Chapter 3. Factors that Influence Microbial Growth

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Evaluation and Definition of Potentially Hazardous Foods

1. Introduction

The factors discussed in this section constitute an inclusive, rather than exclusive, list of intrinsic, extrinsic, and other factors that may be considered when determining whether a food or category of foods requires time/temperature control during storage, distribution, sale and handling at retail and in food service to assure consumer protection.

Many factors must be evaluated for each specific food when making decisions on whether it needs time/temperature control for safety. These can be divided into intrinsic and extrinsic factors. Intrinsic factors are those that are characteristic of the food itself; extrinsic factors are those that refer to the environment surrounding the food. The need for time/temperature control is primarily determined by 1) the potential for contamination with pathogenic microorganisms of concern (including processing influences), and 2) the potential for subsequent growth and/or toxin production.

Most authorities are likely to divide foods among three categories based on an evaluation of the factors described below: those that do not need time/temperature control for protection of consumer safety; those that need time/temperature control; and those where the exact status is questionable. In the case of questionable products, further scientific evidence--such as modeling of microbial growth or death, or actual microbiological challenge studies--may help to inform the decision.

2. Intrinsic factors

2.1. Moisture content

Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (a_w) of the food or environment. Water activity is defined as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (Jay 2000b, p 41):

$a_w = p/p_o,$

where p = vapor pressure of the solution and p_o = vapor pressure of the solvent (usually water). The a_w of pure water is 1.00 and the a_w of a completely dehydrated food is 0.00. The a_w of a food on this scale from 0.00 - 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 - 100%. Thus, % Equilibrium Relative Humidity (ERH) = a_w x 100. The a_w of a food describes the degree to which water is "bound" in the food, its availability to participate in chemical/biochemical reactions, and its availability to facilitate growth of microorganisms.

Most fresh foods, such as fresh meat, vegetables, and fruits, have a_w values that are close to the optimum growth level of most microorganisms (0.97 - 0.99). Table 3-1 shows the approximate a_w levels of some common food categories. The a_w can be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food. Weight for weight, these food components will decrease a_w in the following order: ionic compounds > sugars, polyhydric alcohols, amino acids and other low-molecular-weight compounds > high-molecular-weight compounds such as cellulose, protein or starch (Mossel and others 1995, p 63-109).

Microorganisms respond differently to a_w depending on a number of factors. Microbial growth, and, in some cases, the production of microbial metabolites, may be particularly sensitive to alterations in a_w. Microorganisms generally have optimum and minimum levels of a_w for growth depending on other growth factors in their environments. One indicator of microbial response is their taxonomic classification. For example, Gram (-) bacteria are generally more sensitive to low a_w than Gram (+) bacteria. Table 3-2 lists the approximate minimum a_w values for the growth of selected microorganisms relevant to food. It should be noted that many bacterial pathogens are controlled at water activities well above 0.86 and only *S. aureus* can grow and produce toxin below a_w 0.90. It must be emphasized that these are approximate values because solutes can vary in their ability to inhibit microorganisms at the same a_w value. To illustrate, the lower a_w limit for the growth of *Clostridium botulinum* type A has been found to be 0.94 with NaCl as the solute versus 0.92 with glycerol as the solute (Mossel and others 1995, p 63-109). When formulating foods using a_w as the primary control mechanism for pathogens, it is useful to employ microbiological challenge testing to verify the effectiveness of the reduced a_w when target a_w is near the growth limit for the organism of concern.

Because a_w limits vary with different solutes or humectants, other measures may provide more precise moisture monitoring for certain products. For example, factors other than a_w are known to control the antibotulinal properties of pasteurized processed cheese spreads (Tanaka and others 1986). Also, a_w may be used in combination with other factors to control pathogens in certain food products (section 4.4). Care should be taken when analyzing multicomponents foods, because effective measurements of a_w may not reflect the actual value in a microenvironment or in the interface among the different components. In these cases, the a_w should be measured at the interface areas of the food, as well as in any potential microenvironment.

Table 3-1. Approximate a_w values of selected food categories.	
Animal Products	**a_w**
fresh meat, poultry, fish	0.99 - 1.00
natural cheeses	0.95 - 1.00
pudding	0.97 - 0.99
eggs	0.97
cured meat	0.87 - 0.95
sweetened condensed milk	0.83
Parmesan cheese	0.68 - 0.76
honey	0.75
dried whole egg	0.40
dried whole milk	0.20
Plant Products	**a_w**
fresh fruits, vegetables	0.97 - 1.00
bread	~0.96
bread, white	0.94 - 0.97
bread, crust	0.30
baked cake	0.90 - 0.94
maple syrup	0.85
jam	0.75 - 0.80
jellies	0.82 - 0.94
uncooked rice	0.80 - 0.87
fruit juice concentrates	0.79 - 0.84
fruit cake	0.73 - 0.83
cake icing	0.76 - 0.84
flour	0.67 - 0.87
dried fruit	0.55 - 0.80
cereal	0.10 - 0.20

sugar	0.19
crackers	0.10

Sources: Table 4.6 in Banwart 1979, p 115; Table 2 in FDA 1986; Table 18-3 in Jay 2000, p 367.

