argued that with the new crops farmers will need to apply chemicals only when they are really needed, confident that this treatment will be effective. This should lead to a reduction in the use of herbicides, which would seem to be against the interests of the agrochemical companies. However, many of the modified plants now under test are tolerant of the safest herbicides available, so that farmers will be able to replace older chemicals with ecologically more favourable ones. Agrochemical companies are interested in selling these more advanced products.

Antisense technology

The sequence of bases along only one strand of the DNA double helix directs the production of proteins. This strand is called the "sense" strand.

When proteins are made, an mRNA copy of the appropriate instructions is transcribed from the DNA sense strand and ferried to the ribosomes. There, the mRNA directs the production of proteins from amino acids.

Antisense RNA is an RNA sequence transcribed from the wrong (antisense) strand of the DNA molecule. Antisense and sense RNA combine, preventing the production of proteins encoded by specific genes. For the method to work, an appropriate piece of inverted DNA is inserted into the sense strand of the host cell's DNA. Antisense RNA molecules are formed from the inverted section when the DNA is transcribed (see Figure 9).

Tolerance of the herbicide glyphosate, for example, has been introduced into soya, maize, oilseed rape and sugar beet. Glyphosate is effective at low concentrations, is not toxic to humans or other mammals and is rapidly degraded by soil microorganisms. Unfortunately this herbicide cannot be used on conventional crops because it would kill them. When the modified crops become available farmers could use glyphosate in preference to more hazardous chemicals.

Disease resistance

Viral diseases. Plant viruses cause major reductions in yield and adversely affect the quality of many crops. At present there is no effective chemical control for viral diseases of plants. The conventional response has been to select varieties (using classical plant breeding methods) that are naturally resistant to viral attack. The mechanism of natural resistance is not properly understood, and very few of the genes responsible have so far been identified. However, this does not mean that it is impossible to create virus resistant plants.

For example, hybrids have been made between virus resistant and virus susceptible potato species. Hybrids cannot usually be created between different species. However, in this case protoplasts from two potato species were fused together, and the resultant cells were cultured on a nutrient medium to regenerate whole plants. These plants were resistant to three major virus diseases. Field trials of this type of potato plant have been carried out in several countries since the mid-1980s.

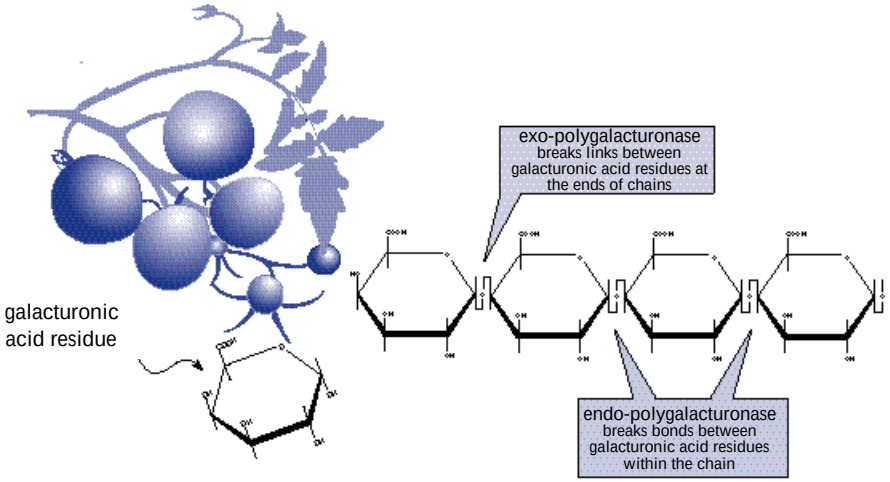
Antiviral "vaccines". The insertion of genes for viral proteins into a plant inhibits subsequent infection by that virus or a closely related one. Potatoes, melons, tomatoes and cucumbers have now been modified to resist viruses in this way.

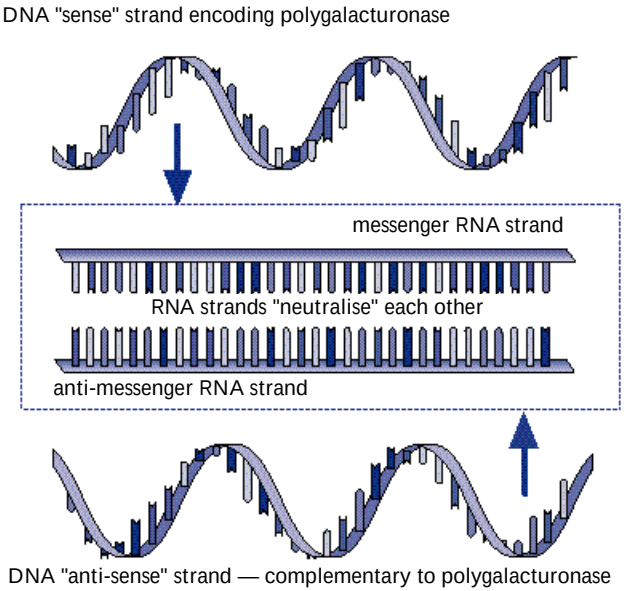
Antiviral antisense. Another route to engineering virus resistant plants is the suppression of viral genes using antisense technology (see box and Figure 9). Here antisense viral genes are inserted into the plant's DNA. The plant then produces antisense RNA to block the RNA of the invading virus (98% of plant viruses have RNA rather than DNA as their genetic material). This approach is still experimental, but initial results show promise.

Genes fight fungi — and locusts! Fungal diseases, particularly of soft fruits such as tomatoes, are being combated by the transfer of chitinase genes. Unlike the cell walls of plants, those of fungi contain chitin. Therefore the novel enzyme allows the plant to fend off fungal infection without damaging its own cells.

FIGURE 9.

Pectin is an essential component of many fruit. It is naturally broken down by polygalacturonase enzymes. Antisense technology can be used to "neutralise" the production of polygalacturonases, delaying the softening of fruits once they have been harvested.





