# CHAPTER 2

# Why Fermented Foods Can Be Safe

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## INTRODUCTION

Over time, the human diet has evolved to exclude materials that are frankly hazardous and for which there is no simple procedure to render them harmless. As a result, our food supply is generally safe, although it can never be entirely devoid of risk. There are a variety of hazards that can be associated with food materials. These can be broadly classified into three categories: intrinsic, extrinsic, and processing/biogenic (Exhibit 2–1).

Some of these hazards can be severe, as in the case of botulinum toxin or bongkrekic acid poisoning, but they can be controlled, and the risk they pose can be reduced to acceptable levels by effective and hygienic processing and preparation. Safety considerations should always be paramount in modern food processing and are also an implicit aspect of traditional methods of processing and preserving food. This is particularly true of fermented foods, which take potentially hazardous raw materials such as raw meat and milk and transform them into acceptable products with better keeping qualities and reduced risk. The bulk of this book addresses particular groups of hazards, their significance in fermented foods, and ways in which they are controlled. This chapter addresses the mechanisms by which fermentation can improve food safety in more general terms.

## PHYSICAL PROCESSING

In Chapter 1, the various physical unit operations associated with fermented food production were described. These operations are common to a number of food production processes and, wherever they occur, they can have some impact on product safety.

### Transport

The manner in which raw materials are transported can affect their quality. Physical damage during transport can breach natural antimicrobial barriers, allowing microorganisms access to nutrient-rich underlying tissues, where they can grow rapidly. Excessive delays during transport can also increase time for microbial growth and for natural processes of senescence to occur.

### Grading and Sorting

Inspection and sorting to remove damaged or obviously infected raw material is a basic protective barrier that can be useful in reducing risk. It can be used to exclude obviously infected meat, damaged or moldy fruit, or mold-infected grains. For example, mechanical or hand sorting of infected nuts has been shown to reduce overall aflatoxin levels considerably; reduced aflatoxin levels were also evident after hand sorting

**Exhibit 2–1** A Classification of Food Hazards

**Intrinsic**—Properties of the food itself, natural toxins (e.g., cyanogenic glycosides, glycoalkaloids)

**Extrinsic**—Contaminants posing a direct hazard (e.g., infectious pathogens—*Salmonella*, *Shigella*, viruses, protozoa; toxic chemical contaminants—pesticide residues, heavy metals)

**Processing/Biogenic**—Hazards produced as a result of processing and/or generated by microbial contaminants (e.g., nitrosamines, biogenic amines, bacterial toxins, mycotoxins)

of figs.[82] This is, however, a relatively blunt weapon because, in many cases, there is no visual indication to help distinguish foods that pose a hazard from those that do not.

### Cleaning and/or Washing

Microbial pathogens can be associated with the surface of fruits and vegetables as a result of contamination in the field. These may be natural soil microorganisms such as the spore-forming pathogens, *Bacillus cereus* and *Clostridum botulinum,* or *Listeria monocytogenes,* or they may be enteric in origin, such as *Salmonella, Escherichia coli,* and *Shigella*, arising from contact with human or animal feces, sewage, or untreated water. Contaminants may also be introduced during harvesting and postharvest handling.

Interior tissues are generally, but not invariably, sterile, and the majority of the surface-associated microflora can be removed by washing. Typically, vigorous washing of plant products in clean water will reduce microbial levels by 10–100-fold, but does not ensure the complete elimination of risk or levels of risk reduction comparable to processes such as pasteurization or canning. The efficacy of washing can be improved slightly by incorporating antimicrobials such as chlorine in the wash water. For example, washing of lettuce leaves with water removed an average of 92.4% of the total microflora. This was increased to 99.5% by including 100 mg $l^{-1}$ of available free chlorine and adjusting the pH to 4.5–5.0.[3] Failure to remove more of the microflora was ascribed to the survival of bacteria in protective hydrophobic pockets or folds in the leaf surface, an effect that will vary between commodities as a result of differences in surface composition and topology. Increased production of ready-to-eat vegetables has heightened interest in the surface disinfection of fruits and vegetables, and the subject has been extensively reviewed.[9]

Washing of animal carcasses with hot solutions of lactic or acetic acid can reduce the total microbial load by 2–3 log cycles, although uniform treatment of all surfaces is difficult and *Salmonella* and *E. coli* show more marked resistance to such treatments.[36]