Table 3-2. Approximate a_w values for growth of selected pathogens in food.			
Organism	**Minimum**	**Optimum**	**Maximum**
Campylobacter spp.	0.98	0.99	
Clostridium botulinum type E*	0.97		
Shigella spp.	0.97		
Yersinia enterocolitica	0.97		
Vibrio vulnificus	0.96	0.98	0.99
Enterohemorrhagic *Escherichia coli*	0.95	0.99	
Salmonella spp.	0.94	0.99	>0.99
Vibrio parahaemolyticus	0.94	0.98	0.99
Bacillus cereus	0.93		
*Clostridium botulinum*types A & B**	0.93		
Clostridium perfringens	0.943	0.95-0.96	0.97
Listeria monocytogenes	0.92		
Staphylococcus aureus growth	0.83	0.98	0.99
Staphylococcus aureus toxin	0.88	0.98	0.99

ICMSF 1996.
* *proteolytic; * non-proteolytic

2.2. pH and acidity

Increasing the acidity of foods, either through fermentation or the addition of weak acids, has been used as a preservation method since ancient times. In their natural state, most foods such as meat, fish, and vegetables are slightly acidic while most fruits are moderately acidic. A few foods such as egg white are alkaline. Table 3-3 lists the pH ranges of some common foods. The pH is a function of the hydrogen ion concentration in the food:

$pH = -\log_{10} [H+]$

Another useful term relevant to the pH of foods is the pK_a. The term pK_a describes the state of dissociation of an acid. At equilibrium, pK_a is the pH at which the concentrations of dissociated and undissociated acid are equal. Strong acids have a very low pK_a, meaning that they are almost entirely dissociated in solution (ICMSF 1980, p 93). For example, the pH (at 25 °C [77 °F]) of a 0.1 M solution of HCl is 1.08 compared to the pH of 0.1 M solution of acetic acid, which is 2.6. This characteristic is extremely important when using acidity as a preservation method for foods. Organic acids are more effective as preservatives in the undissociated state. Lowering the pH of a food increases the effectiveness of an organic acid as a preservative. Table 3-4 lists the proportion of total acid undissociated at different pH values for selected organic acids. The type of organic acid employed can dramatically influence the microbiological keeping quality and safety of the food.

It is well known that groups of microorganisms have pH optimum, minimum, and maximum for growth in foods. Table 3-5 lists the approximate pH ranges for growth in laboratory media for selected organisms relevant to food. As with other factors, pH usually interacts with other parameters in the food to inhibit growth. The pH can interact with factors such as a_w, salt, temperature, redox potential, and preservatives to inhibit growth of pathogens and other organisms. The pH of the food also significantly impacts the lethality of heat treatment of the food. Less heat is needed to inactivate microbes as the pH is reduced (Mossel and others 1995).

Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins.

Titratable acidity (TA) is a better indicator of the microbiological stability of certain foods, such as salad dressings, than is pH. Titratable acidity is a measure of the quantity of standard alkali (usually 0.1 M NaOH) required to neutralize an acid solution (ICMSF 1980, p 94). It measures the amount of hydrogen ions released from undissociated acid during titration. Titratable acidity is a particularly useful measure for highly buffered or highly acidic foods. Weak acids (such as organic acids) are usually undissociated and, therefore, do not directly contribute to pH. Titratable acidity yields a measure of the total acid concentration, while pH does not, for these types of foods.

In general, pathogens do not grow, or grow very slowly, at pH levels below 4.6; but there are exceptions. Many pathogens can survive in foods at pH levels below their growth minima. It has been reported that *C. botulinum* was able to produce toxin as low as pH 4.2, but these experiments were conducted with high inoculum levels (10^3-10^4 CFU/g up to 10^6 CFU/g), in soy peptone, and with the presence of *Bacillus* spp. (Smelt and others 1982). The panel did not consider these results to be relevant to the foods under consideration in this report. It should also be noted that changes in pH can transform a food into one which can support growth of pathogens (ICMSF 1980). For example, several botulism outbreaks have been traced to foods in which the pH increased due to mold growth. These are important considerations when determining the shelf life of a food formulation. Based on a comprehensive review of the literature, the panel concluded that a pH of 4.6 is appropriate to control spore-forming pathogens.

Among vegetative pathogens, *Salmonella* spp. are reported to grow at the lowest pH values; however, in a study by Chung and Goepfert (1970), the limiting pH was greatly influenced by the acidulant used. For example, when tryptone-yeast extract-glucose broth was inoculated with 10^4 CFU/ml of salmonellae, minimum pH values for growth ranged from 4.05 with hydrochloric and citric acids to 5.5 with propionic acid or acetic acid. Additionally, inoculum levels were unrealistically high (10^2 - 10^6 CFU/ml) for salmonellae in food systems. These investigators also noted that these results could not be extrapolated directly to food because the experiment was run in laboratory media under ideal temperature and a_w conditions and without the presence of competitive microorganisms. Similarly, Ferreira and Lund (1987) reported that six out of 13 strains of *Salmonella* spp. representing 12 serovars could grow at pH 3.8 at 30 °C (86 °F) within 1-3 d, and at 20 °C (68 °F) in 3-5 d, when using HCl as an acidulant. Other reports note that certain acids at pH 4.5 inactivate salmonellae. The panel therefore concluded that using a pH minimum of 4.0 for *Salmonella* spp. would not be scientifically substantiated for foods subject to Food Code requirements. Based on a comprenhensive review of the literature data, the panel also concluded that it would be scientifically valid to use a pH minimum of 4.2 to control for *Salmonella* spp. and other vegetative pathogens.

As with other intrinsic properties, when analyzing multicomponents foods, the pH should be measured not only for each component of the food but also for the interface areas among components and for any potential microenvironment.

