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William H. Sperber · Michael P. Doyle
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Compendium of the Microbiological Spoilage of Foods and Beverages

Foreword by R. Bruce Tompkin

Springer

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Foreword

The increased emphasis on food safety during the past two decades has decreased the emphasis on the loss of food through spoilage, particularly in developed countries where food is more abundant. In these countries spoilage is a commercial issue that affects the profit or loss of producers and manufacturers. In lesser developed countries spoilage continues to be a major concern. The amount of food lost to spoilage is not known. As will be evident in this text, stability and the type of spoilage are influenced by the inherent properties of the food and many other factors.

During the Second World War a major effort was given to developing the technologies needed to ship foods to different regions of the world without spoilage. The food was essential to the military and to populations in countries that could not provide for themselves. Since then, progress has been made in improved product formulations, processing, packaging, and distribution systems. New products have continued to evolve, but for many new perishable foods product stability continues to be a limiting factor. Many new products have failed to reach the marketplace because of spoilage issues.

Disruptions in the food supply are more severely felt by countries that depend on readily available low-cost food. For example, the increased diversion of corn to produce fuel, in combination with other factors, led to higher food prices after 2007 and reduced the ability of international agencies with limited budgets, e.g., the Food and Agriculture Organization, to provide food assistance. In addition, certain countries limited exports to ensure a stable food supply for their populations. This experience demonstrates the dependence of many countries on assistance to bolster their food supply and the significance of barriers to international trade.

The world's population continues to increase. In 1960, 1980, and 2000 the population was estimated to be 3.0, 4.5, and 6.1 billion, respectively. It is projected to reach 6.9 and 9.5 billion by 2010 an 2050, respectively.[1] To provide for the population increase, improvements in food production and protection against spoilage will be required.

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[1] U. S. Census Bureau. (2008). International database. Total midyear population for the world: 1950–2050. Accessed on 24 September 2008. http://www.census.gov/ipc/www/idb/worldpop.html

Food production, processing, and distribution systems generally fall into two categories: large or small scale. Large-scale systems incorporate new technologies more quickly and can lead to innovations that bring products of greater variety and convenience to consumers. This segment of the industry is generally more highly regulated and its suppliers are frequently audited by large corporations. Proper coding and inventory control is essential to minimize product loss due to spoilage. Sell-by or use-by dates are commonly applied to indicate the date the food will be acceptable and to facilitate traceability. Larger companies strive to improve control of their incoming raw materials and processing and packaging conditions to ensure compliance with their code-dating procedures and in some cases further delay spoilage. Products that exceed the sell-by or use-by dates are discarded by retailers. The amount discarded is documented by the retailer and can influence future negotiations between supplier and retailer. Continued spoilage problems can lead retailers and others to discontinue the item.

Manufacturers may apply special procedures that enable them to meet the expected demand for their perishable products at certain holidays. For example, this could involve accumulating and holding certain perishable foods at temperatures closer to freezing. As the holiday approaches, the food is released for shipment to retailers. Success requires knowledge of the product, the impact of lowering storage temperature on microbial growth, and validation that the procedure will be successful. Failure to validate the procedures can lead to significant financial losses during a critical season and temporary loss of consumer confidence.

Another characteristic of large-scale systems is that processing occurs in fewer facilities and the products are shipped longer distances. While this may be economically beneficial for the manufacturer, greater control must be exercised to transfer food from the manufacturer to the ultimate user without spoilage.

Considerable advances have been made in delaying or preventing spoilage. For example, this writer spent about 40% of his time solving spoilage issues associated with raw and cooked perishable meat and poultry products from the mid-1960s to the early 1990s. The collective effect of the improvements, for example, in processing conditions, formulation, packaging, control of temperature, and efforts to control *Listeria* and *Salmonella* reduced this time to well below 5%.

It is of interest that as the quantity of foods produced on a larger scale has increased, there is a desire by some consumers to return to foods produced on a smaller, more local scale. This desire is based on the perception that the foods are fresher, less processed, and more wholesome. It has not been documented, however, whether this approach results in a greater or lesser amount of food lost through spoilage on a worldwide basis.

Smaller scale systems are slower to accept and may even reject new technologies. The smaller businesses generally lack the technical knowledge and support available in larger companies. Thus, it is not surprising that many of the authors are involved with large companies and have collaborated with other experts in preparing this text.

LaGrange, IL, USA R. Bruce Tompkin

Preface

Protection of foods and beverages from microbiological spoilage is essential to assure an adequate food supply for the world's population. Several generations of food microbiologists have labored to understand food spoilage and to develop control procedures for its prevention. Because many of these highly experienced food microbiologists are at or near retirement age, we were motivated to organize this *Compendium* in an effort to document and preserve as much of their accumulated knowledge and wisdom as possible. We are pleased that many expert food microbiologists eagerly agreed to contribute to this effort. To our knowledge, this is the first reference and textbook focused exclusively on the microbiological spoilage of foods and beverages.

We also think that this *Compendium* is necessary now because the resources of the food industry and academia have increasingly become focused on food safety initiatives over the past 30 years. To a significant extent, resources previously available to develop an understanding and the means to control food spoilage have been shifted into food safety programs. The emergence of prominent foodborne pathogens, such as *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Campylobacter,* combined with increased competition for limited financial resources, has resulted in decreased attention being given to food spoilage research. Global public health issues such as bovine spongiform encephalopathy and avian influenza H5N1, and their potential impacts on the food supply, have further reinforced the shift toward "mission-oriented" research. The increased number of potential microbiological food safety issues affecting the food supply also fueled a substantial increase in the number of food safety regulations and policies, both at the national and the international levels. Moreover, food regulatory actions are almost always related to food safety controls and requirements, thereby commanding a larger share of the food industry's technical resources to assure regulatory compliance.

The shift in emphasis from food quality research toward various types of food safety programs is understandable and necessary. This shift, however, is not as counterproductive for food quality and spoilage research as might first be suspected. The implementation of numerous new food safety control procedures and regulations can also help to reduce food spoilage and protect product quality through its shelf life as they also provide greater assurance of food safety. For example, pasteurization treatments intended to eliminate pathogens in raw milk also significantly

enhance the quality and shelf life of fluid milk. In fact, the unanticipated enhancement of product quality was a very strong selling point in gaining the food industry's acceptance of the hazard analysis and critical control point (HACCP) system of food safety management in the 1970s. Because of the successful development of HACCP, there remains today a very strong link between food quality and food safety control measures.