### Physical Separation

Physical separation can include peeling or trimming to remove external layers. Because the external layers are where most contamination will be found, removing them can substantially improve overall microbiological quality provided it is done hygienically, avoiding contamination of the freshly exposed surfaces underneath. The interior tissues of plants are generally considered to be sterile. Although this is not always true, substantial reductions in count can be achieved by using only hygienically removed interior tissues. With leafy vegetables such as cabbage, the bacterial load on inner leaves can be 1000-fold lower than on the outer leaves and the interior tissues of the plant.[55]

Chemical hazards can also be reduced by the physical separation of plant components. The removal of green surface layers from potatoes can reduce their glycoalkaloid content, and detaching the 2–3 mm thick parenchymal layer of cassava that underlies the cortex will reduce the overall concentration of the cyanogenic glycosides, linamarin and lotaustralin.[51]

### Moisture Adjustment

Changes in the water activity ($A_w$) of a material can have a profound effect on microbial haz-

ards. Depending on the circumstances, a decrease in $A_w$ that is produced by drying or the addition of solutes such as salt will reduce the potential for microbial growth but also enhance microbial resistance to adverse conditions, thus potentially enhancing the survival of pathogens that may be present. Increasing the $A_w$ has the potential to allow growth of previously quiescent organisms, perhaps allowing them to grow to numbers that are sufficient to initiate an infection once they are ingested or produce enough toxin in the product to cause illness. Under certain circumstances, an increase in the $A_w$ as a result of soaking could also permit the onset of a lactic fermentation, which will help control or eliminate microbial hazards.

### Size Reduction

Comminution of a raw material will disrupt cells, thereby releasing their contents. This, in turn, will increase the supply of nutrients to any microorganisms present, stimulating their growth. This can be beneficial when the growth of those organisms whose dominance is necessary for a successful fermentation is encouraged, but in some circumstances, it could also stimulate growth of any pathogens present. The precise outcome will depend on a number of factors such as relative number of organisms present, their substrate affinities, and their physiological state.

The relative sensitivities of different components of the microflora to antimicrobial factors produced by the plant material may also be important. Many plants have defense systems that are activated by cellular disruption. These systems release compartmentalized enzymes, allowing them to act on their substrates and producing compounds that are active against microorganisms and predators such as insects. Examples of this are the release of isothiocyanate by the myrosinase-glucosinolate system in crucifers[10] and allicin produced during the crushing of garlic.

The reduction of chemical hazards is also sometimes assisted by tissue disruption. The cyanogenic glycosides in cassava are hydrolyzed to produce a cyanohydrin by the endogenous enzyme, linamarase, when cellular damage occurs.[18] Microorganisms appear to play no role in cyanogen reduction in the traditional fermented cassava product, *gari*,[89] although in processes where whole roots or large pieces are fermented, microbial activity does assist the endogenous process by softening the tissues.[91]

### Mixing

Mixing will serve to distribute microorganisms throughout a mass of material. Very often, this distribution will accelerate the desirable fermentation and thereby improve safety, although it also has the potential to transfer pathogens to microenvironments where they are protected from antimicrobial factors that prevail elsewhere in the material.

## MICROBIAL ACTIVITY

As already noted, the production of fermented foods shares many unit operations with other types of food processing, all of which can contribute to product safety. The unique feature of food fermentations, however, is the central role that microbial activity plays in the overall process, contributing a number of desirable properties such as improved product shelf life, increased safety, and improved flavor or texture. In developed countries today, the availability of modern food preservation techniques, such as an efficient cold chain, have diminished the significance of fermentation as a food preservation technology, although it remains of major importance in developing countries.

Improvements in food safety arising from microbial activity during fermentation are largely due to lactic acid bacteria (LAB), which are a group of organisms that predominate in the majority of fermented foods. Their growth and metabolism inhibit the normal spoilage flora of the food material and any bacterial pathogens that it may contain. This inhibition can act in two ways: It can slow or arrest growth of the organism, or it can inactivate or kill the organism. Both procedures can result in an improvement in safety. With toxigenic pathogens, the inhibition

of growth can effectively ensure safety, assuming that initial numbers are below those necessary to produce levels of toxin that can cause illness. With infectious bacterial pathogens, slowing or preventing growth may be insufficient to guarantee safety because the infectious dose of some pathogens can be very low. Inactivation or killing of bacteria does occur, but even quite high levels of inactivation still may not be sufficient to eliminate risk entirely. This will depend on factors such as the pathogen concerned, its initial numbers, and its physiological state.

