CHAPTER 7

Microbiological Hazards and Their Control: Bacteria

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INTRODUCTION

The inactivation or inhibition of undesirable microorganisms is an essential part of food preservation. Foods can be made safe by treatments such as pasteurization or sterilization (e.g., milk, cooked meat products, and canned foods), or they may possess intrinsic properties that contribute to safety, such as structure, low pH, and/or low water activity (A_w) (e.g., fruit, pickles, and honey). One further way of improving shelf life and safety, through the use of a competitive microbial flora, is widely employed in the production of fermented milks, vegetables, meat, fish, and grains, giving products with changed composition and taste as well as a prolonged shelf life. It is estimated that approximately 30% of our food supply is based on fermented products,[40] though not all fermented foods are universally accepted. The modified sensory properties in some fermentation products are acceptable only in certain regions; elsewhere, they would be considered spoiled or unpalatable.

Fermented foods have generally been considered as less likely to be vehicles for food-borne infection or intoxication than fresh foods due to the competitive activity and metabolites of the functional flora[49] (see Chapter 2). All raw materials used in fermentation processes, such as fruits, vegetables, meat, fish, milk, and eggs, can harbor pathogenic bacteria.[60] Depending on the type of fermentation (e.g., lactic acid, alcoholic, or mold), these pathogens may be (partly) eliminated or their growth inhibited by the antibacterial substances that are produced (e.g., lactic acid, acetic acid, alcohol). To ensure a satisfactory fermentation and to control the growth of food-borne pathogens, the use of specific starters is often recommended.

In this chapter, the safety of fermented products will be discussed in relation to the pathogenic bacteria: *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella*, *Staphylococcus aureus*, *Vibrio* spp., and *Yersinia enterocolitica*.

OUTBREAKS RELATED TO FERMENTED PRODUCTS

A great part of food-borne disease is preventable. In addition to good manufacturing practices (GMPs) and the Hazard Analysis Critical Control Point (HACCP) system, domestic hygienic practices have a key role to play in preventing infection.[9,12,52] Most food-borne diseases are attributed to the consumption of, or cross-contamination from, raw or undercooked food products (e.g., vegetables, sprouts, shellfish, and poultry), in many cases with an extended period between preparation and consumption.[14]

The antibacterial factors that are present in fermented foods may affect both the growth and the survival of bacterial pathogens that are present in a raw material. In most fermented foods, the inhibition of growth is more common and can often ensure safety where levels of contamination are low. But with infectious patho-

gens, particularly those with a small minimum infectious dose, some degree of inactivation may be necessary to provide an acceptable level of safety. So, although fermented foods are generally considered to be safe, process failures and contaminated raw materials have resulted in their being involved in food-borne illness. For example, several outbreaks of illness have been attributed to the consumption of fermented sausages contaminated with *S. aureus* and *Salmonella* spp.,[70] and emerging pathogens, such as *L. monocytogenes* and *E. coli* O157:H7, have been identified as causative organisms in outbreaks involving fermented products such as sausages, cheeses, and yogurt.[8,27,47]

These outbreaks have raised questions regarding the safety of fermented food products and have prompted studies using artificially contaminated raw materials to identify critical control points (e.g., fermentation pH, final heating temperature, heating time) and critical limits (e.g., pH 4.6, 55 °C for 20 minutes) that can be integrated into an HACCP plan to ensure safe products.[23]

BACTERIAL PATHOGENS OF CONCERN IN FERMENTED PRODUCTS

There is extensive literature describing the limits for the growth of bacterial pathogens. Some of the data relevant to fermentation processes are summarized in Table 7–1,[61] and a few key features of these organisms are presented in the following paragraphs.

Table 7–1 Limits for Growth of Some Common Bacterial Pathogens

Organism	*Minimum pH*	*Minimum Temperature*	*Minimum A_w (Max % NaCl)*
Aeromonas hydrophila	< 4.5	> 0.0, < 4.0	– (5–6%)
Bacillus cereus	5.0	4.0	0.93
Campylobacter	4.9	30.0	> 0.987 (1.5%)
Clostridium botulinum			
Group 1: Proteolytic	4.6	10.0–12.0	–
Group 2: Nonproteolytic	5.0	3.3	(10%) (5%)
Escherichia coli	4.4	7.0–8.0	0.95
Listeria monocytogenes	4.4	–0.4	0.92
Salmonella	3.8	5.2*	0.94
Shigella	4.9–5.0	6.1–7.9	– (3.78–5.18%)
Staphylococcus aureus			
Growth	4.0	7.0	0.83
Toxin production	4.5	10.0	0.87
Vibrio cholerae	5.0	10.0	0.97 (4.0%)
Vibrio parahemolyticus	4.8	5.0	0.94 (10%)
Yersinia enterocolitica	4.2	–1.3	– (> 5%,< 7%)

*Most serotypes fail to grow below 7 °C.

Aeromonas

Aeromonas are facultatively anaerobic, Gram-negative rods (belonging to the *Vibrionaceae*) that are found in water (including sewage) and in food products that have been in contact with contaminated water (e.g., seafood, vegetables). Some species are well known as fish pathogens; others are human pathogens. *A. hydrophila* seems the most important species; however, its significance in the epidemiology of human illness is still unclear. Symptoms associated with infection include diarrhea, abdominal pain, and headache, and are more severe in children.[58,61]

Aeromonas do not appear to be a serious risk in well-produced fermented foods. The addition of *Aeromonas* to skim milk during lactic fermentation,[57] to yogurt,[6] and to two traditional fermented foods (mahewu and sour porridge) resulted in a sharp decrease in numbers.[65] However, microbiological examination of homemade fermented sorghum porridge[41] and industrially produced fermented sausages (longaniza and chorizo) showed its presence in numbers ranging from 1.0 $\log_{10}$ cfu/g to 4.5 $\log_{10}$ cfu/g. It was concluded that the hygienic status of factories significantly affected the incidence and counts of *Aeromonas*.[24]

Campylobacter

Campylobacter species are micro-aerophilic, Gram-negative curved or spiral rods belonging to the *Campylobacteriaceae*. Members of this genus have been associated with disease in animals for many years. From 1970, the thermophilic campylobacters (growth from 30–45 °C) have been recognized as important food-borne pathogens; they are now the most common cause of gastroenteritis in many developed countries. *C. jejuni, C. coli,* and *C. lari* are responsible for more than 90% of the human illness caused by campylobacters, which are normally characterized by profuse diarrhea and abdominal pain three to five days after consumption. Diarrhea can often be bloody, and more serious sequelae such as reactive arthritis and Guillain-Barré syndrome have also been reported.[1]

Enormous numbers of campylobacters may be found in the gut of chickens (and other birds) and pigs. *C. lari* is often found in seagulls and, as a consequence, in shellfish.[34,61] Raw milk and poorly cooked poultry products are most frequently involved in outbreaks, and cross-contamination from raw poultry to ready-to-eat products may also be an important factor in transmission. Although the infective dose of *Campylobacter* is relatively low (approximately 500 cfu), *Campylobacter* transmission by fermented foods is probably not significant. They do not grow below 30 °C and are sensitive to freezing, drying, low pH, and sodium chloride, and so would not survive well in fermented foods. This has been confirmed by artificial contamination of fermenting products such as salami, weaning foods, and yogurt, which resulted in a rapid decrease in numbers.[39,44,48]

Vibrio spp.