We are further motivated to develop this Compendium because, ultimately, the control of food spoilage means more than simply providing high quality, convenient, processed foods for consumption in economically developed regions of the world. We must think about feeding people in every region of the world. Food spoilage is a significant threat to food security, our ability to provide an adequate food supply to a large and increasing global human population. Shrinking fossil fuel and water reserves, soil erosion, loss of soil fertility, climate change, and political uncertainty are important factors that collectively threaten food security. If food spoilage and other factors that contribute to the waste of food could be substantially reduced, we would be able to feed more people without increasing primary food production. In the opinion of a former World Health Organization official, "This large increasing world population needs food and we have a moral obligation to utilize all our skills and technologies to increase not only food production but also *to limit food spoilage* (italics added for emphasis)."[1] Together with many of our colleagues, we share Dr. Käferstein's sense of professional responsibility. We anticipate that this *Compendium* will play a role in the global reduction of food spoilage and the accompanying enhancement of food security.

In 1958 professor William C. Frazier first published his widely used textbook, *Food Microbiology*. His comprehensive yet concise explanations of food spoilage and food safety were prominent features in the education of several generations of food microbiologists, including this *Compendium*'s editors. It is our sincerest hope that this *Compendium* will provide similar benefits to future generations of food microbiologists.

Minnetonka, MN, USA — William H. Sperber
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[1] Käferstein, F. K. (1990). Food irradiation and its role in improving food safety and the security of food. *Food Control 1,* 211–214.

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Introduction to the Microbiological Spoilage of Foods and Beverages

William H. Sperber

Introduction

Though direct evidence of ancient food-handling practices is difficult to obtain and examine, it seems safe to assume that over the span of several million years, prehistoric humans struggled to maintain an adequate food supply. Their daily food needed to be hunted or harvested and consumed before it spoiled and became unfit to eat. Freshly killed animals, for example, could not have been kept for very long periods of time. Moreover, many early humans were nomadic, continually searching for food. We can imagine that, with an unreliable food supply, their lives must have often been literally "feast or famine." Yet, our ancestors gradually learned by accident, or by trial and error, simple techniques that could extend the storage time of their food (Block, 1991). Their brain capacity was similar to that of modern humans; therefore, some of them were likely early scientists and technologists. They would have learned that primitive cereal grains, nuts and berries, etc. could be stored in covered vessels to keep them dry and safer from mold spoilage. Animal products could be kept in cool places or dried and smoked over a fire, as the controlled use of fire by humans is thought to have begun about 400,000 years ago. Quite likely, naturally desiccated or fermented foods were also noticed and produced routinely to provide a more stable supply of edible food. Along with the development of agricultural practices for crop and animal production, the "simple" food-handling practices developed during the relatively countless millennia of prehistory paved the way for human civilizations.

Less than 10,000 years of recorded history describes the civilizations that provided the numerous advances leading to our modern civilization. Chief among these advances were the development of agricultural and food preservation technologies that permitted large human populations to live permanently in one place and use their surplus time to develop the other technologies we enjoy today, such as writing

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this chapter on a laptop computer while sitting in a heated office on a Minnesota winter evening.

Yet, for most of this 10,000-year period, food preservation was accomplished by quite simple, but not completely effective, technologies. These typically involved the use of the techniques that had been put into practice countless years earlier – drying, salting, smoking, fermentation, and cool storage when possible. Only in the past 200 years of our long existence have we humans developed more advanced technologies for advanced food production, preservation, and distribution. Preservation of some foods by canning began in the early nineteenth century. In the middle of that century, Louis Pasteur and the first microbiologists began to understand and control the microbiological causes of disease, foodborne illness, and food spoilage.

Another century elapsed before the emergence of major advances leading to the widespread availability of fresh and processed foods. The most significant advances, after 1945, were the development of reliable mechanical refrigeration systems, logistical systems for the refrigerated transportation and distribution of food, and widely available home refrigerators and freezers. Numerous refinements continue to improve the microbiological quality of our food supply today. Additional refinements will certainly be made in the future.

In the past several decades, we have also made substantial improvements in food production and management systems. National governments and the food industry promulgated and implemented Good Manufacturing Practices (GMPs) in the United States, which are called Good Hygienic Practices (GHPs) in the rest of the world. In particular, those GMPs related to employee practices, sanitary design of food production facilities and equipment, and cleaning and sanitation procedures have improved food quality. Similarly, the HACCP (hazard analysis and critical control point) system, while developed to assure food safety, has also improved food quality. The HACCP system entails three broad and essential functions – product design, process control, and management accountability (Troller, 1993; Mortimore & Wallace, 1998). These topics will be handled in greater detail later in this chapter and in several of the following chapters.

Additional food regulations and industry practices have been implemented to reduce the public health threat posed by particular foodborne pathogens. While this compendium is focused solely on the microbiological spoilage of foods, regulations and practices that are used to improve public health protection against foodborne pathogens will also improve the microbiological quality of food, thereby reducing the incidence of microbiological spoilage and extending the shelf life of foods.

Food Loss Data

Despite the advanced technologies that support our modern civilization, a large proportion of our food supply is nevertheless lost to spoilage or otherwise wasted. The Economic Research Service (ERS) of the United States Department of Agriculture (USDA) has extensively documented the percentage of food losses in the food chain from primary production through consumption (ERS, 2005). This research was done

Table 1 Percent loss of the United States' food supply from primary production through consumption (abstracted from ERS/USDA, Feb. 1, 2005)