## The LAB

The LAB are a group of gram-positive, nonsporeforming, fermentative anaerobes that are often aerotolerant (Table 2–1). They produce most of their cellular energy as a result of the fermentation of sugars. In the case of hexoses, this can proceed by one of two pathways, providing a useful diagnostic feature as well as playing a critical role in their antimicrobial activity. *Homofermenters* ferment hexoses by the Embden-Meyerhof pathway to produce almost exclusively lactic acid; *heterofermenters* produce less acid overall as a mixture of lactic acid, acetic acid, ethanol, and carbon dioxide using the 6-phosphogluconate/phosphoketolase pathway.[7]

The ability of LAB to inhibit other organisms has been a topic of extensive research over the years, and the field has been reviewed periodically.[4,40,52,72,74] A variety of antimicrobial factors produced by LAB have been identified (Exhibit 2–2); these will be discussed individually. The production of many of these compounds is limited to a few restricted species or strains of LAB. Because these strains/species are not ubiquitous in lactic fermented foods, it is clear that they can only play some contributory role to the overall preservative effect. For so many different LAB to appear in fermented foods, the principal preservative factor must be something that is common to them all. This central and unvarying feature is their use of fermentative pathways to generate cellular energy and the fact that this leads inevitably to the production of organic acids, principally lactic acid, and a decrease in pH. Acidity levels in some fermentations can exceed 100 mM, reducing the pH to less than 4.0 in weakly buffered systems.

### *Organic Acids and Reduced pH*

Low pH and the presence of organic acids are the two principal components of the inhibition of microorganisms under the acid conditions produced by fermentation. The three contributory aspects of acid inhibition were described nearly 50 years ago by Ingram *et al.*[42]

1. the pH
2. the degree of dissociation of the acid
3. the inherent toxicity of the acid anion

Pathogenic bacteria will grow over a pH range of 2–5 units but generally grow best at pH values around neutrality, in the pH range of 6–7. As the

**Table 2–1** Principal Genera of Lactic Acid Bacteria Associated with Food

| | *Rod* | *Coccus* | *Homofermenter* | *Heterofermenter* |
|---|---|---|---|---|
| *Lactobacillus* | + | | + | + |
| *Lactococcus* | | + | + | – |
| *Enterococcus* | | + | + | – |
| *Carnobacterium* | + | | – | + |
| *Leuconostoc* | | + | – | + |
| *Weissella* | | + | – | + |
| *Oenococcus* | | + | – | + |
| *Pediococcus* | | + | + | – |
| *Streptococcus* | | + | + | – |
| *Tetragenococcus* | | + | + | – |

**Exhibit 2–2** Antimicrobial Factors Associated with Lactic Acid Bacteria

| |
|---|
| Low pH |
| Organic acids |
| Bacteriocins |
| Carbon dioxide |
| Hydrogen peroxide |
| Diacetyl |
| Ethanol |
| Reuterin |
| Nutrient depletion and crowding |

pH decreases away from the optimum region, the growth rate declines, eventually reaching zero. At pH values below the minimum that will support growth, the microorganism is progressively inactivated. The rate of inactivation is temperature dependent—the higher the temperature, the faster the rate. This relationship applies for temperatures from chill to lethal, and has been noted in both food and model systems for a number of pathogens such as *Salmonella, E. coli, Listeria,* and *Yersinia enterocolitica*,[33,53,76,88] and also at low $A_w$.[30]

If microorganisms allowed their internal pH to equilibrate to the same value as their environmental pH, they would only be able to function over a very limited pH range close to neutrality. In fact, for most pathogens, growth only ceases once the pH has dropped to less than pH 4.5. This is because bacteria have the ability to maintain their internal pH higher than that of their acidic environment. Both the cell membrane, which has a low permeability to hydrogen ions, and the intrinsic buffering capacity of the cytoplasm help to maintain the cytoplasmic pH, but the cell also possesses a number of active mechanisms for maintaining pH homeostasis. These include the removal of protons in exchange for the uptake of potassium ions, and the uptake and decarboxylation of amino acids to produce neutralizing amines.[11,12,35]

Although weak organic acids such as those produced by fermentation are less effective at decreasing the extracellular pH, they are more effective at inhibiting bacteria than strong acids such as hydrochloric.[15,20,64,83] For example, recent data have shown that for 19 strains of enterohemorrhagic *E. coli*, the minimum growth pH was 4.25 when hydrochloric acid was used as the acidulant and 5.5 when acetic acid was used.[59] This observation is linked to two important properties of acids such as acetic and lactic acid.