Table 3-3. pH ranges of some common foods.		
Food		**pH Range**
Dairy Products	Butter	6.1 - 6.4
	Buttermilk	4.5
	Milk	6.3 - 6.5
	Cream	6.5
	Cheese (American mild and cheddar)	4.9; 5.9
	Yogurt	3.8 - 4.2
Meat and Poultry	Beef (ground)	5.1 - 6.2
	Ham	5.9 - 6.1
	Veal	6.0
	Chicken	6.2 - 6.4

Fish and Shellfish	Fish (most species)	6.6 - 6.8
	Clams	6.5
	Crabs	7.0
	Oysters	4.8 - 6.3
	Tuna Fish	5.2 - 6.1
	Shrimp	6.8 - 7.0
	Salmon	6.1 - 6.3
	White Fish	5.5
Fruits and Vegetables	Apples	2.9 - 3.3
	Apple Cider	3.6 - 3.8
	Bananas	4.5 - 4.7
	Figs	4.6
	Grapefruit (juice)	3.0
	Limes	1.8 - 2.0
	Honeydew melons	6.3 - 6.7
	Oranges (juice)	3.6 - 4.3
	Plums	2.8 - 4.6
	Watermelons	5.2 - 5.6
	Grapes	3.4 - 4.5
	Asparagus (buds and stalks)	5.7 - 6.1
	Beans (string and lima)	4.6 - 6.5
	Beets (sugar)	4.2 - 4.4
	Broccoli	6.5
	Brussels Sprouts	6.3
	Cabbage (green)	5.4 - 6.0
	Carrots	4.9 - 5.2; 6.0
	Cauliflower	5.6
	Celery	5.7 - 6.0

	Corn (sweet)	7.3
	Cucumbers	3.8
	Eggplant	4.5
	Eggs yolks (white)	6.0 - 6.3 (7.6- 9.5)
	Lettuce	6.0
	Olives (green)	3.6 - 3.8
	Onions (red)	5.3 - 5.8
	Parsley	5.7 - 6.0
	Parsnip	5.3
	Potatoes (tubers and sweet)	5.3 - 5.6
	Pumpkin	4.8 - 5.2
	Rhubarb	3.1 - 3.4
	Spinach	5.5 - 6.0
	Squash	5.0 - 5.4
	Tomatoes (whole)	4.2 - 4.3
	Turnips	5.2 - 5.5

Sources: Table 5.5 in ICMSF 1980, p 109-110; Table 3-2 in Jay 2000, p 39.

Table 3-4. Proportion of total acid undissociated at different pH values (expressed as percentages).

Organic Acids	pH Values				
	3	4	5	6	7
Acetic acid	98.5	84.5	34.9	5.1	0.54
Benzoic acid	93.5	59.3	12.8	1.44	0.144
Citric acid	53.0	18.9	0.41	0.006	<0.001
Lactic acid	86.6	39.2	6.05	0.64	0.064
Methyl, ethyl, propyl parabens	>99.99	99.99	99.96	99.66	96.72
Propionic acid	98.5	87.6	41.7	6.67	0.71
Sorbic acid	97.4	82.0	30.0	4.1	0.48

Source: Table 7.3 in ICMSF 1980, p 133.

Table 3-5. Approximate pH values permitting the growth of selected pathogens in food.

Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5 - 5.8	7.2	8.0 - 9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0 -7.0	8.8
Campylobacter spp.	4.9	6.5 - 7.5	9.0
Shigella spp.	4.9		9.3
Vibrio parahaemolyticus	4.8	7.8 - 8.6	11.0
Clostridium botulinum toxin	4.6		8.5
Clostridium botulinum growth	4.6		8.5
Staphylococcus aureus growth	4.0	6.0 - 7.0	10.0
Staphylococcus aureus toxin	4.5	7.0 - 8.0	9.6
Enterohemorrhagic *Escherichia coli*	4.4	6.0 - 7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2[1]	7.0 - 7.5	9.5
Yersinia enterocolitica	4.2	7.2	9.6

Sources: Table 5.3 in ICMSF 1980, p 101.
[1]pH minimum as low as 3.8 has been reported when acidulants other than acetic acid or equivalent are used.

2.3. Nutrient content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Mossel and others 1995, p 47-8, 185-7; Ray 1996, p 62-65; Jay 2000, p 47-8).

Varying amounts of these nutrients are present in foods. Meats have abundant protein, lipids, minerals, and vitamins. Most muscle foods have low levels of carbohydrates. Plant foods have high concentrations of different types of carbohydrates and varying levels of proteins, minerals, and vitamins. Foods such as milk and milk products and eggs are rich in nutrients. The role of water is discussed in section 2.1.

Foodborne microorganisms can derive energy from carbohydrates, alcohols, and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates, such as starch or cellulose found in plant foods, or glycogen found in muscle foods. Some microorganisms can use fats as an energy source.

Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals.

In general, the Gram (+) bacteria are more fastidious in their nutritional requirements and thus are not able to synthesize certain nutrients required for growth (Jay 2000, p 78). For example, the Gram (+) foodborne pathogen *S. aureus* requires amino acids, thiamine, and nicotinic acid for growth (Jay 2000, p 444). Fruits and vegetables that are deficient in B vitamins do not effectively support the growth of these microorganisms. The Gram (-) bacteria are generally able to derive their basic nutritional requirements from the existing carbohydrates, proteins, lipids, minerals, and vitamins that are found in a wide range of food (Jay 2000, p 47-8).

An example of a pathogen with specific nutrient requirements is *Salmonella* Enteritidis. Growth of *Salmonella* Enteritidis may be limited by the availability of iron. For example, the albumen portion of the egg, as opposed to the yolk, includes antimicrobial agents and limited free iron that prevent the growth of *Salmonella* Enteritidis to high levels. Clay and Board (1991) demonstrated that the addition of iron to an inoculum of *Salmonella* Enteritidis in egg albumen resulted in growth of the pathogen to higher levels compared to levels reached when a control inoculum (without iron) was used.

