CHAPTER 10

Biotechnology and Food Safety: Benefits of Genetic Modifications

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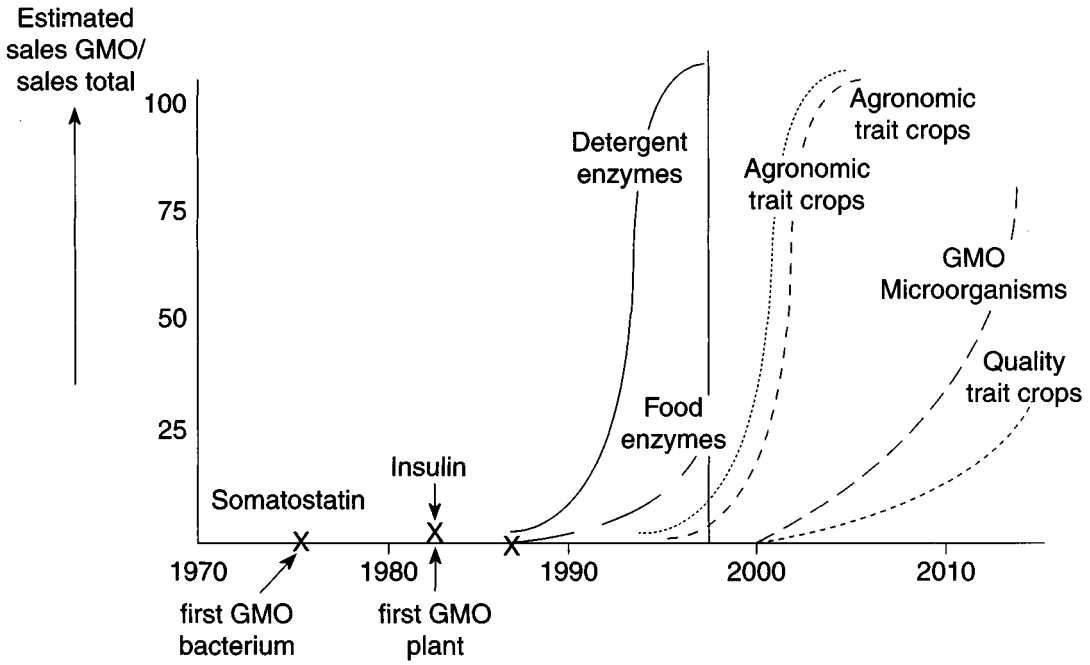
INTRODUCTION

A number of consumer research studies have shown that fermented food products are regarded by consumers as healthy and natural.[9] Factually and from a historic point of view, this is correct. During the approximately 7,000 years that fermented foods have been produced, an enormous selection process has taken place, and the tasty products that are presently on the market are the result of this selection process. It is well known that fermentation of foods and drinks prolongs the shelf life of these products. The historic claims that fermented foods, particularly those derived from milk, prevent diseases are supported by many studies. Those claims related to mucosal health are excellently summarized by Salminen *et al.*[23] Nevertheless, the title of this chapter suggests that at present, there is concern about the safety of fermented food products. This is due to the rapidly growing application of genetically modified food products, including fermented food products. In particular, the penetration on the market of genetically modified plants is remarkable (Figure 10–1 and Table 10–1). In addition, more than 20 food enzymes that are on the market at present are produced using recombinant DNA technology (Table 10–2).

The perception of genetic modification of food products by European consumers is not very positive and varies from country to country. Generally, the attitude of Western consumers is neutral to positive (Figure 10– 2), whereas in most of the developing countries, this technology is seen as an opportunity.[20] However, in spite of the present problems in a number of West European countries, and excluding small groups of dogmatic opponents of genetic engineering, the perception of the consumer will ultimately be determined by the benefits versus risks ratio.

In spite of many efforts, neither scientists nor opinion makers have been able to properly explain the potential risks of genetically modified food products. Therefore, many consumers perceive recombinant DNA technology as an intrinsically dangerous technology, although in the 25 years that this technology has been applied, no unintended dangerous materials have been produced. The main reason for the present perception of this technology by West European consumers is that in many discussions, no clear definitions of the nature of the genetically modified food are used. In an attempt to rationalize the discussions and to avoid inappropriate ethical discussions, let us examine simple decision schemes based on rules of the U.S. Food and Drug Administration (FDA), the United Kingdom, and the Netherlands,[29] and a model for various genetically modified products (Figure 10–3).[30] It should be emphasized that this figure reflects the author's personal view concerning the safety of genetically modified foods that do not contain (antibiotic) resistance markers ("clean"). Genetically modified plants or microorganisms containing antibiotic resistance markers present a very difficult to quantify but realis-



Note: X-axis-penetration as a function of time: The expression of somatostatin in *E. coli* is taken as the starting point and insulin as the first large-scale pharmaceutical product. Y-axis-estimated percent of rDNA product in certain area (Detergent enzymes are nearly 100% rDNA products, about 25% of the food enzymes (e.g., chymosin) are made via rDNA technology).

Figure 10–1 Penetration of rDNA technology in agriculture, pharmaceuticals, and consumer products.

tic risk of spreading genes encoding antibiotic resistance in the environment.[6,10] The chance that these distributed antibiotic-resistant genes are taken up is very low, but not zero, and therefore undermines the argument that genetically modified plants or microorganisms containing a gene that encodes an intrinsically safe protein are safe.

Table 10–1 Rapid Increase of Transgenic Plants

Crop	*1996*	*1997*
Maize	525,000	4,400,000
Soybean	400,000	5,250,000*
Potato/tomato	40,000	500,000†
Oilseed	200,000	1,600,000
Cotton	810,000	1,200,000

*Includes the South Amerian 1996/1997 harvest.
†Includes estimates for China.
Note: In 1994, no transgenic crops were cultivated for usage in the agrofoods industry.

In addition to the lack of proper explanation of the potential risk of genetically modified foods, the benefits for the consumer are either nonexistent or not communicated properly to consumers. This is a major weakness, and only when the benefits to consumers become clear will genetically modified foods be accepted by the majority of consumers. It is obvious that benefits are not absolute values but relative values, strongly influenced by demographic, geographic, and socioeconomic factors.

In this chapter, the supply chains of fermented foods are taken as guides to point out the potential benefits and risks of every step of these chains. The emphasis will be on fermented foods

Table 10–2 Commercial Food Enzymes Made by rDNA Technology (as on the Market at 1.5.1998)

Enzyme	*Commercial Name*	*Producer*	*Application*
α-Galactosidase	α-galactosidase	NOVO N.	Animal feed
Xylanase	Bio-feed wheat	id	id
Lipase	Lipozym	id	Interesterification
ALDC	Maturex	id	Beer
Amylase	Novamyl	id	Bread
1,3 Lipase	Novozym 677	id	Bread
Xylanase	Peptopan	id	Bread
Amylase	Maltogene	id	Starch
Chymosin	Novoren	id	Cheese
Lipase	Novozym 398	id	Interesterification
Lipase	Novozym 435	id	id
Fytase	Pytan	id	Animal feed
Amylase	Termamyl	id	Starch
Transferase	Toryzym	id	Starch
Chymosine	Maxiran	DSM/Gb	Cheese
Phytase	Natuphos	id	Animal Feed
Chymosin	Chymogen	C. Hansen	Cheese
Chymosin	Chymax	Pfizer	Cheese
Xylanase	Biobake 710	Quest	Bread
Invertase		id	Chocolate
Glucanase		id	Beer
α-Galactosidase		id	Guar modification

starting from traditionally bred or genetically modified plants and milk from traditionally bred cattle. The approach of analyzing the safety of fermented products as a function of the supply chain has been adopted, because only with such an approach will all potential risks be included, which is essential to gain the confidence of consumers.