Vibrios are facultatively anaerobic, Gram-negative bacteria belonging to the family of the *Vibrionaceae*, and are commonly found in aquatic environments.

V. cholerae includes the true cholera-producing bacteria ("classic" and "El Tor" biovars) and the so-called "non O1 strains," which lack the somatic "O1" antigen and produce a less severe gastroenteritis. Cholera has been known since ancient times and is still a disease of worldwide significance. The organism is commonly isolated from river waters and coastal marine waters and, as a consequence, from shellfish and other marine animals,[61,71] but its transmission by fermented fish products has not been reported. The use of contaminated water for washing or irrigation may introduce the pathogen onto vegetables. Refrigerated or frozen storage of contaminated food products will not ensure safety because of the good survival of vibrios at low temperatures.[19]

V. parahaemolyticus is a halotolerant pathogen that is commonly found in marine coastal waters in warm water regions. Fish and shellfish are usually associated with outbreaks caused by this pathogen, as are, to a lesser extent, salt-preserved vegetables.[18,61]

V. vulnificus is very similar to *V. parahaemolyticus* but differs from the other pathogenic, halotolerant vibrios in that diarrhea is not a common symptom of infection. *V. vulnificus* is highly invasive, causing septicemia. Illness is clearly associated with liver diseases such as cirrhosis and hepatitis. The majority of cases of illness have been reported after the consumption of raw shellfish, in particular, raw oysters.[38,61]

Vibrios are not acid tolerant and lactic acid bacteria (LAB) isolated from fermented fish products inhibited both *V. cholerae* and *V. parahaemolyticus,* indicating that the antibacterial activity of the functional flora is important in ensuring the safety of fermented fish products.[56] This has also been observed in pickled foods and squid shiokara that were artificially contaminated with *V. parahaemolyticus*.[72,73]

E. coli O157:H7, *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica*

All members of the Enterobacteriaceae family are facultatively anaerobic, oxidase-negative, Gram-negative rods that are capable of fermenting glucose. Some are known pathogens, such as *E. coli* (especially *E. coli* O157), *Salmonella, Shigella,* and *Yersinia,* and a great part of the family, including the food-borne pathogens, are components of the fecal flora of animals. As a result, the whole family or parts of it (coliforms and *E. coli*) are used as hygiene indicators for processed foods.

E. coli O157:H7

On the basis of virulence factors and disease patterns, human pathogenic *E. coli* strains are classified in the following principal groups:

- Enteropathogenic *E. coli* (EPEC), acute watery diarrhea; young children particularly susceptible
- Enterotoxigenic *E. coli* (ETEC), acute watery diarrhea (often in travelers)
- Entero-invasive *E. coli* (EIEC), dysentery-like syndrome
- Enterohemorrhagic *E. coli* (EHEC), bloody diarrhea syndrome

Within the EHEC-group, *E. coli* O157:H7 is the organism that is isolated most frequently in cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Because this pathogen is relatively acid resistant, it is less affected by fermentation and can be of concern in fermented foods such as yogurt and fermented sausages.[22,25,43,61] Several food-borne outbreaks of *E. coli* have been reported in recent years, including some involving fermented meats and cheeses.[17,63,64,67,69]

Salmonella

Salmonella is often found on raw meat (poultry) and is widely distributed in the environment and universally recognized as an important cause of food-borne infections. Salmonellas are present in the gut of contaminated animals (and humans) and are shed in the feces. As a result, a great variety of raw materials can be contaminated with this pathogen. Illness is characterized by diarrhea, abdominal pain, and fever generally 6–48 hours after consumption of the organism.[1] Meat, milk, poultry, and eggs are the principle vehicles for *Salmonella* transmission, and fermented foods derived from these materials, such as salami and cheeses, have occasionally been associated with outbreaks of illness. Recently, acid-resistant strains of *Salmonella* have been implicated in food-borne outbreaks,[42,61] which led to many studies on the fate of salmonellas inoculated in fermenting products such as finger millet,[4] Siljo,[21] and fermented sausages.[23,33,47,62]

Shigella

Shigella is closely related to *E. coli* (DNA homology and biochemical pattern). Shigellas are not natural inhabitants of the environment but originate from humans and higher primates in the acute phase of illness. Both person-to-person infections (due to poor personal hygiene) and the consumption of contaminated water or foods washed with contaminated water give rise to distinct symptoms of the disease, such as bloody

diarrhea. In some countries, shigellas are epidemic.[61] Artificial contamination of products with low pH values (< 5.0) results in a rapid decrease in numbers of shigellas, suggesting that most acid-fermented foods will not be significant as vehicles for this type of infection.[41,53]

Y. enterocolitica

Y. enterocolitica is a widely distributed psychrotrophic bacterium. Certain serotypes (O:3, O:5, 27; O:8, and O:9) present in raw milk, seafood, and raw pork have been associated with food-borne infections. The tonsil area of pigs is a unique ecological niche with a high incidence of *Y. enterocolitica*. Gastroenteritis is the most common symptom; however, sometimes illness is also characterized by fever and abdominal pain (resembling appendicitis). In some cases, this has led to surgery.[61] There are relatively few outbreaks reported with this pathogen; however, in many of these, milk was the incriminated product. The behavior of *Yersinia* has been studied in fermenting milk[57] and yogurt.[11] During the first few hours of the fermentation, growth was observed, followed by a reduction below the detection level after fermentation and storage for four days.

B. cereus

B. cereus is an aerobic, Gram-positive spore-forming rod (belonging to the *Bacillaceae*) that can cause both food-borne infection (characterized by diarrhea) and intoxication (vomiting after ingestion of the toxin cereulide). As a spore former, this bacterium is ubiquitous and may be found in cereals and their products, such as rice and flour, and spices. Small numbers in foods are not considered significant.[61] The behavior of *B. cereus* was studied in fermenting products such as *mageu*, a sour maize beverage;[15] *kinema*, a fermented soya bean food;[50] tempeh;[51] salads;[13] and fish sausage.[5] In all cases, initial growth was observed, but in products where pH decreased due to the growth of LAB, subsequent inhibition occurred that was correlated to the rate of decrease in pH. In products without a lactic acid fermentation (e.g., tempeh), numbers reached 10^8 cfu/g. However, if the soya beans were soaked, resulting in acidification of the beans to pH less than 4.5, the growth of *B. cereus* was prevented.[51] Interestingly, food-borne illness has also been associated with other *Bacillus* species such as *B. subtilis*, *B. licheniformis,* and *B. pumilis,* and strains of many of these are associated with the production of fermented vegetable protein products such as Japanese *natto*, Nigerian *iru* (*dawadawa*), Indian *kinema,* and Thai *thua-nao,* but, as far as we are aware, there have not been any recorded outbreaks of illness associated with them.