The microorganisms that usually predominate in foods are those that can most easily utilize the nutrients present. Generally, the simple carbohydrates and amino acids are utilized first, followed by the more complex forms of these nutrients. The complexity of foods in general is such that several microorganisms can be growing in a food at the same time. The rate of growth is limited by the availability of essential nutrients. The abundance of nutrients in most foods is sufficient to support the growth of a wide range of foodborne pathogens. Thus, it is very difficult and impractical to predict the pathogen growth or toxin production based on the nutrient composition of the food.

2.4. Biological structure

Plant and animal derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes.

Plant and animal foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms. Several factors may influence penetration of these barriers. The maturity of plant foods will influence the effectiveness of the protective barriers. Physical damage due to handling during harvest, transport, or storage, as well as invasion of insects can allow the penetration of microorganisms (Mossel and others 1995, p 204; Jay 2000, p 49). During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers. Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food. For example, *Salmonella* spp. have been shown to grow on the interior of portions of cut cantaloupe, watermelon, honeydew melons (Golden and others 1993), and tomatoes (Lin & Wei 1997), given sufficient time and temperature.

Fruits are an example of the potential of pathogenic microorganisms to penetrate intact barriers. After harvest, pathogens will survive but usually not grow on the outer surface of fresh fruits and vegetables. Growth on intact surfaces is not common because foodborne pathogens do not produce the enzymes necessary to break down the protective outer barriers on most produce. This outer barrier restricts the availability of nutrients and moisture. One exception is the reported growth of *E. coli* O157:H7 on the surface of watermelon and cantaloupe rinds (del Rosario and Beuchat 1995). Survival of foodborne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens (bacteria or fungi). These conditions can also promote the multiplication of pathogens, especially at higher temperatures. Infiltration of fruit was predicted and described by Bartz and Showalter (1981) based on the general gas law, which states that any change in pressure of an ideal gas in a closed container of constant volume is directly proportional to a change in temperature of the gas. In their work, Bartz and Showalter describe a tomato; however, any fruit, such as an apple, can be considered a container that is not completely closed. As the container or fruit cools, the decrease in internal gas pressure results in a partial vacuum inside the fruit, which then results in an influx from the external environment. For example, an influx of pathogens from the fruit surface or cooling water could occur as a result of an increase in external pressure due to immersing warm fruit in cool water. Internalization of bacteria into fruits and vegetables could also occur due to breaks in the tissues or through morphological structures in the fruit itself, such as the calyx or stem scar. Although infiltration was considered a possible scenario, the panel concluded that there is insufficient epidemiological evidence to require refrigeration of intact fruit.

The egg is another good example of an effective biological structure that, when intact, will prevent external microbial contamination of the perishable yolk; contamination is possible, however, through transovarian infection. For the interior of an egg to become contaminated by microorganisms on the surface, there must be penetration of the shell and its membranes. In addition, the egg white contains antimicrobial factors. When there are cracks through the inner membrane of the egg, microorganisms penetrate into the egg. Factors such as temperature of storage, relative humidity, age of eggs, and level of surface contamination will influence internalization. For example, conditions such as high humidity and wet and dirty shells, along with a drop in the storage temperature will increase the likelihood for entry of bacteria. If eggs are washed, the wash water should be 12 °C (22 °F) higher than the temperature of the eggs to

prevent microbial penetration. After washing, the eggs should be dried and then cooled. The Food and Drug Administration (FDA) published a final rule that applies to shell eggs that have not be processed to destroy all live Salmonella before distribution to the consumer. The rule mandates that eggs should be kept dry and chilled below 7.2 °C (45 °F) to prevent growth of *Salmonella* Enteritidis (Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution, 65 FR 76092 [Dec. 5, 2000] [to be codified at 21 C.F.R. parts 16, 101, and 115]).

Heating of food as well as other types of processing will break down protective biological structures and alter such factors as pH and a_w. These changes could potentially allow the growth of microbial pathogens.

2.5. Redox potential

The oxidation-reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential is a measurement of the ease by which a substance gains or loses electrons. The redox potential (Eh) is measured in terms of millivolts. A fully oxidized standard oxygen electrode will have an Eh of +810 mV at pH 7.0, 30 °C (86 °F), and under the same conditions, a completely reduced standard hydrogen electrode will have an Eh of -420 mV. The Eh is dependent on the pH of the substrate; normally the Eh is taken at pH 7.0 (Jay 2000, p 45-7).

The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes, and microaerophiles. Examples of foodborne pathogens for each of these classifications include *Aeromonas hydrophila, Clostridium botulinum, Escherichia coli* O157:H7, and *Campylobacter jejuni,* respectively. Generally, the range at which different microorganisms can grow are as follows: aerobes +500 to +300 mV; facultative anaerobes +300 to -100 mV; and anaerobes +100 to less than -250 mV (Ray 1996, p 69-70). For example, *C. botulinum* is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values. The relationship of Eh to growth can be significantly affected by the presence of salt and other food constituents. For example, in one study with smoked herring, toxin was produced in inoculated product stored at 15 °C (59 °F) within three days at an Eh of +200 to +250 mV (Huss and others 1979). In this case, the major oxidant would be trimethylamine oxide, which becomes the electron acceptor for *C. botulinum*. The anaerobe *Clostridium perfringens* can initiate growth at an Eh close to +200 mV; however, in the presence of increasing concentrations of certain substances, such as salt, the limiting Eh increases (Morris 2000).