By analogy with microbial risk assessments,[28] risk is defined as the probability of a hazard occurring and hazard as a harmful event. This means that when the newly introduced gene encodes a protein that is intrinsically safe and does not provide a selection benefit for any receiving organism, the hazard is considered as zero. Consequently, the risk is zero and the safety of the food product is 100%. If, on the other hand, the newly introduced gene encodes for a protein that is intrinsically safe but provides the receiving organisms with a selection benefit in the open environment, then the health hazard is zero, but the environmental hazard is not zero. Consequently, the probability of transfer of the gene to the receiver has to be estimated. Genes encoding intrinsically unsafe proteins (i.e., health hazard > 0) are not considered because authorities will not give permission for cloning of such genes in plants and/or microorganisms that enter the food chain; neither will the food industry ever use such organisms.

SUPPLY CHAIN OF FERMENTED FOODS AND ITS IMPORTANCE TO ENSURE SAFE PRODUCTS

Table 10–3 provides a selection of fermented foods based on the raw material(s) that is (or may become in the near future) derived from genetically modified plants.

The steps in the supply chains (including potential beneficial and risk aspects) of fermented foods using traditionally bred plant materials or milk from traditionally bred cows with fermen-

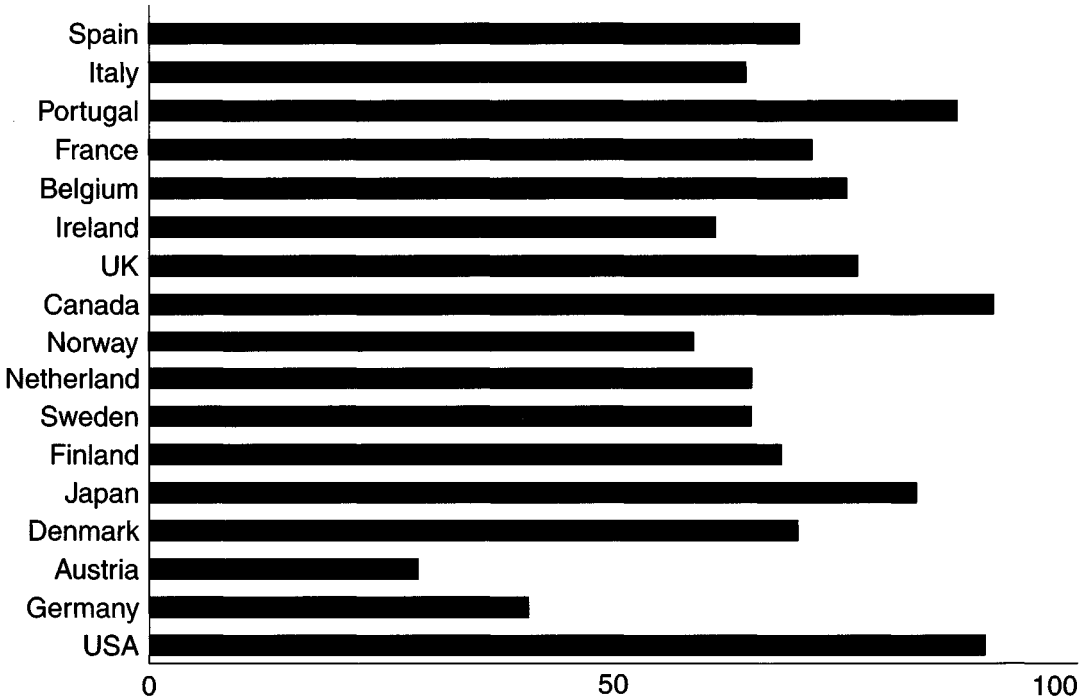


Figure 10–2 Acceptance of rDNA technology for consumer products derived from plants and microorganisms in various countries. Acceptance varies from approximately 90% in Canada, United States, and Portugal to 30 and 45% in Austria and Germany, respectively.

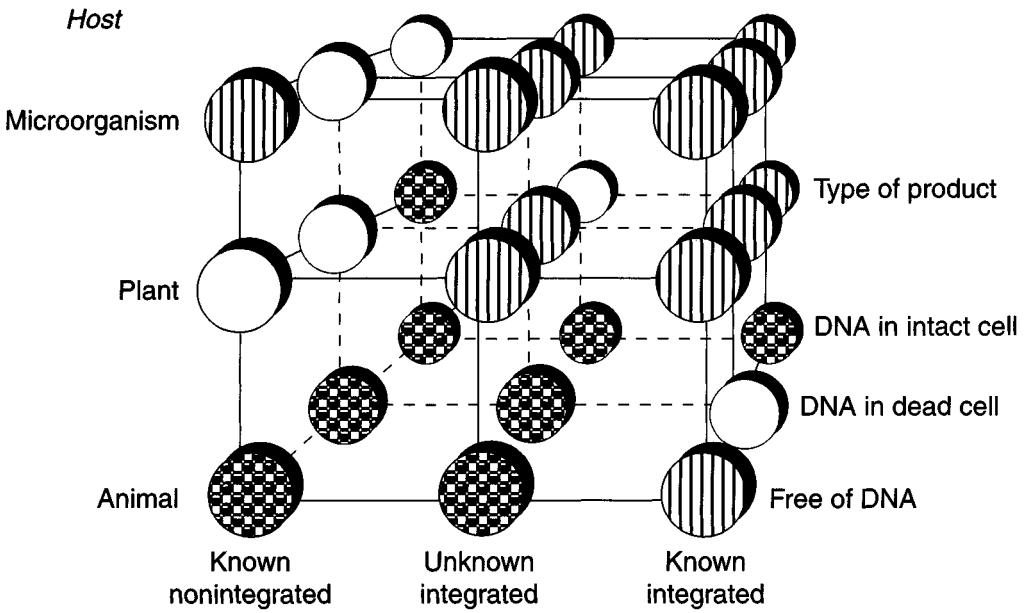
tation processes using genetically modified microorganisms are provided in Figure 10–4. However, Tables 10–1 and 10–3 illustrate that genetically modified plants will become a major source of plant raw material for fermented foods in the near future, and therefore a separate supply chain risk/benefits analysis for fermented products derived from genetically modified plants is provided in Figure 10–5.

It is impossible to deal in this chapter with all of the products in Table 10–3 for both traditional and genetically modified raw materials and/or for traditional and genetically modified microorganisms used in the fermentations. Therefore, only two examples are worked out in more detail.

1. *soy sauce*—a fermented product using genetically modified wheat and/or soybeans, traditional or genetically modified microorganisms, and traditional or recombinant DNA enzymes
2. *cheese*—a fermented product using normal cow's milk, genetically modified functional microorganisms, and/or enzymes made by genetically modified microorganisms

These two examples cover quite well the whole spectrum of fermented food products in which recombinant technology is used at some step in the overall process.