	Data for 2003 based on pounds per capita/year				
Commodity	Primary weight	Retail weight	Consumer weight	Consumed weight	Percent total loss
Meat, poultry, and fish					
Red meat	161	112	104	68	58
Poultry	113	71	66	41	64
Fish, shellfish	16	16	11	11	31
Grain and cereal products	194	194	171	136	30
Sweeteners	142	142	126	101	29
Eggs and egg products	253	250	232	197	22
Dairy products					
Fluid milk, yogurt	194	194	171	137	30
Cheese	28.3	28.3	26	22.1	22
Frozen	26.7	26.7	23.5	18.8	30
Dried	3.8	3.8	3.3	2.6	30
Fats and oils	102	102	82	68	33
Fruits					
Fresh	127	121	106	53	58
Dried	10	2.4	2.2	2	80
Canned	17	13.4	12.6	11.3	33
Frozen	3.9	3.5	3.3	3	24
Vegetables					
Fresh	196	181	160	86	56
Frozen	79	39	37	26	67
Canned	101	47	44	40	60
Dried	16.9	2.3	2.2	2	88
Potato chips	17.2	4.3	4.1	3.7	79
Peanuts and tree nuts	9.3	9.3	8.8	7.9	15
Pounds/year	1811	1597	1396	1037	
Pounds/day	4.96	4.38	3.82	2.84	

to support the development of the Food Guide Pyramid (MyPyramid) serving sizes. The percent losses for all food categories during 2003 in the United States are summarized in Table 1. All data are presented as pounds per capita/year. The Primary Weight column refers to the product weight as it leaves the processing plant, for example, boned meat products, trimmed vegetables, etc. The retail weight is the amount of food purchased at retail, the consumer weight is the amount of food available for consumption at home or at food service establishments, and the consumed

weight is the amount of food actually eaten. Food losses can occur from insect or rodent damage, microbiological spoilage, chemical and physical spoilage, losses in transportation, further processing, product discarded at the end of shelf life, and plate waste. According to these data, about five pounds of food are processed each day for each person in the United States. Only about three pounds are consumed, indicating an average food loss for all categories of about 40%. ERS economists feel that the reported data tend to underestimate the actual amount of food losses.

It is not possible to tell from the current data what proportion of the food losses could be attributed to microbiological spoilage. According to ERS economists, this capability may be developed in the near future. Under any circumstances, it would be difficult to know the proportion of microbiological food spoilage with a high degree of precision. The World Health Organization estimated that in developing countries the loss caused by spoilage microorganisms ranges from >10% for cereal grains and legumes to as much as 50% for vegetables and fruits (Käferstein, 1990). The other food commodities fall within this range. Todd (1987) points out that worldwide postharvest food losses are caused more by insects and rodents than by microorganisms. Of course, microorganisms are still important in food losses, with fungi representing the most important group of spoilage microorganisms responsible for food losses.

Microorganisms and Mechanisms Involved in Spoilage

Sources of Contamination

Preharvest Contamination

The sources of microbiological contamination are practically everywhere in the earth's biosphere, in or on plants, animals, soil, and water. Many types of bacteria, such as pseudomonads, lactics, micrococci, and coliforms, grow readily on agricultural and horticultural plants. Many of these and other types of bacteria, particularly the enterics, also colonize animals, both on the skin or hide and in the gastrointestinal tract. The resident bacteria on both plants and animals can be carried along with the raw materials during harvest, slaughter, and processing and remain in the food products derived from these sources (Frazier, 1958).

Soil is an obvious source of contamination, as a diverse community of microorganisms – bacteria, yeasts, molds, actinomycetes, etc. – thrive in most soils and can grow to very large numbers. Direct contamination with soil microorganisms occurs during production and harvesting. Indirect contamination with soil occurs through the deposition of wind-borne dust particles. Wind-borne mold spores, for example, are a very common cause of mold spoilage of foods, as well as human allergies.

Water can serve as a source and a vector of contamination. Pseudomonads, in particular, grow well in surface waters, whereas the enteric bacteria are present in sewage and waters polluted with sewage. Water can serve as a vector of contamination, especially if polluted surface waters are sprayed onto crops for irrigation or used in primary produce processing.

Postharvest Contamination

Many raw materials and foods have a structural integrity that protects most of their mass from microbial contamination (Frazier, 1958). The endosperm of cereal grains is protected from contamination by a tough bran layer. The shells of eggs and nuts protect the interior of these foods. When intact, the skin or the rind that covers fruits and vegetables keeps the interior of the produce largely free from external contamination. Similarly, most animal flesh is sterile in its natural state, being protected by skin or hide. Therefore, most of the microorganisms in the raw materials of our food supply are present only on the exterior of the food or in the gastrointestinal tract in the case of animals. When you think about it, even the gastrointestinal tract is essentially outside of the animal as well. Therefore, living muscle tissues and other interior structures are usually sterile.

The first steps of primary processing violate the natural sterility of the interior parts of our raw food materials. The milling of cereal grains removes most of the exterior microorganisms with the bran, but some of these microbes will be relocated into the otherwise nearly microbe-free endosperm. Trimming, chopping, or crushing of fruits and vegetables will similarly contaminate the interior portions with those microorganisms existing on the exterior. The most prolific possibility of interior contamination exists in animal slaughter operations. The feces of animals contain exceedingly high numbers of microorganisms, $>10^{11}$ cells/g feces. If the gastrointestinal tract is not carefully removed during slaughter, very high contamination of the muscle tissue could occur. In the case of meat production, the first slaughter operations contaminate the surface of the exposed muscles to some extent. Further fabrication (cutting) of the carcass into prime cuts can spread the initial contamination across larger product areas. The grinding of meat will spread exterior contamination essentially throughout the entire muscle mass.

During further processing, additional contamination can occur when workers handle the food. Contamination can occur from unclean hands or gloves and uniforms. Human contamination of foods can also occur when talking, coughing, or sneezing creates aerosols. In-process foods can be further contaminated by cross-contamination with raw materials and by contact with unclean food-handling utensils and processing equipment. There are also several points of waterborne contamination in food-processing plants. The most direct means of potential contamination is the use of water as a food ingredient. If the food plant's water supply is not potable, significant contamination with spoilage microorganisms could occur. A major indirect source of waterborne contamination may exist during cleaning and sanitation operations, since the use of water is essential for most of these operations. The use of high-pressure hoses to clean floors creates aerosols containing bacteria that were present, and likely growing, on the floor or the process equipment. The bacteria-containing aerosols can drift through the air and directly contaminate raw materials and in-process foods if these are not removed or adequately protected before cleaning commences, or they can indirectly contaminate food after they are deposited on the food-processing equipment. Another inadvertent source of water contamination may be presented by condensate that is formed

in refrigeration units and can be spread by the ventilation systems in the food-processing plants.