C. botulinum

C. botulinum is an anaerobic, Gram-positive, spore-forming rod (belonging to the *Bacillaceae*). Food-borne botulism is a neuroparalytic syndrome caused by the botulinum neurotoxin that is produced by vegetative cells of *C. botulinum* growing in food. This bacterium is ubiquitous, and spores are widely distributed in the soil and on the shores and bottom deposits of lakes and coastal waters, and in the intestinal tract of fish and animals.[61] The human pathogens are classified into proteolytic and nonproteolytic strains; the former being slightly more acid tolerant and mesophilic, whereas the latter can grow down to 3.3 °C. Most outbreaks of food-borne botulism have been caused by home-processed vegetables, fish, or meat products.[59] Although *C. botulinum* spores are present in most raw agricultural products, the number of outbreaks is relatively small, usually occurring after inadequate processing. Nitrite inhibits *C. botulinum* and is an important safety factor in the production of cured and fermented meats. In Japan, botulism caused by nonproteolytic strains producing type E toxin is often associated with the consumption of *izushi*, which is made by the natural fermentation of raw fish and cooked rice.[32] The largest recorded outbreak of food-borne botulism in the United Kingdom was associated with the consumption of contaminated hazelnut yogurt, but in this case, production of

the toxin had occurred in the hazelnut puree that was added to the yogurt rather than in the yogurt itself (see also Chapter 5).[55]

S. aureus

S. aureus is a facultatively anaerobic, Gram-positive coccus belonging to the *Micrococcaceae*. It can produce several heat-stable enterotoxins that, after ingestion of the food, may cause food poisoning with symptoms of nausea and vomiting. The production of staphylococcal enterotoxins occurs mostly in food products that are rich in proteins with a low number of competitive microorganisms (e.g., whipped cream, cooked meat products, smoked fish). *S. aureus* is present on the skin and mucous membranes of warm-blooded animals (including humans). Approximately 50% of the human population are carriers of this pathogen. Contamination of cooked or ready-to-eat food products by a colonized person followed by storage for several hours at ambient temperatures is often implicated in outbreaks of illness.[61] The organism is relatively salt tolerant and will produce toxin down to pH values of 4.5. Fermented sausages[7] and, to a lesser extent, raw milk cheeses[35,36] have been associated with outbreaks of illness, although the organism is generally regarded as a poor competitor and its growth in fermented foods is generally associated with a failure of the normal flora.[54]

L. monocytogenes

L. monocytogenes is a facultatively anaerobic, Gram-positive rod. Species of *Listeria* are ubiquitous and potential food contaminants, but only *L. monocytogenes* is a human and animal pathogen. Approximately 30% of cases of listeriosis are perinatal, and in 20–25% of the cases, infection proves fatal for the fetus or newborn. Most other cases of listeriosis occur in immunocompromised persons, with a death rate varying from 30% to 50%.[61]

Due to its widespread occurrence in the environment, this pathogen can be harbored in low numbers by practically all raw food products, and processed foods can be contaminated from the production environment. *L. monocytogenes* is able to grow at refrigeration temperatures, so a considerable increase in numbers is possible in contaminated products with a long shelf life (e.g., cooked meat products, smoked salmon).

Fermented products such as sausages and soft mold-ripened cheeses have been associated with listeriosis.[26,30] The organism is not particularly acid tolerant, but where mold ripening occurs in a fermented product, the rise in pH can allow surviving *Listeria* to resume growth, causing problems to occur.

PRESENCE OF PATHOGENS IN RAW MATERIALS

Bacteria can enter raw materials or food products at various stages in the food chain. Primary production is a major source of both spoilage and pathogenic organisms. The primary contamination of raw materials includes microorganisms from soil, feces, surface water, and so forth, and will also be influenced by conditions of harvesting, slaughter, storage, and transport. During food production, the extent of secondary contamination will be influenced by factory layout, hygienic design of the equipment, personal hygiene, water, air, (pest) animals, and packaging material.[10]

Many of the bacteria introduced into food are of little concern. They are not well adapted to survival or growth in the product or are easily eliminated in further processing steps. However, a certain proportion of bacteria may cause problems and are a good reason to try to keep the microbial load of raw materials as low as possible, particularly for those products that reach the consumer in a raw state (e.g., meat, poultry, seafood, eggs, and vegetables). Pathogen-free raising of livestock would reduce risk, but this is difficult and expensive to achieve. There is clearly an urgent need for efficient and acceptable methods to decrease the numbers of spoilage microorganisms and pathogens from such products. Ionizing radiation is very effective, but consumer acceptance of this procedure is still a

problem. Decontamination by a lactic acid dip or spray decreases the numbers of some pathogens on carcasses, although acid sprays appear to produce little reduction in *E. coli* (including *E. coli* O157) and *Salmonella*.[20,66] Food legislators in many countries also have objections to this method on the grounds that it may mask poor hygiene or lead to the adaptation of microorganisms to low pH.

Table 7–2 presents the relationship between the pathogens discussed in this chapter and raw materials used in fermentation processes.

It has been estimated that approximately 30% of the human food supply is based on fermented products. Raw materials used are vegetables (e.g., cabbage, cucumber, olives), cereals (e.g., rice, maize, wheat), legumes (e.g., soybean), fruit (e.g., grapes), meat (particularly pork), and milk.[16] From Table 7–2, it is clear that these raw materials may be contaminated with usually low numbers of some of the pathogens listed.

Inactivation, survival, and/or growth during and after fermentation depends on intrinsic factors (e.g., pH and A_w), extrinsic factors (e.g., storage temperature and modified air packaging), implicit factors (e.g., antagonism and the production of antibacterial substances), and process factors (e.g., heat treatment), as well as the physiological characteristics of the organisms themselves.

EFFECT OF FERMENTATION PROCESSES ON THE SURVIVAL OF BACTERIAL PATHOGENS

The effect of fermentation processes on the survival of bacterial pathogens is discussed more extensively in Chapter 2. Before the main fermentation process starts, nonmicrobial process factors such as peeling, washing, soaking, and/or grinding may reduce the numbers of pathogens present in food. Removal of the outer layer (peeling) has the greatest effect, whereas washing of the raw materials usually reduces total counts by 1 log cycle. Sometimes, a higher reduction can be obtained by adding (organic) acids or disinfectants; however, this procedure is not allowed in all countries. Moreover, a major disadvantage to adding acids or disinfectants is the possibility of selecting strains adapted to low pH or to disinfectants if concentrations are too low to be detrimental.[37,66,68] In particular, the acid-adapted pathogens have shown better survival in fermented products.[28,42]

Processes such as mixing and grinding have no lethal effect on bacteria. The only result of these steps is a better distribution of cells in the product and the separation of clumps of cells.