As stated in the introduction to this chapter, fermented foods derived from genetically modified plant material or produced by genetically modified microorganisms will only be accepted if there are clear direct (e.g., significantly better quality or shelf life, more healthy or more convenient) or indirect (e.g., availability, environment) benefits for



Note: X-axis-physical state of foreign gene(s): "known" means that the location of foreign gene(s) is/are known exactly, "integrated" means stable integration of foreign gene(s) on the chromosome of the host; Y-axis-host organism; Z-axis-whether the consumer product is free of DNA (e.g., cheese made with chymosin) or product contains inactivated cells that produced the rDNA product (i.e., tomato paste) or the rDNA producing cells are "alive" present in the product (i.e., fresh tomatoes). Vertical spheres-no risk; white spheres-risk assessment inconclusive; dotted spheres-either risk assessment inconclusive or ethical objections against such consumer products.

Note: This scheme is based on the assumption that the used constructs do not contain an antibiotic resistance marker. Otherwise, the risk to the environment will increase for all cases except contained fermentation of microorganisms.[29]

Figure 10–3 Summary of risk assessment of rDNA products on basis of three parameters.

the consumer. Some potential benefits will be discussed in the following sections.

Some Consumer Benefits of Genetically Modified Plants

Benefits are relative, and for each group of consumers, benefits will be perceived differently. Consumer studies in Western Europe show that the order of consumer criteria to buy products is quality, health aspects, convenience, environment, and price. It is likely that price together with availability will be on top of such a list in developing countries. In the Western world, there is a surplus of food raw materials and improvement of crop yield is not perceived as a benefit by the consumer; neither is the loss of material during transport seen as a problem. However, the maintenance of quality during transport and at home is considered as a benefit by consumers, as was demonstrated by the initial successful introduction of the Flav Sav tomato (with an antisense polygalacturonase gene that ensured delay of softening of the tomato) by Calgene on the United States market in the mid-1990s.

In many developing countries, however, there is at present a shortage of food raw materials. Therefore, an increase in the yield of crops by genetic modification is seen as a major advantage. There is evidence that shows that in the

Table 10–3 Overview of Most Important Fermented Food Products Derived from Plants of which Genetically Modified Varieties Are on the Market and the Main Functional Microorganisms

Raw Materials	*Functional Microorganisms*	*Product*
Cassava	Bacteria	Chickwangne (Congo)
	Corynebacterium, Geotrichum	Gari (Nigeria)
/ sorghum	Lactic acid bacteria, *Saccharomyces, Candida*	Burukutu (Nigeria)
Maize	*Aspergillus*, lactic acid bacteria, yeast	Chicha (Peru)
	Yeasts, bacteria	Jamin-bang (Brazil)
	Lactic acid bacteria, yeasts, molds	Ogi (Nigeria)
/ cassava	Lactic acid bacteria, yeasts	Banku (Ghana)
Rice	*Monascus*	Ang-kak (China)
	Rhizopus	Lao-chao (China)
	Lactic acid bacteria *Saccharomyces*	Puto (Philippines)
	Aspergillus, Bacillus	Sierra rice (Ecuador)
	Hansenula, Candida, Geotrichum	Torani (India)
/ Black gram	Lactic acid bacteria *Torulopsis*	Idli (India)
/ Carrots	*Hansenula*	Kanji (India)
/ Soybeans	*Aspergillus*, Lactic acid bacteria, yeasts	Miso (China, Japan)
or cassava	Yeasts, molds	Tapé (Indonesia)
Soybean	*Mucor, Aspergillus*	Chee-fan (China)
	Aspergillus, Rhizopus	Meju (Korea)
	Actinomucor	Meitauza (China)
	Bacillus natto	Natto (Japan)
	Actinomucor, Mucor	Sufu (China)
	Rhizopus	Tempeh (Indonesia)
/ rice	*Aspergillus*, yeasts Lactic acid bacteria	Miso (China, Japan)
/ wheat	*Aspergillus*, Lactic acid bacteria	Hamanatto (Japan)
/ wheat	*Aspergillus*, yeasts Lactic acid bacteria	Soy sauce (southeast Asia)
/ wheat	*Aspergillus*	Tao-si (Philippines)
Wheat	*Saccharomyces*	Jalebies (India, Pakistan)
	Molds	Minhin (China)
/milk	Lactic acid bacteria	Kishk (Egypt)

next 10 years, the increase in the supply of agricultural products will be less than the expected increase in world population and food shortage, especially in developing countries, will increase[16] (Figure 10–6). It has also been suggested that only when modern biotechnology results in a second green revolution may this global problem be prevented.[17]

Also, the prevention of the substantial losses during transportation of plant raw materials (up

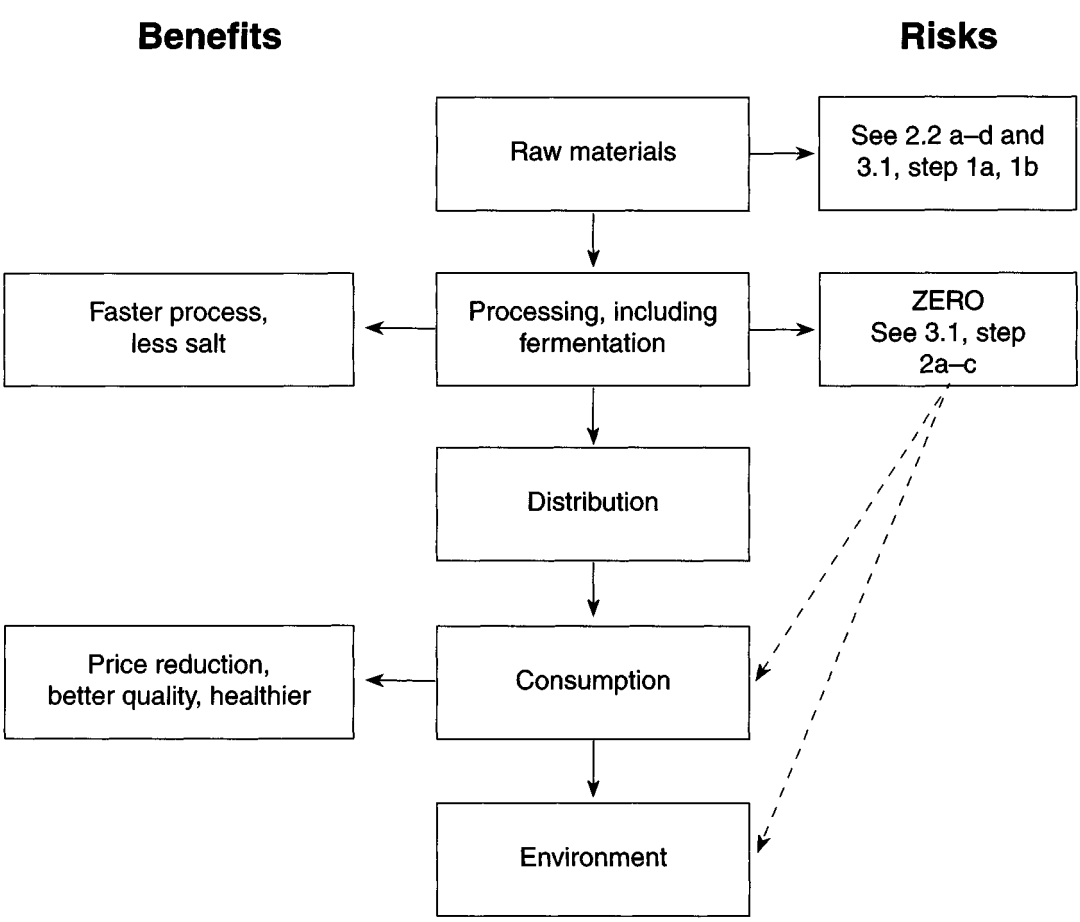


Figure 10–4 Schematic benefit versus risk ratio of rDNA products made by microorganism.

to 30% of harvested plant materials is destroyed due to microorganisms, insects, or uncontrolled endogenous processes) is seen as a major benefit for consumers in that part of the world.