Ecology of Microbiological Spoilage

The many kinds of microorganisms that can grow on food have evolved biochemical mechanisms to digest components of the food, thereby providing energy sources for their own growth. However, in a given type of food, usually only one or a few types of microorganisms will grow sufficiently well to become the predominant spoilage organisms (Mossel & Ingram, 1955). Parameters, such as pH, water activity, and storage temperature to name a few, exert intensive selective pressures on the original food microflora. The driving forces that guide the selection of predominant spoilage microorganisms will be detailed later in this chapter in sections "Intrinsic Factors to Control Microbiological Spoilage" and "Extrinsic Factors to Control Microbiological Spoilage."

Microorganisms Involved in Spoilage

It is useful to consider the types of microorganisms involved in food spoilage in two ways. The first way is a consideration of laboratory tests and biochemical features that are used to broadly characterize and differentiate microorganisms. The second way is to describe the groups of similar microorganisms that are involved in food spoilage.

Means to Characterize and Differentiate Microorganisms

Morphology. A microscope was the first tool with which early microbiologists could begin to understand microorganisms. The microscope enabled the observation of the size and shape, or morphology, of microbial cells. Bacterial cells usually appear as cylindrical rods or spheres. The bacterial rods, often called bacilli, are typically about 1 micrometer (μm) in diameter and 2–6 μm long. The spherical cells, usually called cocci, are typically about 1 μm in diameter. Yeast cells are larger than bacterial cells. They are elliptical and usually about 3–5 μm long. Molds have two predominant morphological features: individual hyphae, which can collectively form a visible mycelial mat, and sporangia, which contain very high numbers of individual spores, each of which is capable of starting a new mold colony. The hyphae are about 15 μm in diameter and are of indefinite length. The individual spores are about 1 μm in diameter.

Gram stain. The Gram stain is a differential staining technique that permits the microscopic determination of a bacterium as either "Gram positive" or "Gram negative." This procedure consists of four steps – initial staining with crystal violet, fixation with iodine, decolorization with ethanol, and counterstaining with safranin. A fundamental difference in the composition of cell walls of bacteria is responsible for the differential results of the Gram stain. Gram-positive cells are not decolorized

by ethanol and retain the original blue color of crystal violet. Gram-negative cells are decolorized by ethanol and take up the red color of safranin.

Ability to form endospores. Several genera of bacteria are able to form an internal structure, or endospore, that is very heat resistant and capable of surviving in quite adverse environments. Yeast and molds form spores, different from bacterial endospores, that are not significantly more heat resistant than their vegetative cells. However, the fungal spores help the fungi survive in dry environments.

Temperature relationships. The temperature range in which they can grow often characterizes microorganisms. Psychrophiles grow well at cold temperatures, as low as 0°C, and often cannot grow above 20°C. Thermophiles grow best at high temperatures, in the range from 45°C to 70°C. Mesophiles grow best at the intermediate temperatures between 20°C and 45°C. There have been some efforts to further divide the psychrophilic microorganisms by creating a category called "psychrotrophs," but such a consideration is beyond the practical needs of this compendium.

Oxygen relationships. Aerobic microorganisms can grow in the presence of oxygen, while anaerobic microorganisms can grow in the absence of oxygen. The use of the term "obligate," as in "obligate aerobe," means that the microbe requires some level of oxygen for growth. The term "obligate anaerobe" refers to a microbe that cannot grow if any amount of oxygen is present. The term "microaerophilic" refers to microorganisms that can grow best when only a small amount of oxygen is available. Facultative with respect to oxygen, a term that applies to most microorganisms, refers to the ability to grow either with or without the availability of oxygen. Additionally, each food has a chemical oxidation–reduction (O/R) potential that is somewhat analogous to the situation described here for atmospheric oxygen content. Aerobic microorganisms grow best at positive O/R values, while obligate anaerobes require negative O/R values for growth.

Type of metabolism. Metabolically speaking, microorganisms can usually be characterized as having either an oxidative or a fermentative type of respiration for the production of energy. This trait is linked both to the oxygen relationships described above and to the evolutionary stature of the particular microorganism. Fermentative metabolism is a relatively primitive anaerobic process in which carbohydrates are metabolized to organic acids and alcohols. Oxidative metabolism is an advanced aerobic process in which carbohydrates may be completely metabolized to carbon dioxide and water. Microorganisms with this capability are usually oxidase positive, possessing the same intracellular electron transport system that is present in higher life forms, including humans. Microbes lacking this capability are often also catalase negative, lacking this enzyme to degrade peroxides that can be formed in anaerobic metabolism.

Water relations. The addition of solutes to a growth medium diminishes a microorganism's ability to grow as the osmotic pressure of the medium is increased, and the water activity of the medium is reduced. Several terms describe microbes that can accommodate reduced water activities. "Osmophile" generally refers to any organism that grows at increased osmotic pressure, and specifically to yeasts that can grow at very high sugar concentrations. "Halophile" refers to organisms, usually

bacteria, which grow at high salt concentrations, even in saturated sodium chloride solutions. "Xerotrophic" refers to organisms that grow under dry conditions. This term is often applied to molds that grow in relatively dry cereal products. The term "osmotolerant" can be used to describe those microorganisms that are capable of growth in reduced water activity, or "intermediate moisture" foods, generally in the range of water activity from 0.85 to 0.95.

pH relations. Most foodborne microorganisms grow best at relatively neutral pH values, in the range of pH 6.0–8.0. None of these grow at extremely high pH values, but some can grow at pH values as low as 0.5–2.0. Those that can grow at such low pH values are called "acidophiles." Those that cannot grow, but can tolerate low pH values without being killed, are called "acidurics."

Groups of Microorganisms Involved in Spoilage

The nearly countless microbial genera that can be involved in food spoilage are organized here in 11 groups. The first two groups are fungi, and the remaining groups are bacteria. Specific information about the fungi can be found in Pitt and Hocking (1997) and Deak and Beuchat (1996). Information about Gram-negative bacteria can be found in Krieg and Holt (1984) and about Gram-positive bacteria in Sneath, Mair, and Sharpe, (1986).

Molds. Capable of growth across a broad range of temperatures, molds are obligate aerobes with oxidative metabolism. Particular genera are also capable of growth across the range of water activity from 0.62 to nearly 1.0. Molds are the most common food spoilage microorganisms at every step of the food chain from field crops to consumer food products. Remarkably, they are even capable of spoiling bottled mineral water (Criado, Pinto, Badessari, & Cabral, 2005). Representative genera of food spoilage molds are *Penicillium, Aspergillus*, *Rhizopus, Mucor, Geotrichum, Fusarium, Alternaria, Cladosporium, Eurotium*, and *Byssochlamys.*

Yeasts can be described in two broad categories: fermentative and oxidative. Yeasts are generally mesophilic and grow best above water activity values of 0.9. Both molds and yeasts grow at slower rates than bacteria. Spoilage of perishable foods by these microorganisms often indicates that the food has simply been "stored too long."