Researchers in the United Kingdom, working with Brazilian scientists, recently isolated a chitinase from a tropical cereal that can degrade fungal cell walls. An unusual feature of this protein is that it also inhibits certain digestive enzymes of locusts. This was the first demonstration of a protein that acts as both an enzyme and an enzyme inhibitor. If the gene encoding this protein could be put into major cereal crops, it might offer an attractive means of combating damage of stored grain.

Improved food quality: The antisense route

A diverse range of food crops with qualities to benefit the food processor, retailer and consumer are currently undergoing field trials. Many of these plants have been modified so that their fruits reach the consumer in peak condition after transportation and storage. Antisense technology has been used to limit or neutralise the action of undesirable genes. The first food plants to be altered in this way were tomatoes.

Ripening research. In the mid-1970s researchers at the University of Nottingham in the United Kingdom started to investigate the complex processes involved in the ripening of fruit. The tomato was selected for this work because it has a relatively small set of genes and is comparatively easy to work with (for example, it can readily be grown by tissue culture). The aim was not to produce a better tomato but to understand the ripening process more fully so that the knowledge acquired could be applied to fruit and vegetables in general. This might allow farmers in tropical countries to benefit from the demand for exotic fruit and out-of-season temperate crops, because the fruit could be transported without refrigeration yet retain its texture and flavour. Food processors might also benefit because they would be able to produce high-quality fruit juices and purees that required less processing and fewer additives.

Pectinase key. Many genes are involved in the development of colour, flavour and texture in fruit. After several years the key to changing texture during tomato ripening was identified, an enzyme called polygalacturonase (PG). As tomatoes ripen, PG breaks down the pectin which holds cell walls together, causing the fruit to soften. A group of industry scientists, working with the Nottingham group, discovered a way to inhibit the PG gene without interfering with other aspects of the tomato's natural ripening mechanism. This meant that the softening process could be slowed down while the tomatoes continued to develop the desirable colour and flavour.

Antisense activity. The PG gene was "neutralised" by the introduction of an antisense gene into the tomato plant (see Figure 9). Although in early experiments some 10% of the PG remained active — sufficient to break down pectin and lead to mushy fruit — the antisense gene was inherited stably, so that cross-pollination between plants with the lowest levels of PG activity produced offspring which had just 1% of the usual enzyme activity. Researchers in the United States made similar use of an antisense PG gene. Their modified tomato is called Flavr Savr™, and has gained approval for sale to U.S. consumers. Pending regulatory approval, European shoppers will have to wait a little longer for a homegrown alternative to watery, flavourless tomatoes.

Other slow-ripening fruit. The gas ethylene is produced by fruit as part of the natural ripening process. An atmosphere of ethylene can be used to ripen fruit artificially. Antisense technology has been used to inhibit the production of ethylene and thereby impede ripening. Among the crops to have benefited from this type of modification are broccoli, raspberries, tomatoes and the "Euromelon" — the result of a joint project among French, Spanish, British and Greek scientists. All of these fruits can be picked when unripe and will remain in that condition until exposed to an atmosphere of ethylene.

Examples of other improvements to food quality

Potatoes pass the acid test. Human beings synthesize only half of the 20 different amino acids that are needed to make proteins. A healthy diet must therefore contain adequate amounts of the remaining 10 amino acids. The risk of malnutrition is enhanced where there is reliance on a single staple food. The proteins in some crops (for example, many of the legumes that are cultivated in Africa and South America) contain only small amounts of certain essential amino acids. These deficiencies could be overcome by inserting into affected crops genes from other organisms which have substantial amounts of these amino acids. Genetically modified potatoes with an improved amino acid content have already been developed in Israel. This work could prove highly beneficial, since potatoes are the world's fourth most important crop and an important source of nutrition in some countries.

Potato production could also be enhanced following the isolation, by Australian researchers, of a gene which doubles the yield of potato tubers from plants into which it has been inserted. Tests of the nutritional status and cooking properties of the potatoes are now underway. Other plant breeders have introduced a bacterial gene into potato plants that increases the proportion of starch in the tubers while reducing their water content. This means that they absorb less fat on frying.

Altered oils. A combination of conventional plant breeding, genetic modification and protoplast fusion has been used by numerous companies and research groups to alter the properties of oil from oilseed rape. The results range from plants that produce a greater proportion of saturated fats (suitable for margarine production) to others which yield a high-temperature frying oil that contains a low proportion of saturated fat.

Sweeter fruit — with no added sugar. Sweeter crops (for example, lettuces and tomatoes) have been produced by transplanting into them the genes for two natural protein sweeteners, monellin and thaumatin. The genes for both proteins came originally from tropical plants. Thaumatin is 3 000 times sweeter than sugar.

Removal of antinutritional factors. Many plants produce chemicals to fend off attack by pests and pathogens. Unfortunately, these natural chemicals can act as antinutritional compounds. Most legume seeds, for example, contain chemicals which inhibit the action of digestive enzymes. Many legumes contain relatively high concentrations of lectins, which, if they are not removed by soaking or destroyed during cooking, can cause severe nausea, vomiting and diarrhoea. In cassava and some legumes which form the staples of several African countries, levels of cyanide-generating compounds can lead to death or chronic neurological disease if these foods are not cooked properly. In some countries, all new varieties of potatoes are screened for levels of the toxin solanine. Genetic modification could be used to produce plant varieties with low levels of these antinutritional factors.

Processed products from genetically modified plants. In January 1995 the United Kingdom government announced the food safety clearance of several processed products obtained from three genetically modified crops: oil derived from oilseed rape that had been genetically modified to confer male sterility, thereby preventing self-fertilization and allowing the production of vigorous high-yielding hybrids; processed products (oil, meal and protein fractions) from soya plants modified to be resistant to the herbicide glyphosate; and tomato paste from tomatoes modified to slow down the process of softening. Unlike the antisense tomatoes described above, these tomatoes had been modified using "sense" technology. They contain a truncated polygalacturonase gene in the "sense" orientation, which, for reasons that are not entirely clear, has the same effect as the antisense polygalacturonase gene.