There is evidence that modern biotechnology can significantly contribute to increasing yields or reducing losses of plants and plant products in the first phases of the supply chain. Monsanto's Roundup herbicide-resistant crops need less herbicides (approximately 10% less) and show increased yields (5–15%) (Farmers Organization Argentina, personal communication, November 1997).

Plants producing *Bacillus thuringiensis* BT-protein (preferably more than one variety of this protein so as to minimize the probability of adaptation) are quite well protected against insects, thereby increasing the yield. Modern biotechnology can also contribute to the reduction of losses due to microbial spoilage during distribution. Plants have a rich arsenal to defend themselves against invading microorganisms.[5] They can change the structure of plant tissue by extensive cross linking catalyzed by redox enzymes, produce low molecular mass antimicrobial agents, and degrade cell walls of invading microorganisms using hydrolases they produce. In particular, plant pathogenic fungi can be effectively destroyed by these enzymes. Some companies like Novartis and Zeneca are very active

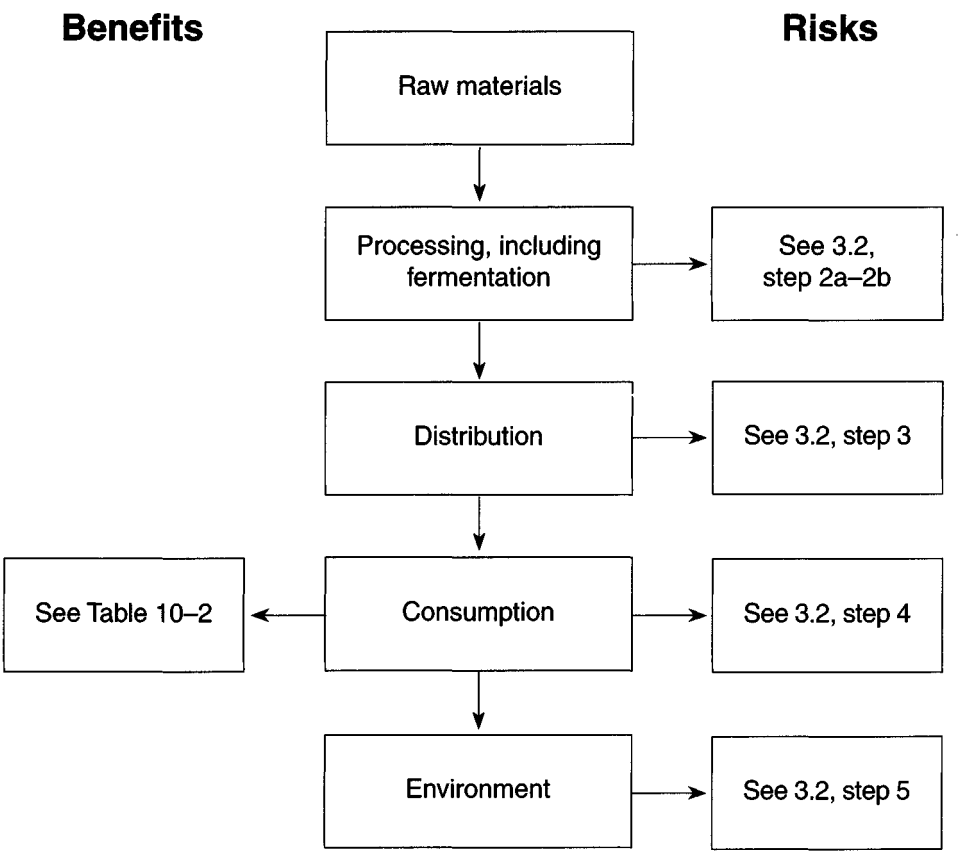


Figure 10–5 Schematic benefit versus risk ratio of rDNA product made by plants.

in this field, as shown by the five and seven patents they filed respectively on this technology between 1980 and 1997. This approach is limited to improving existing plant protection systems. However, recombinant DNA technology can do more. Plant Genetic Systems had a project in the mid-1980s to express potent antimicrobial peptides (e.g., apidaecin)[3] in plants, although with the intention to isolate these antimicrobials from the plant and use them in all type of products. At present, a large number of antimicrobial peptides are known, some of amazing effectivity.[1]

A consumer benefit of currently available genetically modified plants can be a lower price due to higher yield, reduced cost for chemicals, and reduced losses during harvesting and transport. However, an important benefit can be delivered by plants with the right balance of micronutrients that contribute to consumer health. Plants are a well-known and extremely rich source of all kinds of components that may contribute to consumer health, such as antioxidants, organic metal complexes, phytoestrogens, phytosterols, and vitamins. At present, the diet of approximately 2.5 billion people contains insufficient amounts of minerals and vitamins. The consequences of these shortages are dramatic. For example, each year, 2 million young children die and approximately 300,000 children go blind due to a shortage of vitamin A. With the rapid increase in our knowledge of cell and molecular biology, and much better and faster analytical techniques, the number and kind of plant and microbial components with sustain-

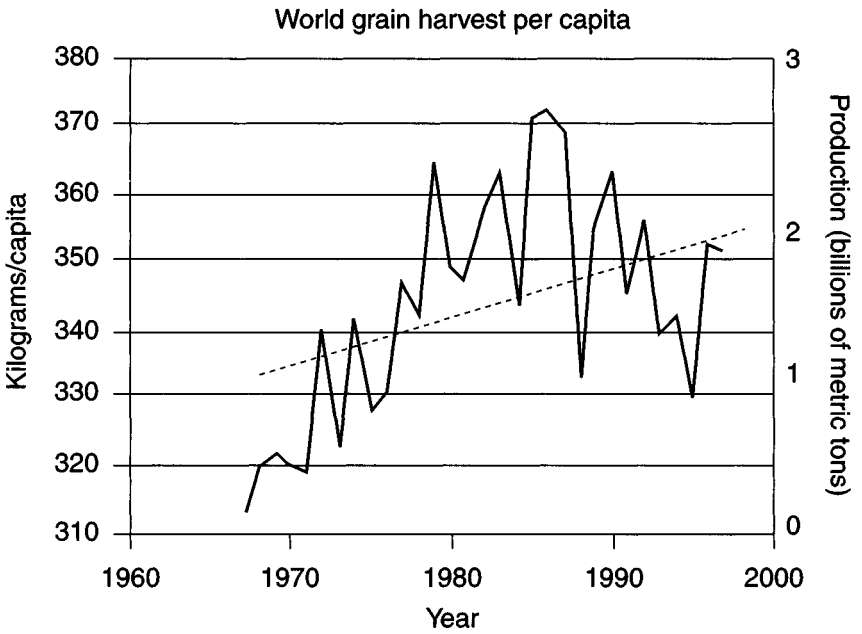


Figure 10–6 World grain harvest during the last 35 years. A steady increase in total production from 1.2 to 2.2 billion of metric tons; a rapid increase in amount per capita from 1966 to 1987, followed by a decrease from 1988 to present.[16] *Source:* Reprinted with permission from FAOSTAT, C.C. Mann, Crop Scientists Seek a New Revolution, *Science,* Vol. 283, pp. 310–314. Copyright 1999 American Association for the Advancement of Science.

able health claims will increase significantly in the near future.