The main factors controlling the survival and/or growth of food-borne pathogens are (low) pH,

Table 7–2 Raw Materials and the Presence of Some Food-borne Pathogens

Pathogen	*Meat*	*Milk*	*Vegetables & Fruit*	*Fish & Shellfish*
Aeromonas	++	+	+++	++
Bacillus cereus	–	++	++	–
Campylobacter	+++	+	–	–
Clostridium botulinum	++	+	++	++
Escherichia coli O157:H7	++	++	+	–
Listeria monocytogenes	+	+	+	+
Salmonella	++	+	+	+
Shigella	–	–	+	+
Staphylococcus aureus	++	++	–	–
Vibrio	–	–	+	+++
Yersinia	+	+	–	–

+ = sometimes present (25%), ++ = likely to be present (25–50%), +++ = usually present (> 50%), and – = not likely to be found in these products.

A_w, heat treatment, and cold storage (see Chapter 2). In Table 7–3, the effect of various fermentation processes is shown on pH and A_w values in the final products and the possible production of specific antimicrobial substances.

It is generally accepted that in well-fermented foods, the production of organic acids and a low A_w (due to the addition of salt or drying) may control the growth of pathogens in these products. However, in view of the values of pH and A_w in the end products (Table 7–3), it can be concluded that many food-borne pathogens, especially in products with relatively high final pH values, can survive. They may even grow if the A_w is also high enough. A critical point for the growth of pathogens is the beginning of the fermentation process, when the pH is still high and the competitive effect of the functional flora is low. Some alcoholic fermentations may be a possible exception, where a low substrate pH value may prevent pathogen growth.[2,46]

The abundance of literature on the inhibition of food-borne pathogens by LAB in fermentation processes is confusing. There are variations in the types of products (e.g., a wide variety of sausages, fermented milks, etc.) and considerable differences in protocols for growth and addition of the pathogen, which makes comparison of the results obtained by different researchers very difficult.

During fermentation, antimicrobial products are produced (see Chapter 2). Most of these, including organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins, are produced by LAB. There is considerable evidence on the antimicrobial activity of organic acids such as lactic acid and acetic acid, but effective concentrations of hydrogen peroxide, carbon dioxide, and diacetyl are rarely present at levels sufficient to make a major contribution to antibacterial activity.

The value of bacteriocins is limited by the fact that they are usually only active against Gram-positive cells, although theoretically this could be overcome by the addition of chelating agents such as Ethylenediaminetetraaceticacid, or EDTA (see Chapter 2). In practice, it might be of more advantage to concentrate on other factors such as choice of raw materials and the use of GMP and the HACCP concept.

THE CONTROL OF MICROBIAL HAZARDS

Although many fermented foods are a less likely vehicle for bacterial food-borne illness than fresh foods, hygienic practices during fermentation (especially in traditional household fermentations), the possible use of contaminated raw materials, and the low stability of these

Table 7–3 Effect of Various Fermentation Processes on the Survival and Growth of Food–borne Pathogens

Type of Fermentation Substrates	*pH*	*A_w*	*Antagonism**
Lactic acid fermentation			
Meat, pork, poultry	4.5–6.0	0.85–0.99	B, H, C, D
Cabbage, vegetables	< 4.0–5.5	> 0.96	B, H, C, D
Milk	< 4.0–5.5	> 0.96	B, H, C, D
Alcoholic fermentation			
Grains	4.0–5.5	> 0.96	E
Fruits	< 4.0	> 0.96	E
Mold fermentation			
Soybeans	4.0–5.0†	> 0.96	–

*By production of other substances than organic acids (e.g., bacteriocins (B), hydrogen peroxide (H), ethanol (E), carbon dioxide (C), diacetyl (D), etc.).

†pH value after soaking, just before inoculation with spores

products compared with canned or frozen foods, make control of microbial hazards necessary. The following factors are of importance:

- use of contaminated raw materials
- prevention of contamination (zoning, cleaning, and disinfection)
- poorly controlled fermentations, including ripening (HACCP)
- consumption without prior heating

The Use of Contaminated Raw Materials

The prevention of contamination of raw materials with pathogenic microorganisms should be the goal of everyone involved in the preharvest and postharvest phases of delivering products to the consumer. This is a "mission impossible" because pathogens are normally present in the soil (and therefore on the surface of fruits and vegetables), in surface water (which results in contaminated fish and shellfish), and in the gut of animals (causing contaminated products of animal origin, such as milk and meat).

To minimize the risk of infection or intoxication, pretreatment of the raw materials might be helpful. The simple practice of washing, for example, removes a portion of the pathogens (up to 90%). Another possibility (not for raw meat) is heat treatment (pasteurization) of the raw materials (e.g., milk). If pasteurization of the raw materials is not possible, a postprocess pasteurization might be necessary to eliminate pathogens in the final product. This type of postprocess pasteurization is sometimes practiced with fermented sausages.

Zoning

Raw materials are the primary source of many of the bacteria that are responsible for foodborne infections and intoxications. But, bacteria may also be transferred to food by the production environment and personnel, either directly or by cross-contamination through surfaces, equipment, utensils, and/or hands that have not been adequately cleaned and disinfected. Zoning, that is, dividing the production area into "dry and wet" and/or "high, medium, and low care" areas, is useful in preventing product contamination. The concept of hygiene zones was initially applied to prevent *Salmonella* contamination of very sensitive products such as milk powder. Nowadays, zoning has evolved into a complex set of measures including the layout and design of equipment, air filtration, personnel hygiene routes, and appropriate cleaning and disinfection procedures. However, zoning is only useful when it is applied logically and to the appropriate degree.

Applied in fermentation processes, there should be areas for

- the storage of the raw materials (low hygiene)
- the preparation of the raw materials, that is, washing, cutting, and adding ingredients (medium hygiene)
- the fermentation of the raw materials (medium hygiene)
- the filling of suitable packages (medium hygiene)
- the storage of the final products (medium hygiene)

An example of an area of high hygiene is the room where starter cultures are prepared for the lactic fermentation of milk. Here, a potential danger is the infection of the cultures with bacteriophages. Zoning, the allocation of an area strictly separated from the production process, will help to prevent the introduction of bacteriophages in starter cultures. However, this will be insufficient if it is not accompanied by other preventive measures such as strict cleaning and disinfection procedures and changing shoes when entering the area. Changing shoes cannot be replaced efficiently by covering shoes with plastic covers (that often break) or by using shoe disinfecting systems (improper disinfection due to rapid inactivation of the disinfectant), which can cause potentially contaminated wet areas around the system.

It is not feasible to discuss zoning for all types of fermentation processes here. In practice, zoning should be embedded in HACCP studies and must be acceptable and practicable for all indi-

viduals. Zoning should be introduced after adequate training so that personnel know why zoning is important and are motivated to follow its requirements.

Cleaning and Disinfection

Inadequate cleaning and disinfection can lead to reduced food quality, resulting in more rapid food spoilage and greater risk of food-borne diseases. Moreover, in food spills (pathogenic) microorganisms might adapt to extreme conditions such as low pH, giving them the opportunity to survive and/or grow in the final product.