Long-term goals

Nitrogen fixation. The capacity to fix atmospheric nitrogen into a form that can be taken up by plants in the same way they use nitrogen in fertilizers — an ability possessed by Rhizobium bacteria that live in close association with leguminous plants — has been a major preoccupation of researchers. Despite decades of intensive study, the goal of a self-fertilizing cereal is still elusive. Doubts have now arisen as to whether this aim is realistic and should be a high priority, for two reasons. The first is that to receive ammonia from its associated bacteria, the plant has to provide the bacteria with "food" so that they can survive in small nodules on its roots. This food is provided at the expense of the plant's productivity. Legumes have had millions of years to evolve a mutually beneficial physiology with their Rhizobia, and it seems unlikely that such relationships could be artificially established with cereals; indeed there is evidence to suggest that nitrogen-fixing cereals could have decreased productivity. The second reason concerns the root nodules: these complex structures would have to be provided on the plant's roots. This is likely to involve the manipulation of very many genes.

A better approach might be to transfer nitrogen-fixing abilities into Agrobacterium, which infects a wider range of plants. Unfortunately this does not include cereals, which form the majority of food crops. For the foreseeable future it would probably be more profitable to try to improve existing nitrogen-fixing species, especially tropical legumes and their associated bacterial strains. Other readily available options include the traditional improvement of crop management, such as rotation between legumes and cereals.

Drought resistance. Drought-resistant crops would bring obvious advantages to farmers in areas of low rainfall. Numerous organisms are able to withstand dehydration without harm and can be resuscitated simply by adding water; dried yeast is a familiar example. A desert plant, appropriately named the resurrection plant, is similarly able to withstand prolonged periods of drought. Both organisms rely for this remarkable ability on a sugar in their cells called trehalose. In 1993, the first successful attempt was made to place the genes involved in the production of trehalose into potato and tomato plants. The results of this work have yet to be evaluated.

Animal biotechnology

Animal nutrition

Probiotics. For many years low levels of antibiotics have been added to animal feed, with the result that the animals gain extra weight. This is thought to be because the antibiotics kill detrimental bacteria in the animal's gut. An alternative approach is to add live, nonpathogenic bacteria to the animal feed. These organisms (termed probiotics) are thought to compete with the detrimental species, leading to a similar weight gain. Genetically modified bacteria might one day be used as probiotics to improve the health and efficiency of feed to weight conversion of farm animals.

Rumen bacteria. The nutrition of ruminant animals (for example, sheep and cattle) is highly dependent on the bacteria which live in their stomachs, enabling them to digest plant material effectively. Genetically modified bacteria could be introduced into the rumen by adding them to animal feed, enabling the animals to make better use of a wider range of food plants.

Silage. Silage is grass which has been fermented by naturally occurring bacteria to enhance its nutritional value. Microbial inoculants are now used to improve the formation of silage, but there is a possibility that genetically modified species could be used to further improve the process.

Bovine growth hormone. Bovine somatotropin (BST) is a protein produced by cows that has a variety of physiological effects, including the regulation of milk yield. BST is naturally present in all cow's milk. The gene for BST has been cloned into bacterial cells so that it can be produced in bulk. Injections of BST can be used to enhance milk yield. The hormone also improves growth rate and protein-to-fat ratios in meat. There has been a moratorium on the general use of BST in countries of the European Union for several years, although it is approved elsewhere, including the United States. BST is probably one of the most severely tested substances in the history of the U.S. Food and Drug Administration, but its use has been opposed in some quarters. Such opposition is based partly on uncertainties over its effects on animal health, and on worries that the hormone might contaminate meat or milk. There has also been opposition on political and economic grounds, as a surplus of milk is already produced in many developed countries. The advantage of BST is that it would allow the same volume of milk to be produced by fewer cattle.

Animal breeding and health

Classical animal breeding has done much to improve the productivity and well-being of farmed livestock. Changes in characteristics such as maturity, fecundity and the distribution of muscle tissue are noticeable in many modern breeds compared with their wild ancestors and old domestic breeds. The achievements of traditional breeding are now being augmented by modern genetic analysis.

The majority of the desirable features in livestock seem to be controlled by many genes, each with a small effect, working in concert. The modification of animals by genetic engineering is still in its infancy, so the genes that should be altered to improve animal productivity or health are still difficult to predict. Much of the work so far has concentrated on simple changes, such as the introduction of growth hormone genes. The results, some of which are described below, were not completely foreseen.

Most of the transgenic animals created to date are mice which are used in medical research. This lies outside the scope of the current discussion. However, this work is likely to benefit human and animal health.

Agriculture in Europe and North America already produces sufficient food for the indigenous population. The real benefits from improved animal production might be seen in the Third World. For example, it may one day be possible to introduce disease resistance into otherwise vulnerable animals. There are well-advanced porcine, ovine and bovine genome projects, which parallel similar efforts to map and sequence the entire human genome. The Bovine Genome Project could result in, for instance, resistance to trypanosomiasis being introduced into more productive breeds of cattle from their naturally resistant African counterparts. However, in the immediate future more benefit is likely to come from the development of new diagnostic agents, vaccines and therapeutic agents than by modifying the animals themselves.

Genetically modified pigs. Genetically modified pigs were first produced in the United States in 1988. Pig embryos were injected with a gene encoding growth hormone. Genetic "switches" were included so that a change in the animals' diet would greatly increase the levels of hormone circulating in the blood. Contrary to some reports, the result was not giant pigs, but animals with very lean muscle tissue. Unfortunately, the high levels of growth hormone led to gastric ulcers, arthritis, kidney disease and other undesirable conditions. Insufficient attention had been paid to what was already known about growth hormone, namely, that it is generally produced only for limited periods during an animal's life, not continuously.

Classical breeding methods supplemented by modern genetic mapping have produced more desirable results. For example, the genes which control leanness in pigs are linked to those that may cause sudden death during a period of stress. Humans also carry similar genes and may, for example, die inexplicably when undergoing routine surgery, although such a genetic predisposition is very rare in humans. Selection for leanness in pigs over many generations has also increased the frequency of the stress-related genes, but modern breeding methods are allowing such genes to be bred out.