What happens to these components with potential healthy properties during fermentation is not very well known, but it is likely that chemical nature and bioavailability of these components will be influenced (for better or worse) by the various steps in a fermentation process. Some literature is available on the conversion of estrogens and phytoestrogens by the microflora of the gastrointestinal system and show that phytoestrogens can be (extensively) converted into more effective compounds, such as estrone into oestradiol.[21] However, knowledge in this area is too fragmented and limited, and this important aspect has to be researched.

Some Potential Hazards and Risks of Genetically Modified Plants

It is outside the scope of this chapter to go into detail on the potential hazards of genetically modified plants. However, the integral supply chain approach followed requires that some aspects of the potential risk are discussed. At present, there are at least four issues in relation to genetically modified plants.

1. Most of the genetically modified plants contain an antibiotic resistance gene as marker and, although the marker gene as such is not expressed, the potential transfer of this marker gene to other crops in the surrounding area has been studied.[11] Although the frequency of such an event is low, it is measurable; therefore, this approach may contribute to a further spread of antibiotic resistance genes in the environment, thereby contributing to an extremely undesirable further increase of antibiotic resistance of human and animal pathogens.
2. The gene providing the plant with a new desired property, such as herbicide resistance, can also be spread in the environment. This

means that there is a small probability that weeds will pick up this property and become resistant to herbicides. This spread of genes providing herbicide resistance is an issue that has to be addressed.

The spread of genes providing plants with new desired properties related to health benefits of consumers will also occur, but in this case, the gene will most probably not provide the recipient with an ecological benefit; therefore, this risk is so low that it can be neglected.

3. It is still not possible to integrate the new genetic information in a predetermined place in the genome of the plant. The random integration may result in the destruction or enhancement of the expression of genes at the locus of integration and therefore may change the metabolism of the plant. To exclude the introduction of a hazard, one should precisely determine the position of integration and use techniques such as DNA microarrays,[4] proteomics,[13] and gas chromatography coupled to mass spectrometry (GC/MS) analysis of metabolites to determine the effects of this random integration on the metabolism of the plant.
4. It is possible that pieces of plant DNA are taken up by the epithelial cells of the gastrointestinal tract (GIT) of consumers. Hard data on this transfer are scarce, but this is a general phenomenon of digestion and uptake of foods and not just an issue related to genetically modified plant material. However, particularly when antibiotic-resistant markers are used in genetically modified organisms (GMOs), it is necessary to determine the probability of this transfer in order to make a good risk assessment. Of course, it would be much better if antibiotic resistance markers were not present at all.

Some Consumer Benefits of Genetically Modified Microorganisms

As stated in the introduction to this chapter, the popularity of fermented food is based on several aspects, of which the enormous variation in appearance, flavor, and texture is the most important for Western consumers. This enables the consumer to select a product out of this enormous range that is close to personal preference and not an "average" product. When fermented foods are made from plant materials that do not have an optimum composition from a nutritional point of view, such as cassava, fermentation contributes to (partial) supplementation of these components (e.g., amino acids such as methionine, lysine, and tryptophan; vitamins, in particular A and B vitamins; minerals). Fermentation and the accompanying physical processes also contribute to the elimination of undesirable components that are present in plants[2] (see Chapter 4). In general, fermented products also have a better microbiological stability, which is a considerable consumer benefit, especially in developing countries with less well-organized (chilled) distribution chains and often no refrigerator in the homes (see Chapter 2).

On top of the general benefits for the consumer described above, certain microorganisms used in fermentation processes, particularly lactic acid bacteria (LAB), may provide additional health benefits (Exhibit 10–1). Recombinant DNA technology may be able to improve these positive aspects of fermented food products. It is even possible to extend this range of potential health benefits to consumers, for example, by oral immunization against pathogens and/or toxins.[31]

Some Potential Hazards and Risks of Genetically Modified Microorganisms or Their Enzymes Used in the Manufacturing of Fermented Foods

There are several aspects related to the safety of fermented foods. Assuming that extensive research and risk assessment have proved that the newly introduced gene produces an intrinsically safe protein and that this protein does not contribute to the biogenesis of any harmful component, either during fermentation or during digestion of the fermented product, the remaining safety aspects are:

Exhibit 10–1 Potential Consumer Benefits of Fermented Foods

- Improved appearance, flavor, and texture
- Conversion of antinutritional and toxic compounds (e.g., removal of cyanogenic glycosides in cassave)
- Increased microbiological keepability
- Enrichment with amino acids, minerals, and vitamins
- Agonistic action against enteric pathogens*
- Improved lactose utilization (important for developing countries)*
- Conversion of potential precarcinogens into less harmful compounds*
- Stimulation of the mucosal immune system†

*Mainly related to foods fermented with lactic acid bacteria.

†Only a small number of lactic acid bacteria may have this property.

- Can the transfer of newly introduced gene(s) to recipient microorganisms occur in the fermentation process or after consumption of the product in the GIT of consumers?
- Can the transfer of newly introduced gene(s) to recipient cells of the GIT of consumers occur?
- Can the transfer of newly introduced gene(s) to recipient microorganisms in the environment occur?

The risk assessment of the aspects mentioned under the first and third bullet have been described in some detail earlier.[30] As the potential for transfer of genetic information from donor microorganisms to recipient microorganisms is a matter of concern, and new data are available, this will be discussed in some detail using the decision tree provided in Figure 10–7.

Provided that the microorganism used in a fermentation process is intrinsically safe and the recombinant version of this microorganism is substantially equivalent and free of any DNA of the marker free (clean) host organisms, the risk related to the use of these recombinant DNA microorganisms during fermentation processes is zero for both consumer and environment.[30]

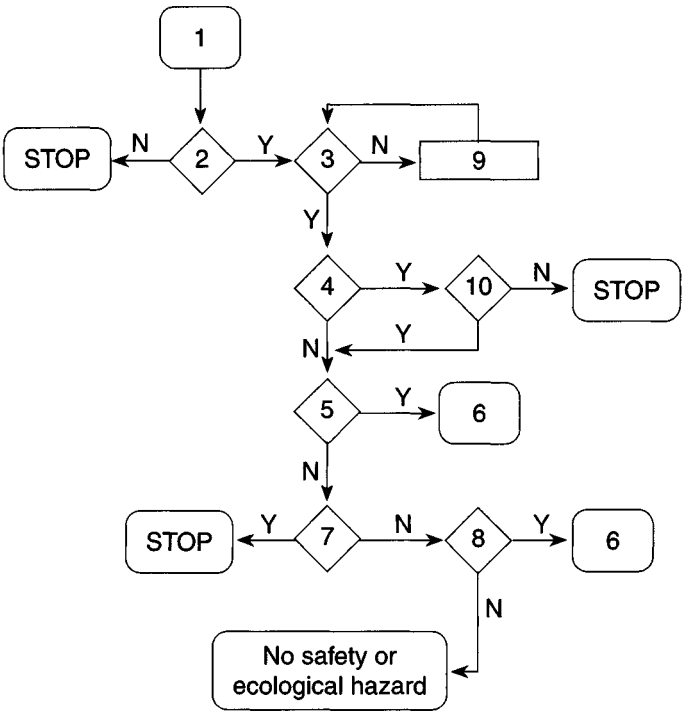
When, during the fermentation process, a chemical or physical treatment is applied that lyses the genetically modified microorganism and destroys the functionalities of the genome, it is not necessary to take into account the aspect of potential transfer of genetic material from these microorganisms to recipient cells in the GIT of consumers or safety aspect (third bullet above).