Cleaning and disinfection are two separate but closely related concepts. *Cleaning* is removing dirt and a proportion of the microorganisms present; *disinfection* is treating the surfaces in such a way that the remaining microorganisms are killed or reduced to an acceptable level. Cleaning should be done first; otherwise, the subsequent disinfection will be less effective.

HACCPs

After zoning has been introduced as part of the prevention plan, attention should be paid to the presence and behavior of pathogens in raw materials, the fermentation process, the final product, and the production environment. Microbiological investigation estimating the presence and numbers of pathogens and spoilage organisms in raw materials and food products forms an important part of control programs for ensuring the safety and quality of food products. Until recently, microbial counts were an important though ineffective method for assessing food quality. Nowadays, food safety systems have been introduced based on HACCPs, in which control is exercised throughout the process itself.

An HACCP study will provide information concerning critical control points (CCPs), that is, raw materials, locations, practices, procedures, formulations, or processes where measures can be applied to prevent or minimize hazards.[45]

It is outside the scope of this contribution to identify all CCPs for known fermentation processes. In general, raw materials (contaminated with pathogens), the fermentation process (time to reach final pH, A_w, and final pH), storage and preparation of starter cultures, and heat treatment (of raw materials or of the fermented products) are the most important CCPs.

An HACCP study will not result in control measures that eliminate all safety problems, but it will provide information that can be used to determine how best to control the remaining hazards. It is then up to management to use that information correctly. If control procedures follow clearly established rules, inspectors can have greater confidence in food producers. Governments are also more able to accept the responsibility taken by industry because they can understand why and how controls are made. This has been recognized internationally, and the application of HACCP principles is recommended by the Codex Alimentarius Commission and is mandatory in many countries. In its present format, the application of HACCP principles is best suited to industrial food processing; small-scale producers of fermented foods, especially in nondeveloped countries, will have greater difficulty following this route. Relatively simple hygiene codes, based on HACCPs, could inform food handlers and households about appropriate fermentation techniques[46,52] (see Chapters 3 and 12).

Quantitative Risk Assessment

In many cases, the concept of HACCPs is used in only a qualitative way. By the implementation of quantitative risk analysis (QRA) in existing HACCP systems, a more quantitative approach is possible. A smart, stepwise, interactive identification procedure for food-borne microbial hazards has been developed by van Gerwen & Zwietering.[29] This procedure is based on three levels of detail ranging from rough hazard identification via detailed to comprehensive hazard identification. At first, the most relevant problems are identified before focusing on less important problems.

- The first level (rough hazard identification) selects pathogens that were involved in food-borne outbreaks with the product in the past. These data can be found in the literature, although not all outbreaks are reported, particularly in nondeveloped countries, and in many cases or outbreaks, the causative organism or the food vehicle is not identified.
- The second level (detailed hazard identification) selects pathogens that were reported to be present on the raw materials and ingredients used in the product. The greater part of these data can be found in the literature.
- The last level (comprehensive hazard identification) selects all (human) pathogens as hazardous, including pathogens that unexpectedly recontaminate the product. In this step, it is possible to estimate the effect of unexpected hazards (i.e., emerging pathogens, or pathogens that adapted to or are resistant to intrinsic factors of the product, such as acid-resistant *Salmonella*). Possible future problems can be anticipated in this way.

After this type of hazard identification, knowledge rules should be used to reduce the probably long list to a manageable list of the pathogens that are most likely present in the final product.

The following three types of rules are used.

1. *Selection of pathogens that are present or able to survive in the end product (e.g., removal from the list of vegetative pathogens present in a product that will undergo pasteurization).* Survival after pasteurization due to inappropriate time/temperature combinations and post-process contamination are not included in this rule. This should be ascertained by GMP and HACCP analysis.
2. *Selection of pathogens that are likely to cause problems.* Pathogens that are very rarely transmitted are not likely to cause health problems and can be removed from the list.
3. *Selection of pathogens that are able to grow or produce toxin in the product.* Estimation of capacity to grow is based on the maximum and minimum growth temperature, pH, and A_w. Other growth-determining factors such as the presence of preservatives and natural antimicrobial systems are not taken into account.

The knowledge rules can only be applied appropriately by experienced microbiologists assisted by the use of literature and/or models predicting the growth or inactivation of pathogens.

Simplified Identification Procedure for Food-Borne Microbial Hazards in Fermented Sausage

Food Science and Technology Abstracts (retrospective 1969–1989 and current 1990–1999) were used as a database for the first levels of hazard identification. The results are presented in Table 7–4. With the rough hazard identification, only three pathogens (*E. coli* O157:H7, *Salmonella*, and *S. aureus*) involved in outbreaks were found. The ingredients used in this product are meat (pork, beef), pork backfat, salt, nitrate, and spices (pepper). With a detailed hazard identification for the raw materials, all pathogens listed in Table 7–2 are selected, even some pathogens that are indicated as not likely to be present (e.g., *B. cereus*). This is because the selection for Table 7–4 was based on literature data for the presence of these pathogens (even in low numbers). Table 7–2 only presents information if there is a strong or a weak relationship between the pathogen and the raw material.

Applying type 1 knowledge rules (survival of pathogens in the end product) only results in the removal of *Campylobacter.* This pathogen will not survive fermentation processes due to the low pH. Using type 2 rules removes *B. cereus*, *Shigella,* and *Vibrio.* These pathogens are of little concern in fermented sausages because growth at low pH is not possible (*B. cereus* and *Vibrio* spp.), or because the presence in the raw materials is only accidental (transmission via contaminated persons).

Table 7–4 Results of the Hazard Identification Procedure Applied to Fermented Sausage

		Knowledge Rules		
Rough Hazard Identification	*Detailed Hazard Identification*	*type 1*	*type 2*	*type 3*
	Aeromonas	x	x	
	Bacillus cereus	x		
	Campylobacter			
Escherichia coli O157:H7	*Escherichia coli O157:H7*	x	x	x
	Listeria monocytogenes	x	x	x
Salmonella	*Salmonella*	x	x	x
	Shigella	x		
Staphylococcus aureus	*Staphylococcus aureus*	x	x	x*
	Vibrio	x		
	Yersinia	x	x	

*Growth and toxin production of *Staphylococcus aureus* is usually restricted to the start of the fermentation process, when there is a rather slow decrease in pH.