The measured Eh values of various foods are given in Table 3-6. These values can be highly variable depending on changes in the pH of the food, microbial growth, packaging, the partial pressure of oxygen in the storage environment, and ingredients and composition (protein, ascorbic acid, reducing sugars, oxidation level of cations, and so on). Another important factor is the poising capacity of the food. Poising capacity, which is analogous to buffering capacity, relates to the extent to which a food resists external affected changes in Eh. The poising capacity of the food will be affected by oxidizing and reducing constituents in the food as well as by the

presence of active respiratory enzyme systems. Fresh fruits and vegetables and muscle foods will continue to respire; thus low Eh values can result (Morris 2000).

Table 3- 6. Redox potentials on some foods.			Presence of air	Eh (mV)	pH
FOOD			**Presence of air**	**Eh (mV)**	**pH**
Milk			+	+300 to +340	NR
Cheese	Cheddar		+	+300 to -100	NR
	Dutch		+	-20 to -310	4.9-5.2
	Emmenthal		+	-50 to -200	NR
Butter serum			-	+290 to +350	6.5
Egg (infertile after 14 d)			+	+500	NR
Meats	Liver, raw minced		-	-200	~7
	Muscle	Raw, post-rigor	-	-60 to -150	5.7
		Raw, minced	+	+225	5.9
		Minced, cooked	+	+300	7.5
	Cooked sausages and canned meat		-	-20 to -150	~6.5
Cereals	Wheat (whole grain)		-	-320 to -360	6.0
	Wheat (germ)		-	-470	NR
	Barley (ground)		+	+225	7
Potato tuber			-	~ -150	~6
Plant juices	Grape		-	+409	3.9
	Lemon		-	+383	2.2
	Pear		-	+436	4.2
	Spinach		-	+74	6.2

Canned foods	"Neutral"	-	-130 to -550	>4.4
	"Acid"	-	-410 to -550	<4.4

NR = Not reported
Reproduced from Mossel and others 1995, p 185 by permission of D.A.A. Mossel.

The measurement of redox potential of food is done rather easily, either for single or multicomponent foods. For multicomponent foods, in addition to measurement of each component, the redox potential of the interface areas and microenvironments should be considered. However, difficulties arise in taking accurate measurements and in accounting for the differences throughout the food and the equilibrium at the point of measurement. According to Morris (2000): "This imposes the further requirements 1) that the measuring electrode be so prepared and calibrated that it gives stable and reproducible readings, and 2) that a foodstuff is tested in a manner that does not cause any change in the potential that is to be measured. ... it would be unwise to use redox potential information in isolation to predict food safety, or to rely exclusively on control of redox potential as the means of preventing growth of specific microorganisms." Redox measurements could possibly be used in combination with other factors to evaluate the potential for pathogen growth. However, the limitations discussed above make it a rather difficult and variable factor that could result in erroneous conclusions in the absence of other comprehensive information.

2.6. Naturally occurring and added antimicrobials

Some foods intrinsically contain naturally-occurring antimicrobial compounds that convey some level of microbiological stability to them. There are a number of plant-based antimicrobial constituents, including many essential oils, tannins, glycosides, and resins, that can be found in certain foods. Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano (Jay 2000, p 266-7). Other plant-derived antimicrobial constituents include the phytoalexins and the lectins. Lectins are proteins that can specifically bind to a variety of polysaccharides, including the glycoproteins of cell surfaces (Mossel and others 1995, p 175-214). Through this binding, lectins can exert a slight antimicrobial effect. The usual concentration of these compounds in formulated foods is relatively low, so that the antimicrobial effect alone is slight. However, these compounds may produce greater stability in combination with other factors in the formulation.

Some animal-based foods also contain antimicrobial constituents. Examples include lactoferrin, conglutinin and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk, and other factors in fresh meat, poultry and seafood (Mossel and others 1995, p 175-214). Lysozyme is a small protein that can hydrolyze the cell wall of bacteria. The lactoperoxidase system in bovine milk consists of three distinct components that are required for its antimicrobial action: lactoperoxidase, thiocyanate, and hydrogen peroxide. Gram (-) psychotrophs such as the pseudomonads have been shown to be very sensitive to the lactoperoxidase system.

Consequently, this system, in an enhanced form, has been suggested to improve the keeping quality of raw milk in developing countries where adequate refrigeration is scarce (Mossel and others 1995, p 188). Similar to the plant-derived antimicrobial compounds, the animal-derived compounds have a limited effect on ambient shelf life of foods.

It is also known that some types of food processing result in the formation of antimicrobial compounds in the food. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. Maillard compounds resulting from condensation reactions between sugars and amino acids or peptides upon heating of certain foods can impart some antimicrobial activity (Mossel and others 1995, p 195-6). Smoke condensate includes phenol, which is not only an antimicrobial, but also lowers the surface pH. Some procesors also lower the surface pH with liquid smoke to achieve an unsliced shelf-stable product.

Some types of fermentations can result in the natural production of antimicrobial substances, including bacteriocins, antibiotics, and other related inhibitors. Bacteriocins are proteins or peptides that are produced by certain strains of bacteria that inactivate other, usually closely-related, bacteria (Lück and Jager 1997, p 251). The most commonly characterized bacteriocins are those produced by the lactic acid bacteria. The lantibiotic nisin produced by certain strains of *Lactococcus lactis* is one of the best characterized of the bacteriocins. Nisin is approved for food applications in over 50 countries around the world (Jay 2000, p 269-72). Nisin's first food application was to prevent late-blowing in Swiss cheese by *Clostridium butyricum*. Nisin is a polypeptide that is effective against most Gram (+) bacteria but is ineffective against Gram (-) organisms and fungi. Nisin can be produced in the food by starter cultures or, more commonly, it can be used as an additive in the form of a standardized preparation (Lück and Jager 1997). Nisin has been used to effectively control spore-forming organisms in processed cheese formulations, and has been shown to have an interactive effect with heat. For example, an F_o process for conventional low acid canned foods may be in the 6 - 8 range, but with the addition of nisin, can be reduced to a F_o of 3 for inactivating thermophilic spores.