Herman the transgenic bull. The world's first transgenic bull was bred in the Netherlands. Herman, the animal in question, became famous in 1992 when the Dutch parliament had to decide whether or not he would be allowed to mate. Scientists at the company that bred Herman wished to reduce cows' susceptibility to mastitis. To do this, they transplanted an extra copy of the gene for a milk protein called lactoferrin into several bovine embryos. Lactoferrin is part of the mammalian (including the human) defence against bacterial infection. Unfortunately, the only embryo to take up the transplanted gene produced not a cow, but a bull. Undeterred, the company hoped that Herman would pass on the gene to his daughters and that they would be less likely to suffer from mastitis. The Dutch parliament eventually gave its consent to the request from the company. Herman has sired several offspring, at least one of which carries the extra lactoferrin gene.

Cloned sheep. Breeders of sheep in the United Kingdom and New Zealand are trying to disentangle the role of genes and the environment in an effort to improve the quality of their livestock. They intend to do this with the help of identical sheep placed in several flocks. The sheep are produced by splitting an embryo when it consists of just eight cells. This happens naturally with the production of identical offspring, such as twins. Each of the eight cells is implanted in a foster mother. Differences between the identical sheep will be due solely to environmental influences (such as diet or exercise). The hope is that this comparison will enable sheep breeders to conduct their work on a more rational basis.

Chickens. Because viral infections are often a problem in intensive poultry production, a lot is known about the viruses which affect chickens. This knowledge has been useful to genetic engineers, because modified versions of the viruses themselves can be used to combat disease. Birds have been given immunity to fatal viruses that proliferate in crowded broiler houses by adding genes for viral proteins to the chicken genome.

Transgenic fish. Compared to mammals and birds, fish are still relatively wild, with little history of improvement through selective breeding. There are very many fish species and consequently a large pool from which genes can be drawn for breeding programmes. Fish, especially when farmed, are prone to infection. Recent genetic research on fish has concentrated on inserting genes that impart resistance to disease. Successful modifications have also been made to genes that influence growth, which could lead to more productive fish for aquaculture. Other possibilities are the introduction of "antifreeze" genes to extend the range of Atlantic salmon into colder waters and to delay the breeding season of fish so that they gain weight. Concerns have been raised about the wisdom of increasing the geographical range of a predator at the top of a food web. Work in this area must proceed with careful consideration of its possible ecological consequences.

EXAMPLES OF THE USE OF ENZYMES IN FOOD PROCESSING

Cheese manufacture

Ancient tradition

In ancient times the nomadic tribes which ranged through eastern Europe and western Asia carried liquids in bags made from animals' stomachs. Milk stored in these containers, warmed by the sun, soured by naturally occurring bacteria and laced with enzymes from the stomach lining, would have been transformed into solid curds and liquid whey. Thus, almost by accident the first cheeses were made.

The Romans were the first Europeans to describe cheese making in detail. To coagulate the milk protein, an enzyme preparation from goat, lamb or even hare stomachs was mixed with sheep's or goat's milk (cow's milk was not produced on a large scale before the 13th century). After the whey had been drained from the coagulated milk, the curds that remained were salted and stored for consumption later in the year. Vegetarian cheeses were also produced using the juices of plants which possessed milk-clotting properties, such as lady's bedstraw (Galium verum) or butterwort (Pinguicula vulgaris).

First commercial rennet

Early cheese makers either placed strips of the kid, lamb or calf stomach directly into warm milk or prepared a crude rennet extract by soaking the strips in salt water. By 1837, some farms were selling rennet extracts in small quantities for the convenience of domestic cheese manufacturers. In 1874, the Danish chemist Christian Hansen founded a laboratory in Copenhagen and started the first industrial production of calf rennet extract. This was obtained from the stomachs of unweaned calves that were slaughtered for veal production and not specifically to obtain the enzyme. World production of rennet now exceeds 25 million litres per year.

Rennet substitutes

In the 1960s the Food and Agriculture Organization of the United Nations predicted a severe shortage of calf rennet. It was anticipated that an increased demand for meat would lead to more calves being reared to maturity, so that less rennet would be available. Over the last 30 years several substitutes for calf rennet have been developed, allowing the supply of enzymes to keep pace with cheese production. Today there are six major sources of protease for coagulating milk (that is, chymosins), three from animals (veal calves, adult cows and pigs) and three from fungi (Rhizomucor miehei [formerly Mucor miehei], Endothia parasitica and Rhizomucor pusillus [formerly Mucor pusillus]). In addition, there are now chymosins derived from genetically modified microbes (Escherichia coli, Kluyveromyces lactis and Aspergillus niger). See Figure 5.

Purity and safety of recombinant Chymosin

Chymosin obtained from recombinant organisms has been subjected to rigorous tests to ensure its purity. Biochemical, toxicological and microbiological tests have also been done. Today at least 50% of cheese in the United States is made with chymosin from genetically modified microbes.

Other enzymes for cheese making

In some cheeses fat-degrading enzymes (lipases) may be added to promote the formation of piquant flavours as the cheese ripens. Many traditional Italian cheeses benefit from such additions to augment the activity of naturally occurring lipolytic microbes. Recently, lipase

from genetically modified microbes has been introduced to accelerate the ripening of other cheeses. Cheddar cheese takes up to 9 months to mature fully. However, with the addition of a suitable lipase, this period can be reduced to a mere 6 weeks. In some countries food additive regulations based on traditional practices prohibit the use of enzymes in cheese other than those used to coagulate milk. These may be revised in the light of current technological developments.

Immobilised enzymes for whey processing

In several countries methods have been developed for the enzymatic treatment of surplus whey from cheese making. The enzyme lactase (β-galactosidase) is immobilised (for example, on porous beads) inside a tall column. Lactase is expensive and immobilisation permits it to be reused many times. Whey is passed over the beads in the column, emerging some 10 minutes later with 80–90% of its lactose sugar split into a mixture of sweeter-tasting glucose and galactose. After enzyme treatment the pH of the whey is adjusted and salt (from the cheese-making process) is removed. Finally the product is concentrated by evaporation to give an opaque, honey-like, protein-rich syrup.

Whey syrup finds a wide range of applications in the confectionery industry, particularly in the manufacture of soft toffee or caramel, where it substitutes for sweetened condensed milk. However, for this application it may be necessary to replace the casein that was precipitated when the whey was formed, since toffee made without this protein may be too fluid. Balanced against this, toffee made with whey syrup is hygroscopic, that is, it absorbs moisture. It is thus especially useful in the manufacture of wafer biscuits, since moisture is absorbed by the toffee, leaving the wafer crisp and extending the product's shelf life.