The pathogens likely to cause problems are *Aeromonas*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *S. aureus,* and *Yersinia.*

To use type 3 rules, it was assumed that the pH of the final product was 5.0 ± 0.1 and the A_w was 0.95 ± 0.01. If all types of knowledge rules are applied, *Aeromonas* and *Yersinia* can be removed because usually high levels are needed for food-borne infection, and growth in fermenting sausage is poor or not possible at all.[61]

The remaining pathogens are those from the rough hazard identification (*E. coli* O157:H7, *Salmonella*, and *S. aureus)* and *L. monocytogenes.*

Under the conditions described, growth and toxin production by these pathogens are usually restricted to the first 6–12 hours of fermentation. This can be followed by a gradual decline or survival of the cells.[25,26,31,33,43,47,56] Because of the presence of competitive microorganisms, growth of *S. aureus* is seldom a problem.[54] The low pH values will affect growth and survival of *L. monocytogenes.*[8,26,27,47] in contrast to the acid-adapted and acid-tolerant strains of *Salmonella* and *E. coli* O157:H7.[2,23,47]

The knowledge obtained from the stepwise identification procedure provides a way to efficiently assess those hazards that need to be controlled during processing.

VALIDATION OF THE SAUSAGE FERMENTATION PROCESS FOR THE CONTROL OF PATHOGENS

As a result of outbreaks of illness caused by *S. aureus* in fermented meat products,[7] the American Meat Institute formulated GMP for fermented dry and semi-dry sausages.[3]

The rapid decrease to low pH values, preferably lower than pH 4.0, that results in a sufficient inactivation of salmonellas and *S. aureus* turned out to be of little effect against *E. coli* O157:H7,[23,47] as was demonstrated by the recent outbreaks of *E. coli* O157:H7 linked to the consumption of fermented sausages. The U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) developed guidelines for validating a 5-log reduction of *E. coli* O157:H7 in fermented sausage.[23] To meet the criteria set in the guideline, challenge tests should be performed to investigate the behavior of this pathogen during the fermentation process and a postpasteurization treatment. The information to perform a microbiological storage test or a challenge trial can be gained from the literature or from laboratory test models (e.g., the Food MicroModel). In most cases, the laboratory models provide a more conservative view than the real product situation, as each parameter

has usually been studied under otherwise ideal conditions.

Microbiological Challenge Tests

Laboratory simulation of what can happen to a food product during processing, distribution, and subsequent handling is an established technique in the food industry. There are two main reasons why this type of testing is carried out. In general, safety is of prime concern because the food manufacturer must ensure that a product presents a minimum risk to the consumer. The other reason is to establish the shelf life or keeping quality of a product. In this case, quality is of most interest. In both cases, the product is normally stored at specified temperatures for at least the anticipated or prescribed shelf life. Depending on the information available on raw materials, process, organisms of interest, and so forth, the product can be inoculated with (low) numbers of spoilage organisms and/or pathogens before processing or storage. With these laboratory simulation tests, which are mandatory in some markets, and data from computer models (e.g., Food MicroModel), it is possible to demonstrate that everything that can be reasonably expected has been done.

Depending on the background for carrying out a laboratory simulation test (quality or safety), and whether the product is likely to contain the types of microorganisms of concern, these tests should be named differently to avoid confusion. In a *challenge test,* the product is inoculated with either pathogenic or spoilage organisms, depending on whether consumer safety issues or product stability are of most concern. In a *storage test,* sometimes called shelf life or keeping quality test, the product is not inoculated with microorganisms and the aim is to check safety and/or quality. The experimental plan and execution of a storage test may, however, differ, depending on which of the factors is to be studied.

Purpose of Microbiological Challenge and Storage Tests

One of the important tools used to ensure the production of safe food is the identification of CCPs in an HACCP study. CCPs have to be controlled in order to eliminate hazards or minimize their likelihood of occurrence. It is essential that the identification and setting of criteria for CCPs are based on sound knowledge and relevant information so that target levels and tolerance limits can be established. CCPs are under control if the criteria are met. Only if sufficient knowledge concerning the effect of intrinsic, extrinsic, and process factors on the safety of the product is available can the setting of criteria at each CCP be done easily.

However, if information is scarce or unavailable, data have to be obtained by storage testing and/or microbiological challenge testing. A *microbiological challenge test* is an exercise to simulate what can happen to a food product during processing, storage, distribution, and subsequent handling, following inoculation with one or more relevant pathogens.

It is important to realize when a microbiological challenge test should not be performed. If it is evident that the pathogen(s) will grow readily in the product, a challenge test will be a waste of time. Such pathogens must be eliminated by proper heat treatment or by preservation of the product.

Challenge testing will provide information on the types of pathogens capable of growth in the product. With the data obtained, the risks of food poisoning can be assessed and the conditions necessary to prevent food poisoning can be determined. As a consequence, the safety of the product can be determined in terms of intrinsic factors. Therefore, the information obtained is the basis for setting criteria at relevant CCPs.

Challenge testing will provide information about the growth of selected pathogens in a product. However, the likelihood of presence of these pathogens in the product should be obtained from literature or databases.

Planning of a Storage Test and a Challenge Test

The factors below, also relevant for the first part of an HACCP study, must be considered when planning storage tests and challenge tests.

- microbiological status of the raw materials
- product composition and limits for critical hurdles
- process design and parameters
- packaging system
- anticipated storage, distribution, and consumer handling conditions
- identification of the risks of food poisoning from the product
- existing knowledge of the types and properties of organisms relevant to the product

Carrying Out a Microbiological Challenge Test

If the decision is made that a challenge test is required, and after deciding which organisms are relevant for the test, a protocol has to be developed. It is important to ensure that enough samples of the product are available at each time point of the investigation. The number of samples in the test depends on the heterogeneity of the product; all of the technical procedures must be reliable and reproducible.

There are two types of microbiological challenge tests: process challenge tests and product challenge tests. The objective of the *process challenge* test is to determine whether or not a selected pathogen survives a process (e.g., fermentation). The results from such tests are available directly after processing by enumeration of surviving microorganisms. In some cases, such as ripening in fermentation processes, analysis must be done after a few weeks. *Product challenge* tests investigate whether or not the pathogens in artificially contaminated products can grow or under what conditions unacceptable levels will be reached.

The test strains used (preferable a cocktail of strains) should be isolated from identical or similar products. During preparation of the inoculum, stress conditions (that might reduce viability) should be avoided. Because in practice, pathogens entering the product from the environment might be adapted to the intrinsic factors of the product (e.g., poorly cleaned product environment), it might be necessary to train the microorganisms to growth at low pH or low A_w in order to ensure reliable results.

Process Challenge Test for Fermented Products Artificially Contaminated with *E. coli* O157:H7

Among the many different types of fermented sausage, pepperoni is one of the most popular varieties. In both the pepperoni and the Lebanon bologna process, *E. coli* O157:H7 survives the fermentation process.[23,47]

Performing challenge tests as described above might lead to the conclusion that other acid-tolerant pathogens will also survive the fermentation process, which makes a postfermentation heat treatment necessary to produce a safe product. Another possibility, less suitable for raw meat but applicable for items such as raw milk, is pasteurization of the raw materials. Zoning, in combination with good manufacturing processes, should prevent postprocess contamination.

In the future, more research is needed to understand the behavior of (acid-adapted) pathogens in fermentation processes. With this knowledge, hygiene codes should be developed for all types of fermentations in order to ensure safe products, both in industrial and in household technologies.