1. They are weak carboxylic acids that only partially dissociate in aqueous solution.
2. In their undissociated form, they carry no net charge and have appreciable lipid solubility, which allows them to diffuse freely through the cell's plasma membrane down a concentration gradient into the cytoplasm.

The fact that the undissociated species is the more active antimicrobial form of these acids is illustrated in Figure 2–1, where the degree of inhibition of *E. coli* is clearly linked with the degree of dissociation of the acid present. In a fermented food, the low pH will increase the proportion of the undissociated form present. When the undissociated acid passes through the cell's cytoplasmic membrane into the higher pH of the cytoplasm, it will dissociate, thereby acidifying the cytoplasm and releasing the acid anion. The increased leakage of protons into the cytoplasm will place a metabolic burden on the cells, which will divert resources away from growth-related functions, thus slowing growth. The cell will also accumulate the acid anion, which can disrupt cellular processes.[79] As the extracellular pH decreases and the total concentration of acid increases, so the burden will increase until growth is no longer possible.

The $pK_a$ of an acid is therefore an important determinant of an acid's antimicrobial activity because it describes the proportion present in the undissociated form at any given pH. Differences in the lipophilicity of the undissociated acid and the intrinsic toxicity of the anion will account for differences in the observed toxicity of acids with similar $pK_a$ values. Acetic acid ($pK_a$ 4.76) is a weaker acid than lactic acid ($pK_a$ 3.86); this could account for the frequent observation that it is a more effective antimicrobial.[12] It may also account for the apparent synergy in the antimicrobial effect of mixtures of lactic and acetic