Fermentative yeasts. The most commonly known spoilage yeasts are facultatively anaerobic fermentative organisms, producing ethanol and carbon dioxide from simple sugars. Some fermentative yeasts are the most osmophilic organisms known, capable of slow growth at water activity 0.60 (Martorell, Fernández-Espinar, & Quereol, 2005). Representative genera include *Saccharomyces* and *Zygosaccharomyces.*

Oxidative yeasts. Less common are the aerobic "film yeasts" which can grow on fermented foods and metabolize organic acids and alcohols. These yeasts seem to occupy an evolutionary middle ground between fermentative yeasts and molds, possessing the morphological characteristics of yeasts and the metabolic characteristics of molds. Representative genera include *Mycoderma, Candida, Pichia,* and *Debaryomyces.*

Pseudomonadaceae. The principal genera in this family of bacteria, *Pseudomonas* and *Xanthomonas,* are Gram-negative rods, nonspore forming, psychrophilic, aerobic, and oxidase positive. They are also completely intolerant of reduced water activity, growing in foods mostly above water activity 0.98. The addition of small amounts of solutes, such as 2% sodium chloride, will substantially restrict their growth. Pseudomonads are primary spoilage microorganisms in fresh meat, poultry, seafood, and eggs.

Neisseriaceae. Like the pseudomonads, the microbes in this family are Gram-negative rods, nonspore forming, aerobic, and catalase positive. The spoilage genera are *Acinetobacter* (oxidase negative) and *Moraxella*(oxidase positive). Some strains of *Acinetobacter* are psychrophilic.

Enterobacteriaceae. This family of Gram-negative rods is facultatively anaerobic, fermentative, mesophilic, nonspore forming, oxidase negative, and catalase positive and is generally incapable of growth below water activity 0.95. All of the 28 genera in this family are commonly called "enteric" bacteria and ferment glucose with the production of acid and gas. A subset of this family, containing about half of the genera, is commonly called "coliform" bacteria, as established by their ability to ferment lactose with the production of acid and gas. Representative spoilage genera include *Escherichia, Erwinia, Enterobacter, Citrobacter, Serratia*, and *Proteus.* Enteric bacteria are often involved in the spoilage of fresh vegetables, meat, poultry, fish, and eggs.

Micrococcaceae. The two principal genera of bacteria in this family are *Micrococcus* and *Staphylococcus*. They are Gram positive, spherical, catalase positive, and mesophilic. *Micrococcus* is oxidative, growing on glucose without the production of acid or gas, while *Staphylococcus* is fermentative, producing both acid and gas from glucose. *Staphylococcus* is osmotolerant. Both the genera are commonly involved in the spoilage of fresh produce and processed meat, poultry, and seafood.

Lactic Acid Bacteria. All members of this group are Gram positive, catalase negative, microaerophilic or facultatively anaerobic, and fermentative. Homofermentative lactics ferment glucose with the production of lactic acid only. Heterofermentative lactics ferment glucose with the production of lactic acid, carbon dioxide, and ethanol or acetic acid. *Lactobacillus* is rod shaped, while *Streptococcus, Lactococcus, Leuconostoc, Enterococcus*, and *Pediococcus* are spherical. The "lactics" are generally mesophilic and grow at water activity values above 0.9. The growth of lactics in meat, vegetable, and dairy products is used to advantage to produce fermented foods such as salami, sauerkraut, and cheese. However, the growth of these bacteria in the same fresh foods, such as luncheon meats, vegetable salads, and fluid milk, constitutes spoilage.

Coryneforms. These microorganisms, of relatively minor importance in food spoilage, are sometimes involved in cheese spoilage. Representative genera are *Corynebacterium* (facultatively anaerobic) and *Brevibacterium* (aerobic). Both are Gram positive and catalase positive. Their sources of contamination are usually soil, animals, or humans.

Spore-forming Bacilli. There are three major genera of bacterial sporeformers important in food spoilage – *Bacillus, Clostridium*, and *Alicyclobacillus*. All

are Gram-positive rods and are generally mesophilic or thermophilic. Because these genera produce heat-resistant endospores, they are the predominant spoilage microorganisms in pasteurized foods in which all vegetative cells have been killed and in improperly sterilized foods.

Bacillus species are aerobic or facultatively anaerobic, catalase positive, and generally not osmotolerant. While most species are mesophilic, individual species cover the entire temperature spectrum for food spoilage. *Bacillus cereus* can spoil pasteurized milk (psychrotrophic), *B. subtilis* can spoil bakery products (mesophilic), and *B. stearothermophilus* can spoil canned foods (thermophilic).

Clostridium species are obligate anaerobes, catalase negative, and not osmotolerant. They are typically involved in the spoilage of foods that have a highly negative O/R potential, such as canned or vacuum-packaged foods. The principal spoilage species are *C. sporogenes* and *C. butyricum* (mesophilic) and *C. thermosaccharolyticum* (thermophilic).

Alicyclobacillus species were discovered in the 1960s and originally classified as *Bacillus* spp. First isolated from acid hot springs in Yellowstone Park, these bacteria typify a significant new ecological grouping of microorganisms called "extremophiles."

Quite unlike all other foodborne bacteria, alicyclobacilli are extreme acidophiles, growing within a pH range of about 2.0–6.0. They are moderate-to-obligate thermophiles, catalase positive, and microaerophilic. Like pseudomonads, the alicyclobacilli cannot tolerate osmotically increased environments, that is, below water activity of 0.98. They have evolved to grow in acid and hot water, and it is these types of foods that they can spoil. The principal spoilage species *A. acidoterrestris* is sometimes involved in the spoilage of pasteurized fruit or vegetable juices that have been improperly cooled or stored at relatively high temperatures, above 30°C.