TWO SETS OF EXAMPLES OF SAFETY EVALUATION OF FERMENTED FOODS FOR WHICH RECOMBINANT DNA IS USED

Supply Chain for Soy Sauce Produced from Genetically Modified Soybeans and/or Wheat and/or Using Genetically Modified Microorganisms and/or Enzymes Produced by Genetically Modified Microorganisms

There are a large number of fermented products that are derived from wheat and/or soybean (see Chapter 1). For the supply chain risk/benefits analysis, the well-known soy sauce was used as an example. A general outline of the benefits and risks of genetically modified soybeans was provided on pages 223 and 227, and for microorganisms and enzymes used in the fermentation process, on pages 228. This outline will now be worked out in detail for soy sauce. A simplified scheme of the soy sauce process is depicted in Figure 10–8.

The safety of soy sauce that is derived from genetically modified soybean will be analyzed as a function of the supply chain. As pointed out on page 227, a genetically modified soybean should not have an antibiotic resistance marker because this will increase the risk and is of no benefit to the consumer. For this example, it is assumed that the genetic modification of the soybean results in a faster fermentation process due to the incorporation of enzymes in the soybean and/or wheat that contribute in the first steps of the process. The faster process is assumed to also contribute to the sensory quality of the soy sauce. Therefore, the



1(E). Generally recognized as safe genetically modified microorganism used in fermentation and still living in the final product
2(Q). Encode the newly introduced gene for an intrinsically safe product?
3(Q). Is the new gene stable integrated in the genome of the host cell?
4(Q). Is an antibiotic resistance marker used during construction of the GMO?
5(Q). Does the newly introduced gene encode for a protein that may result under certain conditions in an ecological advantage for the host cell?
6(A). Carry out full risk assessment.
7(Q). Does the newly introduced gene encode for a protein that after transfer may result under certain conditions in an ecological advantage to recipient mammalian cells?
8(Q). Does the newly introduced gene encode for a protein that after transfer may result under certain conditions in an ecological advantage to recipient microbial cells?
9(A). Integrate new gene, preferably on predetermined locus on the chromosome of the host.
10(Q). Is the antibiotic resistant marker gene eliminated?
(E). Entry or end; Diamonds (Q = questions); Y = yes, N = No

Figure 10–7 General scheme to determine safety to consumers and some environmental consequences of genetically modified microorganisms used in fermentation processes. *Note:* This is a modification of the schemes published in Verrips and Vandenberg.[30]

benefits for the consumer will be better quality and a price reduction.

- **Step 1a (Figure 10–4).** The risk related to the first step of the supply chain (cultivation of the soybeans): Because the newly introduced gene encodes an intrinsically safe protein that does not provide any receiving organism with an ecological advantage, both the environmental and consumer health risks are zero. It should be noted, however, that the environmental hazard is not zero. After studying the frequencies of spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy rape (*B. campestris*) Jorgensen & Anderson[11] concluded that transgenes from oilseed rape may be preserved for many years, and that weedy *B. campestris* with transgenes may present economic (ecological) risks to farmers and plant-breeding companies or biochemical industries. Although their findings of spontaneous hybridization have been confirmed in other studies, unfortunately, they mixed up risk and hazard. Only when the transgene results in a clear ecological advantage for the recipient is there a hazard, and, based on their studies, a realistic probability ($> 10^{-8}$) that the hazard occurs (= risk). Moreover, the transfer of genetic material has happened for millions of years and has not resulted in ecological problems because the transgene did not give the recipient an advantage under a large number of ecological conditions.

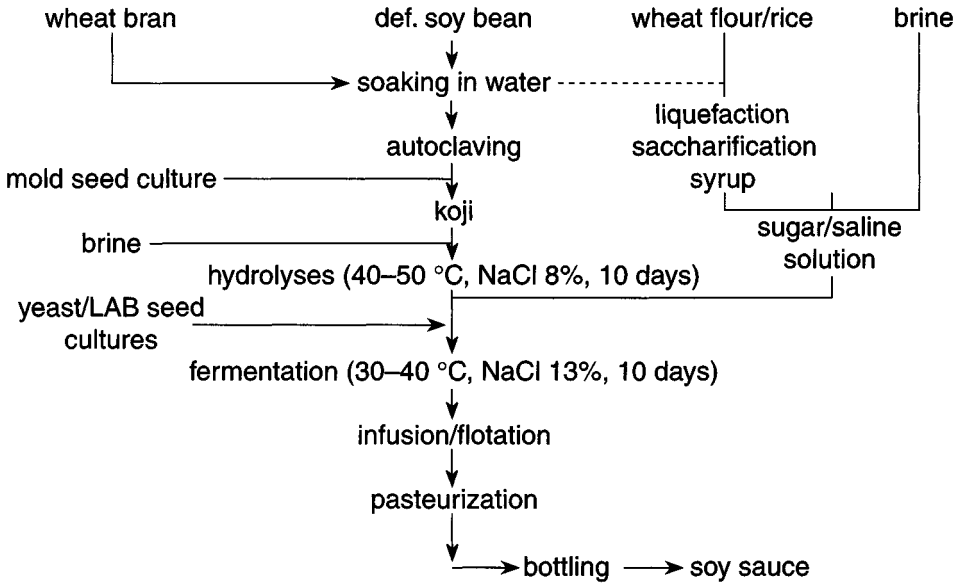


Figure 10–8 Schematic outline of soy sauce process.

It is also important to consider the stability of plant DNA in soil because that DNA can be picked up by microorganisms that are present in the soil. A study showed that "survival" of a neomycin/kanamycin resistance gene from transgenic tobacco was 0.14% after 120 days in soil. Most probably, the persistence is so high because of stabilization of the DNA by soil.[26] Because the stability of DNA in soil is higher than was assumed in the past, the probability that DNA is taken up by soil organisms is also higher. Indeed, various studies have shown that horizontal transfer of plant DNA to microorganisms can occur, but at low frequencies. For instance, based on a number of well-controlled experiments, the transfer of DNA from transgenic potato to the plant pathogenic bacterium *Erwinia chrysanthemi* under natural conditions is calculated to be 10^{-17}, which is much lower than the detection limit of approximately 10^{-12}.[24] Earlier experiments showed that the transfer of hygromycin resistance from various transgenic plants (*B. napus, B. nigra, Datura innoxia, and Vicia narbonensis*) to the fungus *Aspergillus niger* after cocultivation occurred with an unexpectedly high frequency.[10]

- **Step 1b (Figure 10–4).** In cases where the newly introduced gene encodes a protein that can provide an ecological advantage for any recipient organism, the risk is not zero and the risk assessment (Figure 10–7) should be performed before proceeding to step 2.
- **Step 2a (Figure 10–4).** The risk related to the second step of the supply chain (fermentation process): During this process, the soybean is physically denatured; moreover, the chemical composition in certain parts of the process is very hostile for organisms, with the exception of some functional organisms.
- **Step 2b (Figure 10–4).** In the cases that the soy sauce process contains genetically modified microorganisms, it is very likely that the DNA of these microorganisms will also be destroyed during the final steps of the process when the conditions are quite

harsh (but hard evidence is missing). It is again highly recommended to avoid the presence of any nonfunctional foreign genes or genes encoding antibiotic resistance properties in these microorganisms. The hazard of the newly introduced gene product should be analyzed in the same way as described in step 1a for new genes introduced in soybeans and/or wheat.