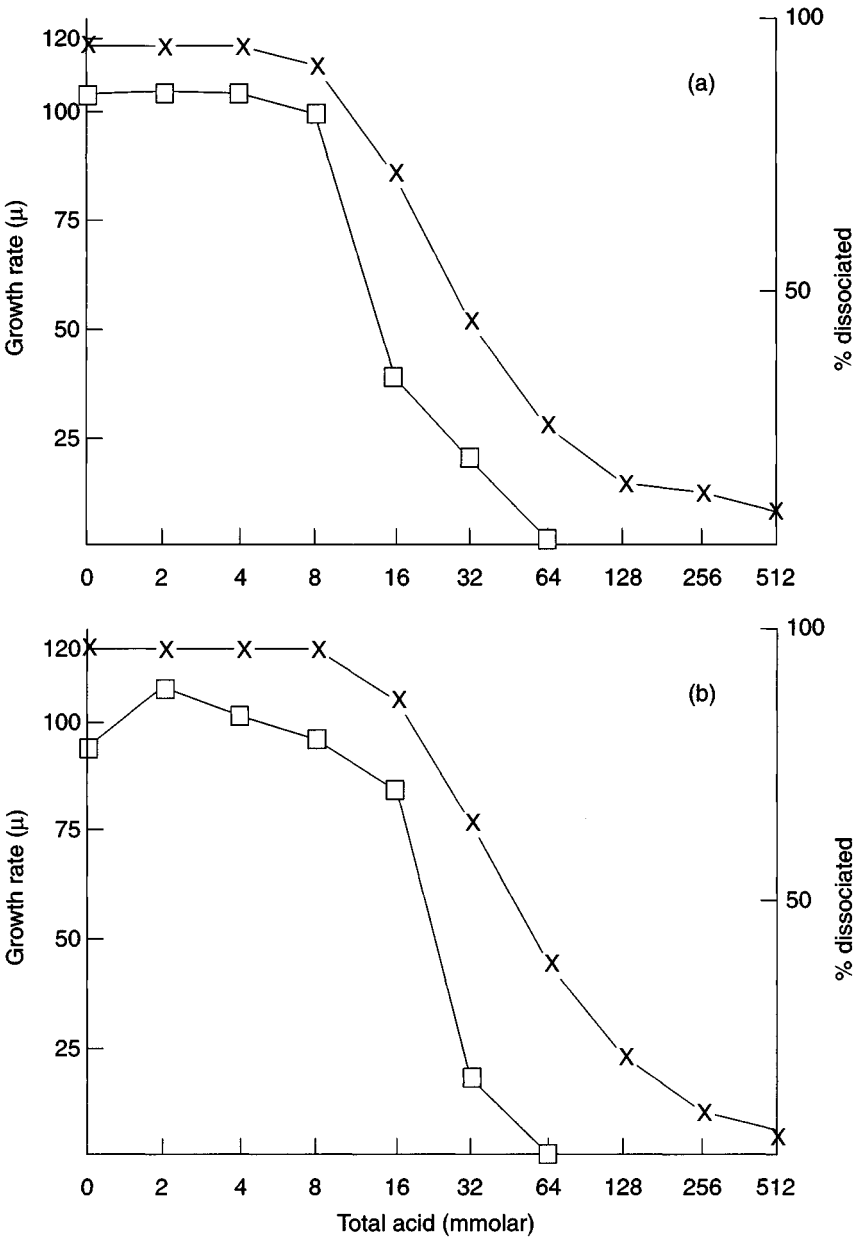


**Figure 2–1** Effect of total acid concentration on the percentage acid dissociation (x) and the specific growth rate of *Escherichia coli* (a) with acetic acid and (b) with lactic acid.

acid, where lactic acid, the stronger acid, decreases the pH, thereby increasing the proportion of acetic acid in the undissociated form and thus potentiating its antimicrobial effect.[2,78] Heterofermentative LAB can produce mixtures of lactic and acetic acid when they have alternative electron acceptors such as oxygen or fructose to help regenerate their nicotinamide adenine dinucleotide (NAD). Although heterofermenters produce less overall acidity than

homofermenters, their early dominance in several natural vegetable fermentations could be very important in providing the rapid inhibition of other organisms present and setting the fermentation on its subsequent course.

The antimicrobial effect will depend on the amount of acid produced, which will depend on the numbers of LAB present. Numerous studies have shown that bacterial pathogens do not survive well when they are added to a prefermented food where the LAB have had the opportunity to grow to large numbers and the pH is already low.[60,61,70,71,81,87] However, when pathogens are present at the start of fermentation, inhibition will be delayed until the LAB have achieved a numerical dominance and produced sufficient acid to achieve an effect. In one instance, when the LAB (in this case, *Lactococcus lactis*) outnumbered the pathogen (*E. coli*) by more than 5 log cycles, the pathogen was still able to grow for five hours, increasing in numbers by 2 log cycles during that time.[95]

### *Bacteriocins*

Bacteriocins are polypeptide antimicrobials that are produced by bacteria and are bactericidal to other, normally very closely related, organisms. In recent years, a considerable research effort has been devoted to the identification and characterization of bacteriocins produced by LAB. The widespread consumption of LAB in foods without any adverse health effects is taken as an indication that they can generally be regarded as safe[1] and therefore their bacteriocins might have potential as "natural" food preservatives. Tangible practical results from this work have been slight, though it has led to the description of numerous new bacteriocins.[43,50,66]

These new bacteriocins have been classified into four main groups (Table 2–2). Under this scheme, Class I bacteriocins are the lantibiotics, which include nisin, the only bacteriocin that is currently approved for use in foods.[29] Nisin is a small (3.4 kDa) heat-stable polypeptide containing some unusual amino acids introduced by post-translational modification of a ribosomally synthesized precursor. It is distinguished from many other bacteriocins by its relatively broad range of activity, being inhibitory to most gram-positive bacteria. It has been used as a food preservative for 50 years and has, more recently, been given "generally regarded as safe" (GRAS) status in the United States.[69] Nisin's principal use in food processing has been to inhibit the outgrowth of spores, which show the greatest sensitivity to nisin. Gram-positive nonsporeforming pathogens such as *Staphylococcus aureus* and *L. monocytogenes* are inhibited, although *S. aureus* is one of the most nisin-resistant gram positives and *L. monocytogenes* has been shown to acquire nisin resistance quite readily.[26,39,62]