Microbiological Food Spoilage Mechanisms

The microbiological spoilage of foods occurs because of the biochemical activity of microorganisms as they grow in the food. The consumer is usually alerted to the existence of spoilage by changes in the food's appearance, odor, texture, or taste. While food spoilage may be universally considered to be undesirable, it affords perhaps one protective advantage for consumers. Food spoilage is usually an indicator that a food has been improperly handled or stored too long. Such mishandling could permit the growth of foodborne pathogens that could cause illness or death if the food were to be consumed. Since foodborne pathogens do not typically give an organoleptic indication of their presence, the organoleptic changes caused by spoilage microorganism serve as a warning to the consumer that the food could be unsafe for consumption. It can be argued that spoilage microorganisms routinely protect millions of people from foodborne illness (Frazier, 1964. personal communication).

The protective feature of food spoilage does not always protect the consumer from the threat of foodborne illness, of course. A main reason for this fact is that microbiological spoilage of foods is not organoleptically detectable until a substantial growth of the spoilage organism has occurred. Typically, the threshold level for observation of food spoilage by odor, taste, or sight is not reached until the spoilage microflora exceeds about 10^7 organisms/g of food. A secondary reason for the failure of the spoilage warning signal to protect consumers is the fact that many people, because of ignorance, frugality, or sheer necessity, will consume even obviously spoiled food.

Spoilage characteristics develop in food as microorganisms digest the food to support their growth. The digestion of sugars, complex carbohydrates, proteins, and fats can all produce undesirable effects in the food if the spoilage microorganisms grow to significant levels.

Sugar fermentation with acid production. A number of catabolic pathways are used by bacteria to metabolize pentoses and hexoses for energy production. Lactic acid is the principal product of these pathways. Its production, often by lactic acid bacteria, yields a sour taste in the food. To a limited extent, some enteric bacteria could also cause this type of spoilage. As a matter of practical interest, the production of lactic acid during spoilage usually lowers the pH of the food, thereby providing further protection against the growth of the foodborne pathogens mentioned above, should any be present. Sugar fermentation by bacteria can occur with (heterolactic) or without (homolactic) the production of a gas, typically carbon dioxide.

Sugar fermentation with gas production. The catabolism of hexoses by fermentative yeasts produces ethanol and carbon dioxide. Relatively low pH and high sugar-containing products would support this type of spoilage. The typical yeast spoilage defect in products such as sugar syrups and tomato products in hermetically sealed packages is caused by gas production. In these instances, flexible product containers will expand, while rigid containers may explode. This type of spoilage is one of the few that violates the common observation that high numbers of microorganisms are required to cause spoilage. It has been observed that over several months of storage, yeast spoilage by gas production can occur in products that never exceed a yeast population of about 10^4 cells/g (Sperber, unpublished data). Nongrowing yeast cells can remain metabolically active, producing ethanol and carbon dioxide.

Protein hydrolysis. Many spoilage bacteria produce proteolytic enzymes that hydrolyze proteins in foods such as milk, meat, poultry, and seafood products. Anaerobic proteolysis by *Clostridium* spp. can result in a noxious putrefaction of the food. Pseudomonads can carry the proteolysis one step further by metabolizing amino acids to produce very foul-smelling compounds, such as the aptly named putrescine and cadaverine.

Digestion of complex carbohydrates. Produce spoilage can be caused by bacteria and molds that produce pectinases. These enzymes digest the pectin layer between the plant cell walls, resulting in a soft or mushy texture. One such spoilage organism that is very appropriately named is *Erwinia carotovora* (carrot eating). When accompanied by proteolytic activity, mushy produce will also develop a foul odor.

Amylolytic enzymes produced by molds and several bacteria digest starches to polysaccharides and simple sugars. This activity will destroy the viscosity of products in which starches are used as thickening agents, such as gravies and pie fillings.

Lipolysis. A wide variety of microorganisms, including pseudomonads, molds, and staphylococci, produce lipolytic enzymes that hydrolyze lipids, producing readily oxidizable substrates that have a rancid odor. As pointed out in Chapter "Microbiological Spoilage of Dairy Products," this type of enzyme activity can be used to develop desirable cheese flavors.

Oxidation of organic acids and alcohols. Many molds and oxidative yeasts can grow on acidified foods and metabolize the organic acid. If substantial growth occurs, the pH of the food could be raised to levels high enough to permit the growth of other types of spoilage organisms.

Guaiacol production. Alicyclobacilli can grow in some fruit or vegetable juices, metabolizing vanillin and other precursor molecules to guaiacol, a product with an asphalt-like or phenolic odor.

Surface growth. Most groups of microorganisms can spoil food by growing on the surface. Refrigerated cured meats and cooked products can become slimy or sticky to the touch because of the growth of yeasts, lactic acid bacteria, and some enterics and pseudomonads. This particular spoilage defect is caused simply by the accumulation of very high numbers of microbial cells and not by any specific metabolic activity of the microbes. Similarly, color changes in food can occur because of the surface growth of microorganisms. Examples include the greening of meats, caused by lactic acid bacteria; fluorescence in milk, caused by pseudomonads; and red spots on breadstuffs, caused by *Serratia marcescens.*

Quorum Sensing

Some of the spoilage mechanisms described above do not involve the steady production and secretion of enzymes as the microbial population increases. The phenomenon called quorum sensing has been discovered to be responsible for many of the effects of large microbial populations (Smith, Fratamico, & Novak, 2004). Quorum sensing has been shown to be active in the production of toxins, invasive factors, dental plaque, biofilms, bioluminescence, bacteriocins, and even food spoilage (Cotter, Hill, & Ross, 2005; Rasch et al., 2005; Gram et al., 2002).

Whether the microorganism in question is an animal or a plant pathogen producing a variety of invasive factors or a food spoilage microbe secreting extracellular enzymes, the production and secretion of these compounds does not occur when the microorganism is present at low levels. Rather, the production of invasive and spoilage factors occurs only when the population reaches high numbers. (This author has not found an estimate of the "high number" necessary to activate quorum sensing, but it is intriguing to speculate that it could be approximately 10^7 cells/g or higher, which is the threshold level for the organoleptic detection of spoilage described above.) It is reasoned that low numbers of microbial cells do not produce

the invasive or digestive factors in order to avoid triggering host defense systems. When high numbers of cells are achieved, quorum sensing enables the coordinated release of such factors, with a better chance to overwhelm the host defenses.