- **Step 2c (Figure 10–4).** In the case that enzymes produced by genetically modified microorganisms are used in the soy sauce process, the regulations require that the enzyme should be intrinsically safe and free of DNA of the microorganism involved.[29] Practice proves that this requirement can be fulfilled without much difficulty in industry. Even though there is evidence that enzymes can be made free of any DNA, as advocated earlier, it is highly recommended to produce enzymes only with food grade microorganisms that are free from nonfunctional foreign genes, in particular, free from genes encoding antibiotic resistance properties. The enzyme as such should be "substantially equivalent" to the wild type enzyme, another requirement that can be met by industry. Consequently, using recombinant DNA enzymes in the soy sauce process does not pose any hazard and therefore *no risk* to consumer and environment.
- **Steps 3–5 (Figure 10–5).** In all of the subsequent steps of the supply chain, including the step in which consumers introduced the (digested) soy sauce in the environment, the risk related to the use of genetically modified soybean and/or wheat or the use of genetically modified microorganisms or enzymes produced by recombinant DNA technology remains zero.

Supply Chain for Cheeses Produced with Genetically Modified LAB and/or Enzymes Produced by Genetically Modified Microorganisms

As stated in the introduction, fermented products, particularly fermented milk products, have a healthy image. Although a number of studies still have to be carried out to substantiate the potential health benefits of LAB, in particular, the effects of LAB and their products on mucosal health, there are already a large number of publications that support these benefits for consumers.[23] The most important potential health and other benefits are summarized in Exhibit 10–1.

Some of the main aspects of cheese processes are provided in Figure 10–9, and the risk assessment of that process and the other steps in the supply chain will be performed similarly to the assessment for soy sauce.

- **Step 1 (Fig 10–4).** Traditional milk will be used for cheese production; therefore, a risk assessment of GMO-related issues in this step is not necessary.
- **Step 2a (Fig 10–4).** In this step, genetically modified LAB are introduced in the cheese process. As the safety of the end product (cheese) for consumers will be discussed in step 4, only the environmental safety aspects are discussed in this step. It is unrealistic to consider the cheese fermentation process as a completely closed process; therefore, there is a probability that genetically modified LAB will enter the environment. Assuming these LAB do not contain any foreign, nonfunctional genes, and that the newly introduced gene encodes an intrinsically safe protein, the hazard will be zero and consequently the risk will also be zero.

  If, however, the LAB contain a gene-encoding antibiotic resistance or a property providing an ecological advantage to the recipient, the environmental hazard is not zero and a formal risk assessment as discussed in detail in an earlier publication[30] should be performed. More recent data[15,18] support the view that horizontal transfer of genetic material from one microorganism to another, either directly by conjugation or transformation or indirectly via transduction, occurs at a much higher frequency than was assumed in the mid-1980s.
- **Step 2b (Figure 10–4).** In this step, enzymes made by recombinant DNA technol-

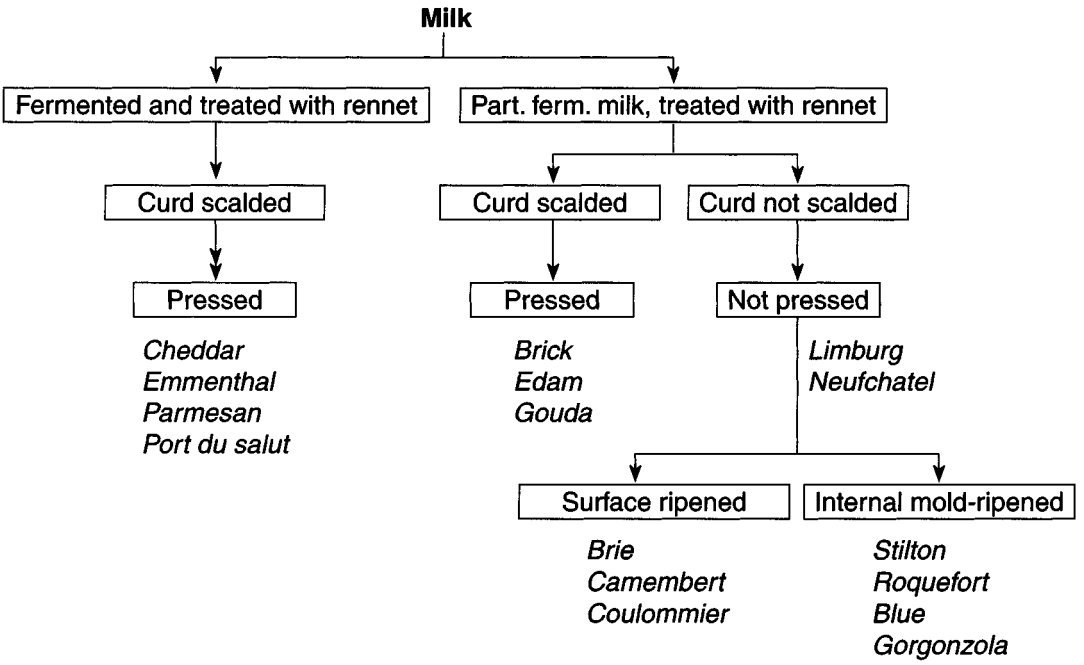
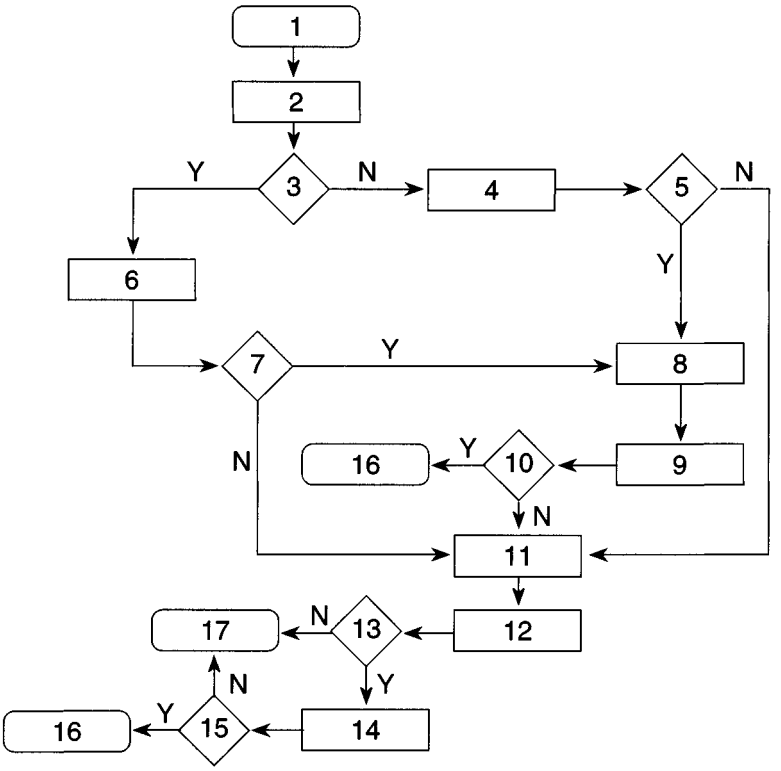


Figure 10–9 Schematic outline of milk fermentation processes and their cheese products.

ogy are introduced.[27] As described in the soy sauce example, an intrinsically safe enzyme produced under proper conditions does not pose a risk to consumer or environment.