This important quality is also conferred on cakes made with whey syrup, since it allows manufacturers to reduce or omit humectants such as sorbitol or glycerol from their recipes. Because of its protein content, whey syrup also acts as an emulsifier, helping bind the cake ingredients and replacing some of the egg protein found in traditional recipes.

New uses for whey syrup are still being developed and may include products as diverse as mousse (in which the syrup's foaming, "mouth feel" and stabilising properties are important) to fermented meat products such as sausages. It may also be possible to use whey syrup as a substrate in microbial fermentations.

Fruit juice production

To appreciate the action of enzymes in fruit juice production, it is necessary to understand something of the structure of fruits and the changes that occur as they ripen.

Changes during ripening

As fruit ripens it becomes progressively softer. Much research has been done to discover exactly which enzymes are responsible for this and other changes in maturing fruit. One hope is that by monitoring the levels of these enzymes, growers might be able to harvest their crops at just the right time or to select varieties with desirable characteristics, such as better storage properties or greater yields and improved quality of juice. Naturally occurring cellulases, acting on the cell walls, play a part in the development of softer fruits such as peaches and tomatoes. Pectin is also broken down as fruits ripen. In unripe fruit, the pectin is bound firmly to the cell walls. Such pectin is insoluble and the liquid within the cells remains fluid, conferring rigidity on them. During ripening, the pectin is altered by enzymes in the fruit. As a result the pectin becomes more soluble, its grip on the surrounding cell walls is loosened and the plant tissue softens.

TABLE 3

Enzymes and their applications in the fruit and vegetable juice industry

Amylase	Removal or prevention of "starch haze" in juice concentrates
Arabanase	Removal of araban haze from juice concentrates (especially pear juice)
Glucose oxidase	Removal of excess oxygen from juices
Naringinase	Reduction in bitterness of citrus juices
Pectinase	Increasing yield of juice from fruit
	Clarification of juice
"Limoninase"	Reduction in bitterness of citrus juices

Dissolved pectin makes juice more viscous and difficult to press from fruit. Pectin also helps retain important colour and flavour compounds within fruit so that juice pressed from it is of inferior quality. Juice may also be difficult to clarify and filter because of suspended pectin particles. In the fruit juice industry pectinases obtained from fungi are used to help overcome all of these problems.

Use of pectinases in the fruit juice industry

Pectinases were first utilized in the commercial preparation of wines and fruit juices in 1930. Before this, juices had been prepared by mechanical means, simply by pressing fruit and filtering the liquid which emerged. The early methods using pectinase were developed by trial and error. Only in the 1960s did the chemical structure of plant tissues become known, and with this knowledge food technologists began to use a greater range of enzymes more effectively.

Enzymes are used to help extract, clarify and modify juices from many plants, including berries, stone and citrus fruits, grapes, apples, pears and even vegetables (Table 3). Where a cloudy juice or nectar is preferred (for example, with oranges, apricots, pineapples or carrots) there is no need to clarify the liquid, and enzymes are used instead to enhance extraction or perform other modifications.

Future developments

The enzyme commonly referred to as "pectinase" is not a single substance but a complex cocktail of enzymes able to attack a variety of bonds in correspondingly diverse pectin molecules. The pectolytic enzymes used to date have been crude preparations, but greater knowledge of the substances they act on has stimulated the demand for purer enzyme preparations with specific, well-defined activities.

Although improvements in enzyme purification processes are important, the focus has turned to gene technology to provide, for example, pectinases with improved temperature stability and lower pH optima. The availability of a range of specific enzymes from genetically modified, food-grade organisms will also lead to new applications for such enzymes. For example, a pectate lyase from the bacterium which causes soft rot in carrots and potatoes (Erwinia carotovora) has recently been cloned in Escherichia coli. This enzyme could be used in tea leaf extraction in the manufacture of instant tea.

Sweetener production

Sugars from starch

During the Napoleonic wars Europe was isolated from its major sources of cane sugar in the tropics. In 1811 German chemists succeeded in producing sugar by breaking down starch with acid, and this process was adopted in several countries until the introduction of sugar beet into European farming. In World War II a gentler enzymatic method of converting starch to sugars was developed which had the advantage of yielding sugar that lacked the bitter compounds characteristic of the acid treatment.

Enzymatic treatments are a major way of producing sweeteners today, including syrups derived from sucrose or starch that contain mixtures of glucose, maltose, fructose and other sugars. High-fructose syrup (HFS) from corn (maize) starch has now eclipsed sucrose as the major sweetener used in the U.S. food industry. More than 8 million tonnes of HFS are sold annually, although the production and use of HFS in the countries of the European Union has been limited by quotas intended to protect European sugar beet growers.

HFS is an excellent alternative to sucrose or invert sugar in food processing. It is used in many products, including soft drinks, jam, ice cream, cakes, canned fruit, pickles and sauces. Unlike sucrose, HFS remains stable in chilled, frozen and acidic foods without forming crystals or undergoing conversion to other sugars.

High-fructose syrup

HFS is made from a low-cost raw material, starch. The starch is converted into syrup by several enzymes, which are used in three distinct stages:

Liquefaction. Starch is obtained as a by-product after valuable oil and protein have been extracted from maize. The starch solution is boiled and treated with α-amylase, an enzyme from Bacillus. This treatment gelatinizes and dissolves the starch and starts to break it down. The partly degraded starch molecules are known as dextrins.

Saccharification. Depending on the carbohydrate composition required in the finished product, a cocktail of various fungal enzymes is then added to the dextrins. For syrups with a high glucose content a mixture of a amylase or pullulanase with amyloglucosidase is used. Over 1–3 days these enzymes break down the dextrins progressively to glucose. Evaporation of water yields a viscous glucose syrup.