Early work suggested that nisin resistance in several *Bacillus* species was due to the production of a nisinase enzyme that specifically reduced the C-terminal dehydroalanyl lysine to

**Table 2–2** Classification of Bacteriocins Produced by Lactic Acid Bacteria

| *Class* | *Subclass* | *Description* |
|---|---|---|
| I | | Lantibiotics (e.g., nisin); small, heat stable, containing lanthionine |
| II | | Small (<10 kDa), heat stable, non-lanthionine-containing, membrane-active peptides |
| | IIa | Pediocin-like peptides with Y-G-N-G-V-X-C- near the amino terminus; anti-listerial |
| | IIb | Two-peptide bacteriocins |
| | IIc | Thiol-activated peptides |
| III | | Large (>30 kDa) heat labile proteins |
| IV | | Complex bacteriocins: protein with lipid and/or carbohydrate |

alanyllysine.[45–47] More recently, attention has focused on the composition of the cytoplasmic membrane where nisin exerts its effect by forming pores. Studies have seen differences in the phospholipid content of membranes in nisin-resistant and nisin-sensitive strains of *L. monocytogenes*[63,90] and changes in the fatty acid composition leading to less fluid membranes in resistant strains.[57] This appears not to be the complete explanation, however, as several studies have implicated other features of the cell envelope in resistance. For example, it has been shown that the hydrophobicity of *L. monocytogenes* cells correlates with their nisin sensitivity,[27,63] that nisin-resistant strains show different sensitivities to cell wall-acting compounds,[22] and that removal of the cell wall of resistant strains abolishes resistance.[27] Some clarification of these findings and the observation that cells possess limited nisin-binding sites[26] has come from the work of Breukink *et al.*[17] They confirmed an observation made 20 years previously[75] that nisin binds with high affinity to Lipid II, a membrane-anchored cell wall precursor, and proposed that differences in nisin sensitivity may reflect different amounts or availability of Lipid II.[17]

Nisin is used commercially as a partially purified concentrate but may be produced *in situ* during a fermentation. Its production during cheese fermentations and the consequent inhibition of other starter organisms led to its first isolation and description. Studies of the effect of nisin-producing cultures on the safety of fermented foods have produced mixed results. *In situ* nisin production has been shown to inhibit the growth of *L. monocytogenes* in Camembert cheese,[56] although levels of 150 IU $g^{-1}$ produced by *Lc. lactis* in fermented rice made no discernible contribution to the inhibition of *S. aureus,* which was ascribed entirely to the lactic acid produced.[95] However, there have been several reports of work employing LAB-producing bacteriocins other than nisin to inhibit *L. monocytogenes* in fermented sausages, cottage cheese, smoked salmon, and bean sprouts.[8,34,58,68]

The value of nisin and other LAB bacteriocins as aids to bacteriological food safety is limited by their range of activity. Gram-negative bacteria, which constitute the majority of food-borne bacterial pathogens, are generally resistant to nisin due to its inability to penetrate the outer membrane. If, however, the outer membrane permeability is increased by treatment with chelators such as ethylenediaminetetraacetic acid (EDTA) or thermal injury, then they do display sensitivity.[14,23,85,86] Prospects for using this approach to inhibit gram negatives in fermented foods are not promising because the pH drop during fermentation does not appear to produce appreciable outer membrane injury and reduces the ability of EDTA to chelate metal ions and permeabilize the outer membrane.[13]

### *Carbon Dioxide*

Heterofermentative LAB produce 1 mole (22.4 liters) of carbon dioxide for every mole of hexose they ferment. This can help establish anaerobic conditions, thereby preventing the growth of obligate aerobes such as molds and slowing the growth of facultative organisms such as the *Enterobacteriaceae.* Anaerobic conditions will also give the LAB a competitive edge and encourage a successful fermentation. An increased partial pressure of carbon dioxide also has its own specific antimicrobial activity. Molds and oxidative gram-negative bacteria are most susceptible, whereas lactobacilli and some yeasts show high tolerance. There is considerable scope for improving the understanding of the inhibitory mechanisms of carbon dioxide and the relative importance of factors such as the ability of carbon dioxide to decrease intracellular pH, its inhibition of enzymatic reactions, and the disruptive effects of its interaction with cell membranes.