- **Step 3 (Figure 10–5).** During distribution, the probability of the escape of LAB into the environment is extremely low, and as horizontal transfer also occurs with low frequencies, this probability is considered to be effectively zero.
- **Step 4 (Figure 10–5).** In this step, the consumer will be in direct contact with the genetically modified LAB and/or the enzyme. For the enzyme, a relatively straightforward procedure has to be followed to prove that the enzyme is intrinsically safe, originates from a well-known source, and is substantially equivalent to the wild type enzyme.[29] If that is the case, the enzyme will not pose a hazard and consequently no risk. For the genetically modified LAB, a formal decision scheme has been developed.[30] Two aspects should be analyzed: the general safety of the fermented food product made with genetically modified LAB and the probability of transfer of genetic information from the LAB to other bacteria in the GIT of consumers (Figure 10–10). In this figure, the following aspects related to a quantitative risk assessment are considered: (1) probability of transfer of genetic material from the GMO to other microorganisms in the GIT as a function of the residence time of the GMO in the GIT, (2) whether the GMO remains alive, (3) whether DNA from lysed GMOs are taken up by normal inhabitants of the GIT, (4) the probability that transfer of genetic information of the GMO to normal inhabitants results in an ecological advantage of the transformed inhabitants and, if so, (5) whether that may pose a health risk to the consumer, or environmental risks. Unfortunately, insufficient data are available to carry out such risk assessment in the proper way.



1(E). Genetically modified lactic acid bacterium (GMO)
2(A). Determine the distribution of the residence time of GMO in the gastrointestinal tract (GIT) of consumers. Take the time corresponding with 95% of this distribution curve as *t(r)*.
3(Q). Will the GMO lyse with *P(b)> b* in the GIT within *t(r)*?
4(A). Determine the probability *P(c)* that intact cells of the GMO transfer genetic information to normal inhabitants of the GIT. In these studies, use *t(r)* as contact time and the conditions of the GIT.
5(Q). Is *P(c)>c*?
6(A). Determine the probability *P(f)* that DNA originating from lysed GMO transform normal inhabitants of the GIT (resulting in transformed inhabitants).
7(Q). Is *P(f)>f*?
8(A). Determine whether the transformed inhabitants obtain an advantage over untransformed inhabitants in the GIT: *A(i)*. Define *A(i)* in either faster growth rates *t'(g)*, better adhesion to epithelial cells *h'*, or higher production of certain metabolites *{p(x)', x=1,....}*.
9(A) Determine the probability *P(e)* that any of the events described under *action 8* will result in the formation of a hazardous (=transformed inhabitant produce toxin or that will replace beneficial microorganisms in the GIT) microorganism.
10(Q). Is *{P(c) + P(f)}*P(e) > e*?
11(A). Determine also the probability *P(d)* that the GMO will be transformed by genetic material originating from the common GIT microorganisms (=modified GMO).
12(A). Determine whether the modified GMO gains an advantage over untransformed GMO: *B(i)*. Define *B(i)* in either faster growth rates *t''(g)*, better adhesion to epithelial cells *h''*, or higher production of certain metabolites *{p(x)'', x=1,....}*.
13(Q). Is *P(d)> d*?
14(A). Determine the probability *P(g)* that any of the events described under *action 12* will result in the formation of a hazardous GMO.
15(Q). Is *P(d) * P(g) > g*?
16(E). This risk is unacceptable and the GMO should not be released.
17(E). This risk is acceptable and the GMO can be released.
A = action; E = entry or end; Q = question

Figure 10–10 A proposal for a structured assessment of the risk related to the introduction of genetically modified lactic acid bacteria in (or as) food products.[29]

In fact, only fermented products of which the safety has been demonstrated, ecological hazards have been proven to be zero, and consequently, the risk is zero, should be approved. However, assuming that the ecological hazard is not zero, which means that the newly introduced gene confers an ecological benefit on recipient cells, both risk assessments should result in an acceptably low risk. This can only be achieved if the probability of transfer of genetic information from the donor to recipient cells is very low. For horizontal transfer of genetic material between microorganisms, conjugation, transformation, and transduction show a higher probability for plasmid DNA than for chromosomal DNA. Therefore, it is recommended that the foreign DNA be integrated (preferably on a preknown locus) into the chromosome of the microorganism. Integration at preknown loci therefore forms an important component of the risk cube presented in Figure 10–3.

A parameter that is essential for proper risk assessment of events in the human GIT is the residence time of genetically modified microorganisms in the GIT, whether they lyse, whether they conjugate, or whether the DNA liberated during lysis can transform other cells, including human cells. Although the number of reliable data sets have increased during the last decade, they are still not sufficient to solve all of the questions of the decision scheme given in Figure 10–10. Nevertheless, the data available indicate that LAB can survive passage through the GIT.[19] The functional microorganism in many cheese fermentations, *Lactococcus lactis,* survives up to three days, although the survival rate is only 1–2%. Another noteworthy observation in this study was that the PCR method could detect special DNA stretches of this bacterium for up to four days after ingestion,[12] indicating that either the feces contained nonviable *L. lactis* or that these stretches of DNA were liberated during lysis of the bacterium. Experiments with phage M13 DNA ingested by mice showed that a small but measurable percentage of this DNA reached peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and could be covalently linked to mouse DNA.[25] Even taking into account that these experiments are rather artificial, for a proper risk assessment, they have to be included.

From these studies, it can be concluded that there is a probability that genetically modified microorganisms, including LAB, can transfer genetic information to other microorganisms in the GIT. This information is another strong argument to ban genetically modified microorganisms that contain an antibiotic resistance gene. In cases where a nongenetically modified microorganism has such a gene, it should be deleted because further unnecessary spread of antibiotic-resistant genes creates very serious health and environmental problems.

- **Step 5 (Figure 10–5).** The release of feces of consumers into the environment is the final step to be assessed. If the newly introduced gene does not encode for a protein that provides an ecological advantage for the recipient cells, then a risk assessment of this step is not necessary. If this is not the case, then the decision scheme outlined in an earlier publication[30] can be applied for this step. However, this step may become more complex because it should take into account whether the transfer of genetic information from the LAB to recipient microorganisms has occurred in the GIT of consumers and, if so, these modified recipients should be evaluated as well.

In this example, LAB are the functional microorganisms in the fermented product, but for other organisms that are generally recognized as safe (GRAS), such as *Saccharomyces cerevisiae, Kluyveromyces lactis, Penicillium roqueforti, P. camenberti,* and *A. niger,* or *A. awamori,* the same hazard and risk assessment can be applied with similar results.

CONCLUSION

Fermented foods are considered by consumers as natural and healthy.[7,8,14,22] The introduction of genetically modified plants, microorganisms, or enzymes into these products needs careful discussion with authorities and consumer organizations. Many fermented foods contain living microorganisms, and if these organisms are genetically modified, one should consider them as a potential source of DNA for horizontal gene transfer because a number of studies provide circumstantial evidence that, in rare cases, genes have been laterally transmitted among *Eubacteria, Archaea,* and *Eukarya.* However, extensive studies by Puhler's[6] group showed that this transfer occurs at extremely low frequency.

In these discussions, the proven benefits to the consumer should be explained in clear terms. The risk assessment should show that the safety hazard related to the newly introduced gene(s) is zero, and consequently, the risk for consumer is zero. Whenever possible, without losing the consumer benefit, the environmental hazard should also be zero. If that is not possible, the risk assessment should show that the risk is extremely low, preferably less than 10^{-8} per product unit produced. Consumer studies show that provided there is a clear benefit and risk is absent, most consumers will accept these products. To gain the confidence of the consumer for fermented foods in which recombinant DNA technology plays a role, clear and transparent labeling and public relations will be required.

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