Isomerization. Glucose shares its chemical composition with fructose but has a different molecular structure. This makes glucose about half as sweet as fructose. The enzyme glucose isomerase converts glucose to fructose, thereby increasing the sweetness of the syrup. Immobilised glucose isomerase is packed into a column, and glucose syrup heated to 60˚C is passed continuously over it. At this temperature, the glucose syrup has a low viscosity, microbial spoilage is prevented and conversion occurs swiftly. Typical HFS has a dissolved-sugar composition of 42% fructose and 53% glucose (the remainder being other sugars). Should syrups with a greater fructose content be required, glucose can be separated from the liquid leaving the column. This sugar can be recycled over the enzyme column to achieve a greater overall conversion rate.

Better enzymes from nature

Over the last 25 years much research has been directed towards finding better enzymes for HFS production. In 1974 a Danish company introduced a bacterial α-amylase from Bacillus licheniformis that catalysed the breakdown of starch at 100˚C or more. This led to

significant improvements in the initial liquefaction process. A diverse range of dextrin-degrading enzymes has also become available to satisfy the demand for specialised sugar syrups, for example, for baby food, for diabetic confectionery and for use in brewing and wine making. These developments have resulted from careful selection of microorganisms that produce the enzymes. Finding the ideal production strain takes many years, however, and is largely a matter of luck. A microbe with one attribute in its favour will likely lack another equally important characteristic. Modern molecular biology has started to reduce this dependence on trial and error.

Modified microbe

Bacillus stearothermophilus produces an α-amylase which is well suited to sugar syrup production. Unfortunately, this species makes only small amounts of the desirable enzyme. Several copies of the appropriate gene were transferred into a closely related species, Bacillus subtilis, enabling commercial production of the superior enzyme. After extensive safety tests, this amylase became the second enzyme in the world (after chymosin) from a genetically modified organism to be approved for food processing in the United States.

A designer enzyme

At the relatively high temperatures (60˚C) used in immobilised enzyme columns, glucose isomerase rapidly becomes inactive. Typically the enzyme's activity halves every 55 days, so that after several months the expensive enzyme has to be replaced. In 1986, a company in the Netherlands in cooperation with a Belgian company initiated an ambitious research programme to improve the stability of glucose isomerase. The research team first sought to understand the reasons for the decline in enzyme activity.

Stronger links. Careful examination of crystals of the enzyme revealed its structure. Glucose isomerase was found to consist of four identical subunits joined together by fragile bonds. At raised temperatures the protein broke apart at these bonds and linked instead to glucose molecules in the syrup as they passed through the column. This explained the inactivation of the enzyme.

Further investigations showed that of the many hundreds of amino acid residues making up glucose isomerase, just two were responsible for the weak links. By substituting these amino acids with others that bound more tightly to their neighbours, the "protein engineers" were able to produce a more stable enzyme. This was done by subtly altering a small section of the DNA that coded for glucose isomerase, so that one out of the 20 lysine residues in each subunit of the protein was replaced by an arginine residue.

Production processes. The remaining technical hurdles of fermentation, product recovery and immobilisation were gradually overcome, and by 1993 a new product was created. The improved glucose isomerase has a half life that is roughly double that of the original form, resulting in a doubled productivity in the enzyme column. A further benefit could come from the new enzyme's greater thermostability. With standard glucose isomerase there is a limit to the proportion of fructose that can be produced in the syrup leaving the column. At higher temperatures, a greater proportion of fructose is formed; the new enzyme may therefore permit one-step production of HFS at very high temperatures.

Product registration. Before the new enzyme can be sold to HFS producers, both it and the production process must undergo extensive testing and gain the approval of regulatory bodies. The U.S. Food and Drug Administration has already been consulted, and it has accepted the description, purpose and conditions of use for this glucose isomerase.

REGULATORY, SAFETY AND SOCIOECONOMIC CONSIDERATIONS

To date, relatively few products of genetic modification have reached the supermarket shelves. Genetic modification of farm animals is in its infancy, and work with the major crop plants, especially cereals, has proved more difficult than was at one time anticipated. Outside the laboratory, modified plants and microbes have so far been restricted to closely monitored field trials. Nevertheless, during the coming century the impact of food biotechnology is likely to be both profound and far reaching.

For this reason, there has been much debate about the potential social and economic implications of biotechnology. The safety of new developments has been examined with rigour, and legislation has been drafted to protect the interests of the public.

Food safety

In addition to issues of environmental safety, the implications of modern biotechnology for food safety need to be considered. Recognising the numerous benefits to health, nutrition, food preservation and food production that biotechnology could bring, the Organization for Economic Cooperation and Development (OECD) established a working group of national experts to consider the safety implications of modern food biotechnology. The intention was to exchange ideas, data and information among experts to enhance international cooperation in the field. The working group considered numerous examples of how the safety of novel foods and food components had been evaluated in the past and established some concepts and principles that underpin the safety evaluation of foods derived by modern biotechnology. These principles have been widely accepted and are similar to recommendations made by other influential groups such as the World Health Organization and the Food and Agriculture Organization of the United Nations. The major conclusions of the OECD report are summarised below.

Food is considered safe if there is reasonable certainty that no harm will result from its consumption under anticipated conditions. Historically, food prepared and used in traditional ways is considered safe on the basis of long-term experience, even though it may naturally contain harmful substances. In principle, food is presumed to be safe unless a significant hazard has been identified.

Modern biotechnology broadens the range of genetic changes that can be made to food and widens the range of possible sources of food, although this does not inherently lead to food that is less safe than that developed by conventional techniques. Therefore the evaluation of foods derived from modern biotechnology does not require a fundamental change in established principles of food safety, nor does it require a different standard of safety.

Furthermore, modern biotechnology provides precise techniques for the direct and focused assessment of safety (for example, by the detection of minute amounts of contaminating material), which can be usefully applied to foods derived from both modern and traditional methods.

Substantial equivalence

The OECD report said that the most practical method to establish food safety was to consider whether a novel food (or food component) was substantially equivalent to an analogous conventional food product, where one existed. Account should be taken of the processing (such as cooking) that the food might undergo, as well as how much food was to be consumed, by whom and the dietary pattern.

To demonstrate substantial equivalence, a number of factors have to be considered, such as:

- the characteristics and composition of the conventional food to which the new one is to be compared
- knowledge of the component parts of the new product or organism, such as any introduced genes, the method used to introduce the new genetic material and how that new genetic material is expressed
- the characteristics and composition of the new product or organism compared with the existing food or food component.