### *Hydrogen Peroxide*

In the presence of oxygen, LAB produce hydrogen peroxide through the activity of flavoprotein oxidases. Because LAB are catalase negative, the hydrogen peroxide can accumulate in the medium. Hydrogen peroxide is a strong oxidizing agent and exerts its antimicrobial effect directly or through its degradation products such as superoxide, $O_2^-$, and hydroxyl radicals.

LAB appear to be more resistant to hydrogen peroxide than many other bacteria. A reported minimum inhibitory concentration for *L. lactis* was 125 mg $l^{-1}$, whereas that for *S.aureus* was 5–6 mg $l^{-1}$.[25,92] However, the amounts accumulated by LAB in culture are quite variable. Lactococci growing in milk and the meat starter organism, *Pediococcus cerevisiae,* both produce levels below 1 mg $l^{-1}$ in the bulk environment.[73,77] Increased amounts of hydrogen peroxide can be produced by improving aeration through shaking and at low temperatures when the oxygen solubility is greater.[21,25] It has been suggested that hydrogen peroxide production by LAB in refrigerated foods is a significant antimicrobial factor.[16,37] However, this seems an unlikely scenario in the production of most fermented foods. It is possible that hydrogen peroxide plays a significant role during the early stages of fermentation while residual dissolved oxygen is removed, or that microenvironments with higher levels may persist. In general, though, the contribution of hydrogen peroxide is likely to be relatively minor.

### *Diacetyl*

Diacetyl (2,3-butanedione) is produced by strains of several LAB and plays an important role in the sensory properties of many fermented foods, particularly dairy products. It can accumulate when the organism reaches the stationary phase of growth or when there are alternative sources available from which to generate the key intermediate pyruvate, such as citrate. Antibacterial activity of diacetyl has been demonstrated against a number of bacteria, including *Aeromonas hydrophila, Bacillus* spp., *Enterobacter aerogenes, E. coli, Mycobacterium tuberculosis, Pseudomonas* spp., *Salmonella, S. aureus,* and *Y. enterococolitica.*[31,44,48,65] Gram negatives generally show greater sensitivity and the LAB are most resistant.

However, diacetyl does have an extremely sharp odor, and the acceptable sensory threshold in dairy products is 2–7 mg $kg^{-1}$.[54] The levels of diacetyl reported to produce appreciable microbial inhition are usually quite high (e.g., for gram-negative bacteria, approximately 200 mg $kg^{-1}$), well in excess of this level. Some studies have suggested that lower concentrations of diacetyl can be effective, particularly at lower temperatures,[6] but others have failed to see any appreciable contribution of diacetyl at 10 mg $kg^{-1}$ to the inhibition of *E. coli* in refrigerated dairy foods.[38] Overall, the contribution of diacetyl to bacterial inhibition in fermented foods appears to be relatively minor.

### *Ethanol*

Ethanol is a well-established antimicrobial. It is the principal fermentation product of the yeast *Saccharomyces cerevisiae* and plays a central role in the microbiological safety of alcoholic beverages, where it is often the most important single hurdle in a series that can also include heat treatment, low pH, anaerobic conditions, and elevated carbon dioxide levels. Yeast activity also removes the mycotoxin patulin from apple juice (see Chapter 5).

Heterofermentative LAB will also produce ethanol, but in lower concentrations. The levels produced are inversely related to the amount of acetic acid produced because these compounds represent alternative fates of acetyl phosphate in the heterofermentation pathway. In addition to any direct inhibitory effect, ethanol has also been shown to augment the lethal effect of low pH and lactic acid in *E. coli* O157, though at higher concentrations than would be likely to occur in lactic fermentations.[49] Heterofermenters dominate during the initial phase of several natural fermentations and ethanol may, like other products of heterofermentation, contribute in these early stages to setting the fermentation on its desirable course.