Quorum sensing depends upon the synthesis of a biochemical signal molecule, followed by its accumulation in the growth environment and recognition by other cells of the same microbial species. *N*-Acylhomoserine lactones (AHLs) produced by Gram-negative bacteria are the most common quorum-sensing signal examined to date. Numerous different quorum-sensing signals, too complex to be described here, are produced by both Gram-negative and Gram-positive bacteria. Novel means to control microbiological problems ranging from food spoilage to biofilms to human illness would be the development of techniques to interfere with the molecular quorum sensing between bacteria (Gram et al., 2002).

Associations Between Groups of Spoilage Microorganisms

All of the above discussions are written in the typical style as if the individual microorganisms under consideration were growing alone in pure cultures. In reality, of course, the situation is quite different. Whether a microorganism is growing in a food or in a natural environment, it is in a steady ecological struggle to maintain its existence and dominate the ecosystem in which it is growing. Faster growing microorganisms have a distinct advantage over slower growing organisms. In general, bacteria grow faster than yeasts, which grow faster than molds (Frazier, 1958). Yeasts and molds, however, possess growth characteristics that permit them to dominate harsh environments in which bacteria grow very slowly or not at all. The types of ecological interactions between microorganisms have been grouped into three categories – antagonisms, synergisms, and metabiosis.

Antagonisms

Most of the associations between microorganisms are antagonistic, in which each microbe is trying to gain an advantage over a multitude of competitors. Many microorganisms produce organic acids and alcohols that are inhibitory to some of their competitors. Some produce antibiotics or bacteriocins, which possess highly specific antimicrobial activity, often against closely related species. Some microbes can gain a competitive advantage by using or hoarding an essential mineral or vitamin that is needed by its competitors. Some pseudomonads produce siderophores, an iron-chelating compound, thereby preventing the growth of competitors that require iron (Gram et al., 2002).

Synergisms

A synergistic association exists when two or more microorganisms grow together, producing an effect that none of the individual microbes could produce alone. Few genuine synergisms have been documented. It has been known for a long time that

Pseudomonas syncyanea and *Lactococcus lactis* will produce a blue color in milk only when growing together (Frazier, 1958).

Metabiosis

Metabiotic associations are essentially "sequential synergisms," in which the growth of one microorganism produces environmental conditions favorable for the growth of a second microorganism, which in turn can create favorable conditions for a third microorganism, and so on. Raw milk provides an excellent example of extended metabiosis. *Lactococcus lactis* and some coliforms are the first bacteria to grow in raw milk. They produce lactic acid, which creates a favorable environment for aciduric lactobacilli. When the accumulated acidity of the milk stops the growth of lactobacilli, oxidative yeasts and molds begin to grow and oxidize the lactic acid, thereby raising the pH of the milk and permitting the growth of proteolytic bacteria (Frazier, 1958).

The production of sauerkraut is also an excellent example of metabiosis. It will not be described here because it is not considered to be a food spoilage process.

The growth of aerobic, oxidative microorganisms can remove oxygen and reduce the O/R potential of a food, thereby creating anaerobic conditions that favor the growth of vastly different microbes. Even a seemingly simple spoilage pathway can exemplify a metabiotic association. The amino acid arginine can be metabolized by lactic acid bacteria to ornithine, which in turn is metabolized by enteric bacteria to the foul-smelling amine putrescine (Edwards, Dainty, & Hibbard, 1985).

Intrinsic Factors to Control Microbiological Spoilage

There are a number of inherent, or intrinsic, food properties, such as water activity, pH, preservative compounds, and O/R potential, that affect the type and rate of microbial spoilage. Each of these properties, while present in the food at some "natural" level, can be manipulated during food product formulation to better control food quality and safety. The past century of advances in food science and technology have led to a very large increase in the number and quantity of food products available to consumers. It is estimated that in ca. 1900, the US consumers had their choice of about 100 different kinds of food. Nearly a century later, about 12,000 kinds of food were available (Todd, 1987).

The increasing complexity of food products has forced greater emphasis on the ability of food processors to better manage food quality and safety. Product design is an essential feature of the HACCP system of food safety. It is during this product design phase of research and development activities that validated control measures must be tested and incorporated into the food formulation (Sperber, 1999; Mortimore & Wallace, 1998).

To a large extent, the intrinsic properties of foods determine the expected shelf life or perishability of foods. Several terms to describe this relationship are commonly used, albeit imprecisely defined (Frazier, 1958):

Perishable. Most fresh foods, such as milk and dairy products, meats, poultry, seafood, and produce, have shelf lives ranging from several days to about 3 weeks.

Semiperishable. Some fresh foods such as whole vegetables, fruits, and cheeses may be stored without spoilage for about 6 months under proper storage conditions (refer to section "Extrinsic Factors to Control Microbiological Spoilage" for proper storage conditions).

Nonperishable or shelf stable. Many natural and processed foods have an indefinite shelf life. They can be stored without microbiological spoilage for periods of several years or longer. Examples of shelf-stable foods are dry beans and nuts, flour, sugar, canned fruits and vegetables, mayonnaise and salad dressings, and peanut butter.

The following intrinsic factors are important in the control of microbiological spoilage of foods and beverages. While each of the factors is known to exert its individual effect on spoilage microbes, the food processor must be aware that combinations of the intrinsic factors interact in all foods. Thus, moderate reductions of water activity and pH, along with moderate usage of chemical preservatives, can accomplish the same antimicrobial effect as major alterations of any single intrinsic factor. Moreover, the intrinsic factors interact with the extrinsic factors for food preservation described in section "Extrinsic Factors to Control Microbiological Spoilage" of this chapter.

Water Activity

The determination of the water activity (a_w) value of a food has replaced the percent moisture determination as the most accurate means to determine the growth potential of microorganisms. Some of the water present in foods is chemically bound by hydrogen bonds, by the constituent food molecules, and by added solutes. The a_w value indicates the proportion of the food's moisture that is physically available for microbial growth.

The a_w can be determined manometrically by dividing the vapor pressure of the food by the vapor pressure of water. It can be estimated mathematically for individual solutes by dividing the moles of water by the moles of water plus the moles of solute. It is most easily determined by measuring the food's equilibrium relative humidity (ERH) and dividing it by 100 (Scott, 1957). This last procedure is most commonly used today, as practical instrumentation for rapid a_w determinations has been commercialized. Since ERH values can range from 0 to 100%, a_w values will range from 0 to 1.0.