REFERENCES

1. Adams, M. R. & Moss, M. O. (2000). *Food Microbiology,* 2nd edn. Cambridge, England: Royal Society of Chemistry.
2. Adams, M. R. & Nicolaides L. (1997). Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control* 8, 227–239.
3. American Meat Institute. (1982). *Good Manufacturing Practices: Fermented Dry and Semi-Dry Sausage.* Washington, DC: American Meat Institute.
4. Antony, U., Moses, L. G. & Chandra, T. S. (1998). Inhibition of *Salmonella typhimurium* and *Escherichia coli* by fermented flour of finger millet (*Eleusine coracana*). *World J Microbiol Biotechnol* 14, 883–886.
5. Aryanta, R. W., Fleet, G. H. & Buckle, K. A. (1991).

The occurrence and the growth of microorganisms during the fermentation of fish sausage. *Int J Food Microbiol* 13, 143–156.

6. Aytac, S. A. & Ozbas, Z. Y. (1994). Survey on the growth and survival of *Yersinia enterocolitica* and *Aeromonas hydrophila* in yogurt. *Milchwissenschaft* 49, 322–325.
7. Bacus, J. N. (1986). Fermented meat and poultry products. In *Advances in Meat Research.* Vol. 2, *Meat and Poultry Microbiology,* pp. 123–164. Edited by A. M. Pearson & T. R. Dutson. Westport, CT: AVI Publishing.
8. Beumer, R. R. (1997). Listeria monocytogenes: *detection and behaviour in food and in the environment.* PhD diss., Wageningen University Research Center, The Netherlands.
9. Beumer, R., Bloomfield, S., Exner, M., Fara, G. M. & Scott, E. (1999). The need for a home hygiene policy and guidelines on home hygiene. *Annali di Igiene* 11, 11–26.
10. Beumer, R. R., Vrouwenvelder, T. & Brinkman, E. (1994). Application of HACCP in airline catering. *Food Control* 5, 205–209.
11. Bodnaruk, P. W., Williams, R. C. & Golden, D. A. (1998). Survival of *Yersinia enterocolitica* during fermentation and storage of yogurt. *J. Food Sci* 63, 535–537.
12. de Boer, E. & Hahné, M. (1990). Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *J Food Prot* 53, 1067–1068.
13. Bonestroo, M. H., Kusters, B. J. M., de Wit, J. C. & Rombouts, F. M. (1993). The fate of spoilage and pathogenic bacteria in fermented sauce-based salads. *Food Microbiol* 10, 101–111.
14. Bryan, F. L. (1988). Risk of practices, procedures and processes that lead to outbreaks of foodborne diseases. *J Food Prot* 51, 663–673.
15. Byaruhanga, Y. B., Bester, B. H. & Watson, T. G. (1999). Growth and survival of *Bacillus cereus* in mageu, a sour maize beverage. *World J Microbiol Biotechnol* 15, 329–333.
16. Caplice, E. & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *Int J Food Microbiol* 50, 131–149.
17. Centres for Disease Control and Prevention. (1993). Update: multi-state outbreak of *Escherichia coli* O157:H7 infections from hamburgers: Western United States, 1992–1993. *MMWR* 42, 258–263.
18. Chan, K. Y., Woo, M. L., Lam, L. Y. & French, G. L. (1989). *Vibrio parahaemolyticus* and other halophilic vibrios associated with seafood in Hong Kong. *J Appl Bacteriol* 66, 57–64.
19. Corrales, M. T., Bainotti, A. E. & Simonetta, A. C. (1994). Survival of *Vibrio cholerae* O1 in common foodstuffs during storage at different temperatures. *Lett Appl Microbiol* 18, 277–280.
20. Cutter, C. N. (1999). Combination spray washes of saponin with water or acetic acid to reduce aerobic and pathogenic bacteria on lean beef surfaces. *J Food Prot* 62, 280–283.
21. Dessie G., Abegaz, K. & Ashenafi , M. (1996). Fate of *Salmonella enteritidis* and *Salmonella typhimurium* during the fermentation of siljo. *East Afr Med J* 73, 432–434.
22. Dineen, S. S., Takeuchi, K., Soudah, J. E. & Boor, K. J. (1998). Persistence of *Escherichia coli* O157:H7 in dairy fermentation systems. *J Food Prot* 61, 1602–1608.
23. Ellajosyula, K. R., Doores, S., Mills, E. W., Wilson, R. A., Anantheswaran, R. C. & Knabel, S. J. (1998). Destruction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Lebanon bologna by interaction of fermentation pH, heating temperature, and time. *J Food Prot* 61, 152–157.
24. Encinas, J. P., Gonzales, C. J., Garcia-Lopez, M. L. & Otero, A. (1999). Numbers and species of motile aeromonads during the manufacture of naturally contaminated Spanish fermented sausages (longaniza and chorizo). *J Food Prot* 62, 1045–1049.
25. Faith, N. G., Wierzba, R. K., Ihnot, A. M., Roering, A. M., Lorang, T. D., Kaspar, C. W. & Luchansky, J. B. (1998). Survival of *Escherichia coli* O157:H7 in full- and reduced-fat pepperoni after manufacture of sticks, storage of slices at 4° or 21 °C under air and vacuum, and baking of slices on frozen pizza at 135, 191 and 246 °C. *J Food Prot* 61, 383–389.
26. Farber, J. M, Daley, E., Holley, R. & Usborne, W. R. (1993). Survival of *Listeria monocytogenes* during the production of uncooked German, American and Italian-style fermented sausages. *Food Microbiol* 10, 123–132.
27. Farber, J. M. & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev* 55, 476–511.
28. Gahan, C. G. M., O'Driscoll, B. & Hill, C. (1996). Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. *Appl Environ Microbiol* 62, 3128–3132.
29. van Gerwen, S. J. C. & Zwietering, M. H. (1998). Growth and inactivation models to be used in quantitative risk assessments. *J Food Prot* 61, 1541–1549.
30. Griffiths, M. W. (1989). *Listeria monocytogenes*: its importance in the dairy industry. *J Sci Food Agric* 47, 133–157.
31. Hinkens, J. C., Faith, N. G., Lorang, T. D., Bailey, P., Büge, D., Kaspar, C. W. & Luchansky, J. B. (1996). Validation of pepperoni processes for control of *Escherichia coli* O157:H7. *J Food Prot* 59, 1260–1266.
32. Hisa, K., Hayasi, K. & Sakaguchi, G. (1999). Proposal of HACCP systems for prevention of foodborne botulism. II. theoretical considerations in izushi production to prevent *Clostridium botulinum* type E botulism based on HACCP. *J Antibact Antifung Ag – Jap* 27, 3–11.

33. Ihnot, A. M., Roering, A. M., Wierzba, R. K., Faith, N. G. & Luchansky, J. B (1998). Behaviour of *Salmonella typhimurium* DT104 during the manufacture and storage of pepperoni. *Int J Food Microbiol,* 40 117–121.

34. Jacobs-Reitsma, W. F., Bolder, N. M. & Mulder, R. W. A. W. (1994). Cecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: a one year study. *Poultry Sci* 73, 1260–1266.