If the novel food is judged to be substantially equivalent to an existing food, it is treated in the same manner as its conventional counterpart.

Where new classes of foods or food components are introduced it is more difficult to apply the concept of substantial equivalence. Here experience gained in the evaluation of similar materials is taken into account. Where a product is thought not to be substantially equivalent to an existing one, further investigations focusing on the identified differences are required. Totally new foods, where no similar materials have ever been consumed, must be evaluated solely on the basis of their own composition and properties.

Genetically modified potatoes

As an example of the application of the principle of substantial equivalence, potatoes are an established part of the human diet. They can contain toxic alkaloids, but people generally know how to prepare them and avoid eating green potatoes, which contain significant amounts of alkaloids. Potatoes are often infected by naturally occurring viruses, but these do no harm to humans and have a long history of human consumption. If a potato were genetically modified with one of these viruses so that it produced viral protein at levels comparable to those from naturally infested potatoes, it would be considered to be substantially equivalent to the infected potatoes that have a long history of safe use and consumption. This theoretical analysis applies only to viral proteins in the parts of the potato plant that are traditionally consumed. It also assumes that the insertion of the viral coat protein gene does not lead to secondary effects through, for example, interruption of coding sequences within the plant's genome.

Chymosin from genetically modified bacteria

As described in the section on cheese manufacture, the enzyme chymosin can be obtained from genetically modified microorganisms. Chymosin from the bacterium Escherichia coli is obtained from a completely different organism and by a method that is completely different from its traditional counterpart, which comes from an animal. Thus the types of potential impurities differ and the characteristics of the enzymes may differ, too. To determine whether the two preparations were substantially equivalent, the U.S. Food and Drug Administration compared the activities of the two enzymes and evaluated whether impurities in the product from the modified organism affected its safe use. After a battery of tests, both enzymes were found to be substantially equivalent in safety and function.

Safety implications of marker genes

The current generation of genetically modified organisms frequently contains marker genes, some of which encode resistance to antibiotics. In such cases it is important to consider whether food derived from an organism with such a gene would be substantially equivalent to a conventional product. Among the questions that must be asked are whether the marker gene encodes a protein such as an enzyme which catalyses the breakdown of an antibiotic and, if so, what levels of that protein (if any)

would be expected in the food. Does the gene encode resistance to a clinically useful antibiotic, and if so, would ingestion of the food while someone was being treated with that antibiotic interfere with the drug's effectiveness? Finally, is it at all likely that the gene could be transferred to other organisms such as microbes in the food or in the intestine of the consumer? For food enzymes produced by genetically modified organisms, the Organisation for Economic Cooperation and Development (OECD) considered that levels of marker genes or their products in food would almost always be biologically insignficant. Would the same be true of plants with marker genes?

With these concerns in mind, the Food Safety Unit of the World Health Organization (WHO) organised a workshop. WHO recognised that there was a need for marker genes, which may have no function in the final product, but that at present it was impractical for such genes to be removed from modified organisms after they had fulfilled their function. The presence of marker genes per se in food was not thought to constitute a safety concern. It was also noted that the number of marker genes in plant varieties approaching commercialisation was restricted to two antibiotic resistance markers and to a few herbicide tolerance markers. The workshop concluded that there is no recorded evidence for the transfer of genes from plants to microorganisms in the gut. This is highly unlikely to occur for theoretical reasons, since the mechanism for controlling gene expression is fundamentally different in bacteria and plants. Unless the gene transferred into the plant were under the control of a bacterial system (and therefore unable to be expressed in the plant), there would be no mechanism for expression of the gene, even if it were transferred to the gut bacteria.

Regulatory approval

European Union. Within the countries of the European Union, regulations have been proposed to cover the use of novel foods or food ingredients. In particular, the regulations will apply to modified or new molecules, to any products that have not previously been eaten by humans to a significant degree, to genetically modified organisms and their products, and to novel processing methods. This proposal is still under discussion by the European Union Council of Ministers.

United States. In the United States, foods are regulated by three bodies. The Food and Drug Administration (FDA) is concerned with food safety relating to new plant varieties, dairy products, seafood, food additives and processing aids, whereas the Department of Agriculture regulates meat and poultry products and field tests all genetically modified plants. The Environmental Protection Agency is responsible for pesticide chemicals, and it may therefore have to approve new plant varieties resistant to attack by pests. Of the three agencies, the policy of the FDAwith respect to new plant varieties is most clearly defined at present.

The FDA regards the key factors in reviewing safety to be the characteristics of the food and its intended use, rather than the fact that new methods have been used in its production. Novel food products are not subject to regulatory approval if the constituents of the food are the same or substantially similar to substances currently found in other foods (such as proteins, fats, oils and carbohydrates). For example, if a gene from a banana were transferred to a tomato, approval would not ordinarily be required before that food was placed on the market. However, if a sweetening agent that had never been an ingredient of any other food were added to a variety of grapefruit, the novel food would need regulatory approval. The sweetener would be regarded as a food additive and therefore be subject to other, more stringent regulations.

Deliberate release of modified organisms

The conduct of laboratory-based genetic modification of microorganisms, while requiring care, presents few hazards other than those normally associated with the use of similar microorganisms. Such risks are readily contained by well-established and tested laboratory procedures. However, when a proposal is made to use a modified organism (microbe or plant) beyond the confines of the laboratory, particularly a robust organism capable of withstanding the rigours of the natural environment, a different set of safety questions arises.

What might the risks be? The deliberate planting or dispersal of genetically modified organisms outside the laboratory could cause unforeseen effects if, for example, modified plants or microbes were able to:

- avoid limiting factors that regulate naturally occurring populations and thereby change the usual balance between populations (for example, an increased tolerance of drought, high temperatures or high salt concentrations could achieve this)
- produce new compounds, (for example, insecticides, herbicides)
- transfer their newly inserted genetic material to other plants.

Note that these changes, should they occur, would not necessarily be harmful; indeed some, such as the production of toxins or improved nitrification, might be the purpose of the modification in the first place. The nature of the potential environmental impact falls into two broad categories: effects which result from the activities of the modified organism itself and those which might arise should genetic material be transferred into other organisms.