There are a number of other bacteriocins and natural antimicrobials that have been described, however, these have found very limited application in commercial use as food preservatives because of their restricted range of activity, limited compatibility with the food formulation or their regulatory status.

In addition to naturally-occurring antimicrobial compounds in foods, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination. Table 3-7 lists some of the most frequently used preservatives in the United States by food category (Lück and Jager 1997). The selection and use of these preservatives is typically governed by food law regulation of a country or region of the world. A number of criteria should be followed when selecting a preservative for a specific food application. Ideally, the preservative should have a wide spectrum of activity against the target spoilage organisms and pathogens expected to be encountered in the food. The preservative must be active for the desired shelf life of the food and under the expected formulation conditions in the food. It should cause minimal organoleptic impact on the food and should not interfere with

desirable microbiological processes expected to occur in the food, such as the ripening of cheese or leavening of baked goods.

Added antimicrobial compounds can have an interactive or synergistic effect with other parameters of the formulation. One example is the interaction with pH. Many preservatives have an optimum pH range for effectiveness. Other factors include a_w, presence of other preservatives, types of food constituents, presence of certain enzymes, processing temperature, storage atmosphere, and partition coefficients. The effective use of combinations of preservatives with other physico-chemical parameters of a food formulation can stabilize that food against spoilage organisms or pathogens. Leistner systematically developed the "hurdle concept" to describe these effects (Leistner 1995). The hurdle concept states that several inhibitory factors (hurdles), while individually unable to inhibit microorganisms, will, nevertheless, be effective in combination. A classic example of applying the hurdle concept is the anti-botulinal stability of certain shelf-stable processed cheese formulations. Combinations of moisture, total salt, and pH have been shown to allow for the safe storage of these products at room temperature for extended time even though the individual factors, taken singly, would not support that practice (Tanaka and others 1986). In combination products, the effectiveness of an antimicrobial may be altered by other factors including the potential for migration of the antimicrobial to other components of the food and the different food parameters at the interface areas.

There are a number of food formulations that, either by addition of preservatives or through the application of the hurdle concept do not require refrigeration for microbiological stability or safety. However, in the absence of a well-defined and validated microbiological model, it is usually difficult to evaluate the microbiological safety of these products. In the majority of these cases, the application of appropriate microbiological challenge testing is the most effective tool for judging the suitability of these formulations for non-refrigerated storage.

Table 3-7. Preservatives frequently used in conjunction with main groups of foods in the U.S.

Foodstuff	Nitrate, Nitrite	Sulfur Dioxide	Acetic Acid	Propionic Acid	Sorbic Acid	Benzoic Acid	BHA and BHT	Smoke	Nisin	Parabens
Fat Emulsions	-	-	+	-	++	+	+	-	-	+
Cheese	-	-	-	+	++	(+)	-	-	+	-
Meat products	++	-	-	-	+	-	-	++	-	-
Seafood products	+	+	++	-	+	+	-	++	-	(+)
Vegetable products	-	+	++	-	++	++	+	-	-	-
Fruit products	-	++	+	-	++	++	(+)	-	-	+
Beverages	-	(+)	-	-	++	++	+	-	-	+
Wine	-	++	-	-	++	-	-	-	-	-
Baked goods	-	-	+	++	++	-	-	-	-	(+)
Confectionery	-	-	-	-	++	(+)	(+)	-	-	-

Source: Adapted from Davidson and Branen 1993; Table 11 in Lück and Jager 1997, p 61;
++ used frequently
 + used occasionally
(+) used in exceptional cases only
 - not used

2.7. Competitive microflora

The potential for microbial growth of pathogens in temperature-sensitive foods depends on the combination of the intrinsic and extrinsic factors, and the processing technologies that have been applied. Within the microbial flora in a food, there are many important biological attributes of individual organisms that influence the species that predominates. These include the individual growth rates of the microbial strains and the mutual interactions or influences among species in mixed populations (ICMSF 1980, p 221-31)

2.7.1. Growth

In a food environment, an organism grows in a characteristic manner and at a characteristic rate. The length of the lag phase, generation time, and total cell yield are determined by genetic factors. Accumulation of metabolic products may limit the growth of particular species. If the limiting metabolic product can be used as a substrate by other species, these may take over (partly or wholly), creating an association or succession (ICMSF 1980, p 222). Due to the complex of continuing interactions between environmental factors and microorganisms, a food at any one point in time has a characteristic flora, known as its association. The microbial profile changes continuously and one association succeeds another in what is called succession. Many examples of this phenomenon have been observed in the microbial deterioration and spoilage of foods (ICMSF 1980, p 226).

As long as metabolically active organisms remain, they continue to interact, so that dominance in the flora occurs as a dynamic process. Based on their growth-enhancing or inhibiting nature, these interactions are either antagonistic or synergistic.

2.7.2. Competition

In food systems, antagonistic processes usually include competition for nutrients, competition for attachment/adhesion sites (space), unfavorable alterations of the environment, and a combination of these factors. Early studies demonstrated that the natural biota of frozen pot pies inhibited inoculated cells of *S. aureus*, *E. coli* and *Salmonella* Typhimurium (Jay 2000, p 52). Another example of this phenomenon is raw ground beef. Even though *S. aureus* is often found in low numbers in this product, staphylococcal enterotoxin is not produced. The reason is that the *Pseudomonas-Acinetobacter-Moraxella* association that is always present in this food grows at a higher rate, outgrowing the staphylococci (ICMSF 1980, p 222).