### *Reuterin and Other Low Molecular Weight Compounds*

A number of low molecular weight compounds with antimicrobial activity have been isolated and identified from culture filtrates of LAB. The most studied to date has been reuterin, β-hydroxypropanal. This is present in stationary phase cultures of strains of the heterofermenter, *Lactobacillus reuteri,* when grown anaerobically on a mixture of glucose and glycerol or glyceraldehyde. Reuterin exists in three forms—the alde-

hyde, its hydrate, and a cyclic dimer—and has very broad spectrum antimicrobial activity against bacteria, fungi, protozoa, and viruses. The relative activity of the different forms is not known, although they are thought to act by inhibiting sulfhydryl-containing enzymes.[24]

The addition of reuterin to foods such as minced beef, milk, and cottage cheese has been shown to control coliform growth and to inactivate *L. monocytogenes* and *E. coli* O157.[24,32] However, its role in fermented foods is uncertain. One study where *L. reuteri* and glycerol were added to herring fillets to stimulate reuterin production *in situ* showed some potential to reduce growth of the gram-negative flora present. In other cases where the incorporation of *L. reuteri* in foods has been described, in a fermented milk and an Emmental-type cheese, its presence has been for its potential probiotic effect rather than for reuterin production in the food.[28,84]

Pyroglutamic acid, 2-pyrrolidone-5-carboxylic acid, is a natural constituent of some plant foods and fermented products such as soy sauce and can be produced by the thermal dehydration of glutamic acid.[5,94] It has been shown to be produced by a range of LAB, including strains of *L. casei, L. helveticus, Streptococcus bovis,* and unspecified pediococci.[19,41,93] Pyroglutamic acid is active primarily against gram-negative bacteria such as *Pseudomonas* and *Enterobacter.* Its activity against gram-negative pathogens has not been reported, although it is inactive against the gram-positive pathogens *L. monocytogenes* and *S. aureus.*[41]

A number of other low molecular weight compounds with antimicrobial properties have been identified in culture filtrates from *L. plantarum* cultures. These include mevalonolactone, methylhydantoin, and benzoic acid.[67] They have been shown to act synergistically with lactic acid against *Pantoea (Enterobacter) agglomerans,* but their significance in food fermentations remains to be established.

### *Nutrient Depletion and Overcrowding*

The absence of inhibitory agents is a necessary but insufficient condition for microbial growth. The microbial cell must also have sufficient space and nutrients available to it. Foods will rarely offer microorganisms a uniform medium, and numerous microenvironments may exist where conditions differ substantially from the bulk properties of the material. This is a critical factor in the microbiological stability of butter and similar spreads, where microorganisms are confined in aqueous droplets dispersed in the continuous fat phase. The presence of large numbers of LAB in a fermented food will deplete it of readily available nutrients and occupy space that might have been available to other less desirable organisms. This is supported by the recent observation that filtration of a fermented broth to remove cells of *Lc. lactis* decreased the subsequent inhibition of *E. coli.*[13] It is an area that is only just beginning to be explored.

## OVERALL SIGNIFICANCE OF DIFFERENT ANTIMICROBIAL FACTORS

To describe the combined effect of a number of antimicrobial factors, the analogy of a hurdle

**Table 2–3** Shelf Life and Composition of Fermented Fish Products from Thailand[80]

| *Product* | *Salt (%)* | *pH* | *Shelf Life* |
|---|---|---|---|
| Pla-som | 2.3–5.9 | 4.0–4.6 | 3 weeks |
| Som-fug | 2.5–5.8 | 4.1–5.0 | 2 weeks |
| Pla-chom | 3.8–4.8 | 5.0–6.1 | 2 weeks |
| Pla-chao | 4.4–9.5 | 4.0–5.3 | 1–2 years |
| Pla-paeng-daeng | 4.5–9.2 | 3.9–5.2 | 6–12 months |
| Pla-ra | 7.8–17.9 | 4.7–6.2 | 1–3 years |

race has been used. Perhaps nowhere in food preservation is the concept of multiple barriers or hurdles more appropriate than in fermented foods. The LAB themselves can contribute a number of different antimicrobials, and microbial activity is often combined with other inhibitory factors such as salting or partial drying.

It is possible to make some generalizations regarding the relative importance of these various factors. Certainly the predominant microbial factor is the production of organic acids and the reduction in pH, although the others described here could all contribute to the aggregate effect, particularly to ensuring a successful early dominance of LAB. Reduction in $A_w$ generally plays a much more significant role in the inhibition of microbial growth compared to bacterial acid production. This is illustrated by data on the shelf life of fermented fish products in Thailand and their composition, where differences in salt content have greater impact on product shelf life (Table 2–3). Microbial activity is, however, probably more important and effective at reducing numbers of microbial hazards.

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