The influence of a solute on water activity varies inversely with its molecular or ionic size. Therefore, smaller molecules or ions will be more effective than larger molecules in reducing water activity in food formulations. Sodium chloride (ionic weight = 29.25) is theoretically 11.7 times more effective than sucrose (molecular weight = 342) on an equal weight basis in reducing water activity.

Table 2 Minimum water activity (a_w) values to support the growth of representative spoilage microorganisms (derived from Christian, 2000; Deak & Beuchat, 1996; Sperber, 1983)

Microorganism	Minimum a_w for growth
Alicyclobacilli	0.97
Pseudomonads	0.97
Enteric bacteria	0.95
Lactic acid bacteria	0.92
Saccharomyces cerevisiae	0.92
Spoilage yeasts	0.90
Bacillus subtilis	0.90
Spoilage molds	0.84
Xerotrophic molds	0.62
Osmophilic yeasts	0.60

Microorganisms vary greatly in their ability to grow in foods with increased osmotic pressure or reduced water activity values (Table 2). The alicyclobacilli and pseudomonads are hardly osmotolerant, while some species of molds and yeasts are the most osmotolerant organisms known. The type of spoilage organism likely to spoil a particular food can be estimated by the determination of the food's water activity (Table 3).

Table 3 Water activity values of foods (derived from Christian, 2000; Sperber, 1983)

Food	Water activity
Fresh meat, poultry, and seafood	0.99
Bread	0.94
Mayonnaise	0.90
Icing, frosting	0.80
Dried fruit	0.65–0.75
Pancake syrup	0.70
Wheat flour, freshly milled	0.65
Wheat flour	0.60
Dry pasta, spices, milk	0.40–0.60

Water moves freely across the cytoplasmic membranes of microbial cells. When a microbial cell is subjected to high external osmotic pressure, the cell will be dehydrated, resulting in its inability to grow or even in its death. The more osmotolerant microbes indicated in Table 2 cope with the increased external osmotic pressure by greatly increasing their internal concentration of small solute molecules or ions (Csonka, 1989; Sperber, 1983). Enteric bacteria accumulate potassium ions, enabling their growth at a_w 0.95. Further accumulation of potassium ions beyond this point, however, is toxic to the cells, so a_w 0.95 represents the lower limit for the growth of enteric bacteria. The more osmotolerant microbes accumulate "compatible solutes" that do not readily poison the cells as they accumulate to higher concentrations. The most common compatible solutes are proline and glycerol. Therefore, when a food or a growth medium is osmotically adjusted with glycerol rather than

Table 4 Influence of solute type on the minimum water activity to support the growth of spoilage microorganisms (derived from Christian, 2000; Deak & Beuchat, 1996; Sperber, 1983)

	Water activity achieved by		
Microorganism	Sodium chloride	Glycerol	Sucrose
Pseudomonas fluorescens	0.957	0.940	–
Clostridium sporogenes	0.945	0.935	–
Bacillus megaterium	0.94	0.92	–
Lactobacillus helveticus	0.963	0.928	–
Streptococcus thermophilus	0.965	0.94	–
Saccharomyces cerevisiae	0.92	–	0.89
Candida dulciaminis	0.86	–	0.81

sodium chloride, bacteria are able to grow at lower minimum a values (Table 4). Similarly, yeasts are able to grow at reduced a_w values when sucrose, rather than sodium chloride, is used as the solute.

pH

The pH value of foods is another important intrinsic value that determines what types of microorganisms can spoil a food. pH is expressed as

$$\mathrm{pH} = -\log_{10}[\mathrm{H}^+] = \log_{10} 1/[\mathrm{H}^+]$$

where $[H^+]$ is the hydrogen ion concentration.
Since pH is a logarithmic function, doubling or halving the $[H^+]$ will alter a substrate's pH value by 0.3 units ($\log_{10}2 = 0.3$). This means that as the acidity of a system increases, the pH value will decrease. The pH of pure water is 7.0. Values <7.0 are acidic, while values >7.0 are alkaline or basic.

Some microorganisms have developed elaborate acid tolerance responses to cope with reduced pH environments. In general, many kinds of foodborne spoilage microorganisms can grow collectively over most of the pH range, from 0.5 to 11.0 (Table 5). Most foodborne bacteria can grow in the pH range of 4.5–9.0. Most foods range in pH from slightly acidic to strongly acidic (Table 6).

Table 5 Microbial pH range for growth (derived from Jay, 2000; Sperber, unpublished data)

Microorganism	Minimum pH	Maximum pH
Molds	0.5	11.0
Yeasts	1.5	8.5
Alicyclobacilli	2.0	6.0
Lactic acid bacteria	3.5	9.0
Enteric bacteria	4.5	9.0

Table 6 Food pH values (derived from Lund & Eklund, 2000; Sperber, unpublished data)

Food	Typical pH value
Carbonated beverages	2.0
Vinegar	3.0
Apple juice	3.1
Orange juice	3.6
Tomato juice	4.2
Cheddar cheese	5.2
Ground beef	6.2
Milk	6.4
Peas, sweet corn, honeydew melons	6.5
Fresh fish	6.7
Surface-ripened cheeses	>7.0
Hominy	8.5
Nixtamalized corn	10.0

Table 7 Influence of acidulant on the minimum pH for growth of *Salmonellae* (derived from Chung & Goepfert, 1970)

Acidulant	Minimum pH for growth
Hydrochloric	4.05
Citric	4.05
Malic	4.30
Lactic	4.40
Acetic	5.40
Propionic	5.50

The ability of microbes to grow at lower pH values varies greatly with the type of acid that is used to establish the pH (Table 7). The reader should note that it seems obvious that some organic acids, such as acetic and propionic acids in Table 7, have an inhibitory effect that exceeds the inhibition that would be expected solely by pH reduction. Such enhanced inhibitory effects are a large part of the basis for the use of chemical preservatives, as discussed below.

Chemical Preservatives

Chemical Properties of Organic Acids

In addition to the microbiostatic action of their pH effect, organic acids exert various internal metabolic effects. Only undissociated acids, however, can enter the microbial cell by migrating through the cytoplasmic membrane. Therefore, the preservative activity of organic acids is dramatically affected by the pH of the food.

The acid's dissociation constant (pK_a) is the pH value at which the acid is 50% dissociated. Therefore, the proportion of undissociated acid is inversely related to