Making predictions. Predicting the influence of any organism (genetically modified or not) introduced into a new habitat is difficult. However, it is important to recognise that nearly all agricultural species are introductions which have been cultivated by humans, often for many thousands of years. In this time they have been subjected to enormous random genetic change — selection by nature and by human intervention. It is unlikely that the deliberate and targeted genetic modification of such species would result in hazards any greater than those that have already occurred.

Field trials. More than a thousand field trials of genetically modified crops have been conducted worldwide. Extensive research with the specific aim of determining the ecological effects of modified crops has been carried out in the United Kingdom. The Planned Release of Selected and Modified Organisms research took 3 years, and was funded by European industry and the United Kingdom government. Independent public sector ecologists conducted the work as they saw fit. The results suggested that the genetically modified plants (potatoes, maize, oilseed rape and sugar beet) presented no intrinsic environmental threat. Genes were not readily transferred from the modified plants to their wild relatives, and there was no increase in the ability of the modified crops to invade natural habitats. The ecologists warned that their findings should not be simply extrapolated to other crops, but suggested that a cautious step-by-step approach to planned releases has much to commend it. Similar work involving more than 200 field trials and more than 500 locations and transgenic lines has been carried out in France.

European regulations governing deliberate releases. All European Union nations are party to a European Council directive governing the deliberate release of genetically modified organisms (GMOs) into the environment. This includes provisions for marketing food products containing or made from GMOs. Under this directive all

of the member states have agreed to put into place laws and regulations to ensure that common policies on GMOs are adopted throughout the union. The regulations do not apply to organisms obtained by conventional breeding techniques (for example, mutagenesis) that have been applied over a long time.

The directive requires that proposed releases should be considered individually. People intending to make a release must first seek permission from the appropriate national authority. The application must contain a technical dossier including information about the personnel concerned and their training and a full environmental risk assessment with details of appropriate safety and emergency responses.

In certain cases interest groups or the public may be consulted about a proposed release. Action has been taken in several countries to ensure that there is public interest representation on the relevant advisory bodies and that the public has access to information about releases. Companies or others may ask for some information to be kept confidential on grounds of commercial competitiveness, but in such cases they must give verifiable justification. Following the destruction of some field trials in the Netherlands, there are now provisions in Dutch law allowing for the location of field test sites to be kept secret, but normally this is not done.

A general principle is that releases should be conducted a step at a time and that the scale of release should be increased gradually only as it becomes apparent that it is safe to do so. Once a release has been made, a report on it must be sent to the appropriate authorities, with particular reference to any risk to human health or the environment. The directive makes provisions for the exchange of information on releases between member states and for the publication of details.

It should be emphasised that the European directive discussed above is based on a precautionary approach. As more experience has been gained, the risks presented by genetically modified plants appear to be smaller than was first anticipated. International research in this area is leading increasingly to the conclusion that there is no greater risk from introducing genetically modified organisms into the environment than is presented by similar introductions of organisms produced by conventional breeding techniques.

Social, economic and other effects

Biotechnology could bring considerable benefits to world agriculture, but there could be accompanying disadvantages. Insufficient space permits only an indication of some of the main issues here.

Many of the consequences of modern biotechnology could be similar to those produced by existing trends, such as the shift towards larger farms and more capital intensive farming systems. This tends to favour wealthy farmers in the Northern Hemisphere who can invest in new technologies rather than those in the impoverished South. Biotechnology may reduce developed countries' dependency on products imported from developing countries, with adverse effects in the producer nations.

Concerns in the Western world about overproduction are unlikely to be shared by countries whose population growth far outstrips the capacity of farmers to provide sufficient food. However, if biotechnology brings greater agricultural productivity to developing nations, there could be major shifts in employment patterns, with adverse effects on the countries' stability.

Some people also question whether developments provided by the new biotechnology are really needed — the so-called fourth hurdle (after the safety, efficacy and quality of a new product have been demonstrated).

However, the only place where the market need for a product can be assessed properly is the marketplace. The requirement to demonstrate need in advance could severely restrict innovation.

Biodiversity

Biotechnological methods (including the genetic modification of plants) have already led to plants with greatly improved characteristics compared to the old cultivars produced by conventional methods. Excessive planting of a limited number of cultivars would lead to increased genetic uniformity. This might be accompanied by a greater risk of widespread epidemics of plant diseases, and it could lead to a reduction in biodiversity (in crop plants, weed species, insects and microflora in the fields in question).

Conversely, biotechnology might encourage the production of a far wider variety of new crops, increasing biodiversity. In the developed nations, the "green revolution" of the 1960s and 1970s has been so successful in increasing the yield of food that farmers in the United States and Europe are paid to take land out of cultivation, again increasing diversity. The biotechnological revolution may further accelerate this trend.

On a wider scale, biotechnologists depend on the genetic resources of the world for their raw materials, and thus have a vested interest in maintaining biodiversity. The techniques of plant tissue culture might be used to help maintain rare and endangered species. Some argue in favour of patents on living organisms, as they might allow Third World countries to obtain payment for the use of their genetic resources.

The need for public involvement

Although the potential of genetic engineering in the food sector is often exaggerated, many improvements are possible in the areas of food quality, variety and safety. The economic effects of such developments could be considerable, and there are opportunities to reduce the environmental impact of food processing and production, making a positive contribution towards more sustainable development. Much will depend on the attitudes of consumers. Some of the new foods will be indistinguishable, at least on the supermarket shelf, from those currently available. In other products, genetic engineering will lead to significant and noticeable improvements, such as better flavour. The provision of appropriate information and public understanding are therefore a crucial concern. Although concerns about some aspects of biotechnology are justifiable, it is important that these be judged against the enormous benefit that technologies such as genetic engineering could bring.

In a democratic society public involvement is essential. The strong case for the involvement of the informed lay public on decision-making bodies is reflected in the legislative framework for biotechnology devised in several countries. Some sectors of the food industry are willing to search for applications of genetic modification that are mutually acceptable to consumers, legislators, food producers and processors. It is to be hoped that this monograph will contribute to that discussion.

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