Organisms of high metabolic activity may consume required nutrients, selectively reducing these substances, and inhibiting the growth of other organisms. Depletion of oxygen or accumulation of carbon dioxide favors facultative obligate anaerobes which occur in vacuum-packaged fresh meats held under refrigeration (ICMSF 1980, p 222).

Staphylococci are particularly sensitive to nutrient depletion. Coliforms and *Pseudomonas* spp. may utilize amino acids necessary for staphylococcal growth and make them unavailable. Other genera of Micrococcaceae can utilize nutrients more rapidly than staphylococci. Streptococci inhibit staphylocci by exhausting the supply of nicotinamide or niacin and biotin (ICMSF 1980, p 222). *Staphylococcus aureus* is a poor competitor in both fresh and frozen foods. At temperatures that favor staphylococcal growth, the normal food saprophytic biota offers protection against staphylococcal growth through antagonism, competition for nutrients, and modification of the environment to conditions less favorable to *S. aureus* (Jay 2000, p 455). Changes in the composition of the food, as well as changes in intrinsic or extrinsic factors may either stimulate or decrease competitive effects.

2.7.3. Effects on growth inhibition

Changes in growth stimulation have been reported among several foodborne organisms, including yeasts, micrococci, streptococci, lactobacilli and Enterobacteriaceae (ICMSF 1980, p 224). Growth stimulating mechanisms can have a significant influence on the buildup of a typical flora. There are several of these mechanisms, a few of which are listed below (ICMSF 1980, p 224):

- Metabolic products from one organism can be absorbed and utilized by other organisms.
- Changes in pH may promote the growth of certain microorganisms. An example is natural fermentations, in which acid production establishes the dominance of acid tolerant organisms such as the lactic acid bacteria. Growth of molds on high acid foods has been found to raise the pH, thus stimulating the growth of *C. botulinum*.
- Changes in Eh or a_w in the food can influence symbiosis. At warm temperatures, *C. perfringens* can lower the redox potential in the tissues of freshly slaughtered animals so that even more obligately anaerobic organisms can grow.
- There are some associations where maximum growth and normal metabolic activity are not developed unless both organisms are present.

This information can be used in the hurdle concept to control microorganisms in temperature-sensitive foods.

3. Extrinsic factors

3.1. Types of packaging/atmospheres

Many scientific studies have demonstrated the antimicrobial activity of gases at ambient and sub-ambient pressures on microorganisms important in foods (Loss and Hotchkiss 2002, p 245).

Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that are directly toxic to certain microorganisms. This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. Oxidizing radicals generated by O_3 and O_2 are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms. A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. This effect may have positive or negative consequences depending upon the native pathogenic microflora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity (Loss and Hotchkiss 2002, p 245).

A variety of common technologies are used to inhibit the growth of microorganisms, and a majority of these methods rely upon temperature to augment the inhibitory effects. Technologies include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage (Loss and Hotchkiss 2002, p 246).

Controlled atmosphere and modified atmosphere packaging of certain foods can dramatically extend their shelf life. The use of CO_2, N_2, and ethanol are examples of MAP applications. In general, the inhibitory effects of CO_2 increase with decreasing temperature due to the increased solubility of CO_2 at lower temperatures (Jay 2000, p 286). Carbon dioxide dissolves in the food and lowers the pH of the food. Nitrogen, being an inert gas, has no direct antimicrobial properties. It is typically used to displace oxygen in the food package either alone or in combination with CO_2, thus having an indirect inhibitory effect on aerobic microorganisms (Loss and Hotchkiss 2002, p 246). Table 3-8 shows some examples of combinations of gases for MAP applications in meat, poultry, seafood, hard cheeses, and baked goods (Farber 1991, p 67).

Table 3-8. Examples of gas mixtures used for various MAP products.			
Product	**% CO_2**	**% O_2**	**%N_2**
Fresh meat	30	30	40
	15 - 40	60 - 85	0
Cured meat	20 - 50	0	50 - 80
Sliced cooked roast beef	75	10	15
Eggs	20	0	80
	0	0	100
Poultry	25 - 30	0	70 - 75
	60 - 75	5 - 10	&gte 20
	100	0	0
	20-40	60-80	0
Pork	20	80	0
Processed Meats	0	0	100
Fish (White)	40	30	30
Fish (Oily)	40	0	60
	60	0	40

Hard cheese	0 - 70		30 - 100
Cheese	0	0	100
Cheese; grated/sliced	30	0	70
Sandwiches	20 - 100	0 - 10	0 - 100
Pasta	0	0	100
	70 - 80	0	20 - 30
Baked goods	20 - 70	0	20 - 80
	0	0	100
	100	0	0

Source: Table 9 in Farber 1991

The preservation principle of antimicrobial atmospheres has been applied to fruits and vegetables, raw beef, chicken and fish, dairy foods including milk and cottage cheese, eggs, and a variety of prepared, ready-to-eat foods.

There are several intrinsic and extrinsic factors that influence the efficacy of antimicrobial atmospheres. These factors-including product temperature, product-to-headspace gas volume ratio, initial microbial loads and type of flora, package barrier properties, and biochemical composition of the food-all interact to determine the degree to which the microbial quality and safety are enhanced (Loss and Hotchkiss 2002, p 255).

Temperature, the most important factor affecting the efficacy of antimicrobial atmospheres, directly affects growth rate, but also indirectly affects growth by affecting gas solubility. At practical food storage temperatures, packaging configurations, especially the product-to-headspace volume ratio, play a major role in determining the magnitude of microbial inhibition.