35. Johnson, E. A., Nelson, J. H. & Johnson, M. (1990). Microbiological safety of cheese made from heat-treated milk, part I: executive summary, introduction and history. *J Food Prot* 53, 441–452.

36. Johnson, E. A., Nelson, J. H. & Johnson, M. (1990). Microbiological safety of cheese made from heat-treated milk, part II: microbiology. *J Food Prot* 53, 519–540.

37. Jyhshiun, L., In-Soo-Lin, Frey, J., Slonczewski, J. L. & Foster, J. W. (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli. J Bacteriol* 177, 4097–4104.

38. Kaspar, C. W. & Tamplin, M. L. (1993). Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Appl Environ Microbiol* 59, 2425–2429.

39. Kingamkono, R., Sjoegren, E., Svanberg, U. & Kaijser, B. (1996). Inhibition of different strains of enteropathogens in a lactic-fermenting cereal gruel. *World J Microbiol Biotechnol* 11, 299–303.

40. Knorr, D. (1998). Technology aspects related to microorganisms in functional foods. *Trends Food Sci Technol* 9, 295–306.

41. Kunene, N. F., Hastings, J. W. & von Holy, A. (1999). Bacterial populations associated with a sorghum-based fermented weaning cereal. *Int J Food Microbiol* 49, 75–83.

42. Leyer, G. J. & Johnson, E. A. (1992). Acid adaptation promotes survival of *Salmonella* spp. in cheese. *Appl Environ Microbiol* 58, 2075–2080.

43. Massa, S., Altieri, C., Quaranta, V. & DePace, R. (1997). Survival of *Escherichia coli* O157:H7 in yogurt during preparation and storage at 4 °C. *Lett Appl Microbiol* 24, 347–350.

44. Morioka, Y., Nohara, H., Araki, M., Suzuki, M. & Numata, M. (1996). Studies on the fermentation of soft salami sausage by starter culture. *Animal Sci Technol* 67, 204–210.

45. Mortimore, S. & Wallace, C. (1998). *HACCP: A Practical Approach,* 2nd edn. Gaithersburg, MD: Aspen Publishers.

46. Motarjemi, Y. & Nout, M. J. R. (1996). Food fermentation: a safety and nutritional assessment. *Bull WHO* 74, 553–559.

47. Nissen, H. & Holck, A. (1998). Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella kentucky* in Norwegian fermented, dry sausage. *Food Microbiol* 15, 273–279.

48. Northolt, M. D. (1983). Pathogenic micro-organisms in fermented dairy products. *Neth Milk Dairy J* 37, 247–248.

49. Nout, M. J. R. (1994). Fermented foods and food safety. *Food Res Int* 27, 291–298.

50. Nout, M. J. R., Bakshi, D. & Sarkar, P. K. (1998). Microbiological safety of kinema, a fermented soya bean food. *Food Control* 9, 357–362.

51. Nout, M. J. R., Beernink, G. & Bonants-vanLaarhoven, T. M. G. (1987). Growth of *Bacillus cereus* in soya bean tempeh. *Int J Food Microbiol* 4, 293–301.

52. Nout, M. J. R. & Motarjemi, Y. (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control* 8, 221–226.

53. Nout, M. J. R., Rombouts, F. M. & Havelaar, A. (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic micro-organisms. *Int J Food Microbiol* 8, 351–361.

54. Nychas, G. J. E. & Arkoudelos, J. S. (1990). Staphylococci: their role in fermented sausages. *J Appl Bacteriol Symp Supp* 19, 167S–188S.

55. O'Mahony, M., Mitchell, E., Gilbert, R. J., Hutchinson, D. N., Begg, N. T., Rodhouse, J. C. & Morris, J. E. (1990). An outbreak of foodborne botulism associated with contaminated hazelnut yoghurt. *Epidemiol Inf* 104, 389–395.

56. Ostergaard, A., Embarak, P. K. B., Wedell-Neergaard, C., Huss, H. H. & Gram, L. (1998). Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products. *Food Microbiol* 15, 223–233.

57. Ozbas, Z. Y. & Aytac, S. A. (1996). Behaviour of *Yersinia enterocolitica* and *Aeromonas hydrophila* in skim milk during fermentation by various lactobacilli. *Z Lebensmittel Forsch* 202, 324–328.

58. Poffe, R. & de Beeck, E. O. (1991). Enumeration of *Aeromonas hydrophila* from domestic wastewater treatment plants and surface water. *J Appl Bacteriol* 71, 366–370.

59. Rhodehamel, E. J., Reddy, N. & Pierson, M. D. (1992). Botulism: the causative agent and its control in food. *Food Control* 3, 125–143.

60. Roberts, D. (1990). Sources of infection: food. *Lancet* 336, 859–861.

61. Roberts, T. A., Baird-Parker, A. C. & Tompkin, R. B., eds. (1996). *Microorganisms in Foods, 5, Microbiological Specifications of Food Pathogens.* London: Blackie Academic and Professional.

62. Sauer, C. J., Majkowski, J., Green, S. & Eckel, R. (1997). Foodborne illness outbreak associated with a semi-dry fermented sausage product. *J Food Prot* 60, 1612–1617.
63. Sharp, J. C. M., Reilly, W. J. & Coia, J. E. (1995). *Escherichia coli* O157 infection in Scotland: an epidemiological overview, 1984–1994. *PHLS Microbiol Digest* 12, 134–140.
64. Shay, B. & Souness, R. (1995). Recent regulations impacting on the small goods industry. *Food Aust* 47, 491–494.
65. Simango, C. & Rukure, G. (1992). Survival of bacterial enteric pathogens in traditional fermented foods. *J Appl Bacteriol* 73, 37–40.
66. Smulders, F. J. M. & Greer, G. G. (1998). Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: prospects and controversies. *Int J Food Microbiol* 44, 149–169.
67. Swinbanks D. (1996). Outbreak of *E. coli* infection in Japan renews concern. *Nature* 282, 290.
68. Thomas C. & Beirne D. (1999). Microbiological safety in processed vegetables. *Int Food Hyg* 10, 17–23.
69. Verotoxin-producing *Escherichia coli* O157. (1997). *CDR Weekly* 7, 409.
70. Warburton, D. W., Weiss, K. F., Purvis, U. & Hill, R. W. (1987). The microbiological quality of fermented sausage produced under good hygienic practices in Canada. *Food Microbiol* 4, 187–197.
71. West, P. A. (1989). The human pathogenic vibrios: a public health update with environmental perspectives. *Epidemiol Inf* 103, 1–34.
72. Wu, Y. C., Kimura, B. & Fujii, T. (1999). Fate of selected food-borne pathogens during the fermentation of squid shiokara. *J Food Hyg Soc Jpn* 40, 206–210.
73. Yu, S. L. & Chou, C. C. (1987). Survival of some food poisoning bacteria in pickled vegetables stored at different temperatures. *Food Sci China* 14, 296–305.