In MAP, package barrier properties have a major effect on the microbial growth by influencing the time in which the selected modified atmosphere gases remain in contact with the product and the rate at which oxygen enters the package.

Water activity, salt content of the aqueous phase, pH, and fat content of foods also play a role in overall inhibitory effects of antimicrobial gases. As with temperature, the physical and chemical characteristics of the food have an effect on the solubility of the inhibitory gas. For example, increasing salt concentrations decreases CO_2 solubility.

The major safety consideration in extending shelf life of foods by MAP or related technologies is the loss of sensory cues to spoilage provided by bacterial growth. Without spoilage bacteria indicators, it is conceivable that a food could have acceptable organoleptic quality, but be unsafe. The effect of loss of competitive inhibition by spoilage bacteria is most pronounced on the

facultative anaerobic pathogenic bacterial populations in foods under altered atmospheres (Loss and Hotchkiss 2002, p 261).

By combining antimicrobial atmospheres with other techniques, hurdle technology strategies may be generated that can further enhance food quality and safety.

3.2. Effect of time/temperature conditions on microbial growth

3.2.1.Impact of time

When considering growth rates of microbial pathogens, in addition to temperature, time is a critical consideration. Food producers or manufacturers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from when the product is produced until the time it is intended to be consumed or used. Several factors are used to determine a product's shelf life, ranging from organoleptic qualities to microbiological safety. For the purpose of this report, the key consideration is the microbiological safety of the product. The Uniform Open Dating Regulation requires the shelf life of a perishable food product to be expressed in terms of a "sell by" date (NIST 2000). The "sell by" date must incorporate the shelf life of the product plus a reasonable period for consumption that consists of at least one-third of the approximate total shelf life of the perishable food product.

At retail or foodservice, an additional period of time referred to herein as "use-period" should also be considered. As an example, fast food locations may find it operationally desirable to hold processed cheese slices at ambient temperatures for a complete shift or meal period, which may be in excess of 4 h. This practice provides operational efficiency by allowing the cheese to melt faster on a hot sandwich as well as providing a better quality sandwich. Although refrigeration may be required for safety under long-term storage conditions, for use-periods measured in hours, storage at ambient temperatures may be acceptable.

Under certain circumstances, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question. For refrigerated food products, the shelf life or use-period required for safety may vary depending on the temperature at which the product is stored. For example, Mossel and Thomas (1988) report that the lag time for growth of *L. monocytogenes* at 10 °C (50 °F) is 1.5 d, while at 1 °C (34 °F) lag time is ~3.3 d. Likewise, they report that at 10 °C (50 °F) the generation time for the same organism is 5-8 h, while at 1 °C (34 °F), the generation time is between 62 and 131 h. Figure 1 shows the effect of temperature and pH on lag times of *L. monocytogenes*. The data were obtained by using the USDA Pathogen Micromodel Program (version 5.1) at a NaCl concentration of 2% and a_w of 0.989. It should be noted that this model was developed in broth under various salt and pH combinations, and that growth of bacteria in food systems will likely differ. According to the model results, a temperature shift from 10 (50) to 25 °C (77 °F) decreases the lag time of *L. monocytogenes* from 60 to 10 h. In a similar manner, a pH increase from 4.5 to 6.5 decreases the lag time from 60 to 5 h. In conclusion, the safety of a product during its shelf life may differ, depending upon other conditions such as temperature of storage, pH of the product, and so on. This study by Mossel

and Thomas (1988), along with numerous others, illustrates that various time/temperature combinations can be used to control product safety depending on the product's intended use.

Figure 1

Effect of temperature or pH on lag times of *Listeria monocytogenes* from USDA PMP ver 5.1 (2% NaCl, a_w 0.989)
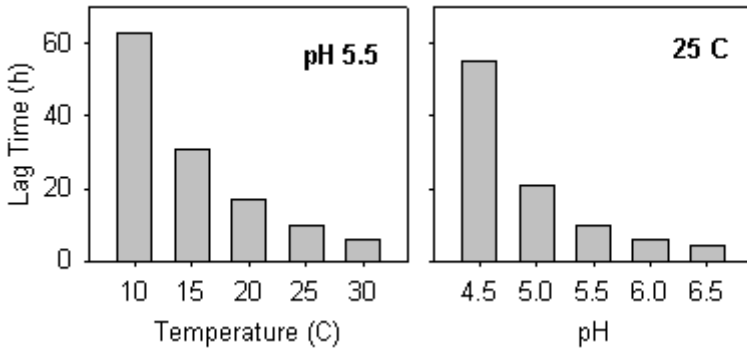
As stated earlier, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question.

3.2.2. Impact of temperature

All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. An understanding of the interplay between time, temperature, and other intrinsic and extrinsic factors is crucial to selecting the proper storage conditions for a food product. Temperature has dramatic impact on both the generation time of an organism and its lag period. Over a defined temperature range, the growth rate of an organism is classically defined as an Arrhenius relationship (Mossel and others 1995, p 79-80). The log growth rate constant is found to be proportional to the reciprocal of the absolute temperature:

$G = -\mu / 2.303 \, RT$ where,

G = log growth rate constant
μ = temperature characteristic (constant for a particular microbe)
R = gas constant
T = temperature (°K)

The above relationship holds over the linear portion of the Arrhenius plot. However, when temperatures approach the maxima for a specific microorganism, the growth rate declines more rapidly than when temperatures approach the minima for that same microorganism. A relationship that more accurately predicts growth rates of microorganisms at low temperatures follows (Jay 2000, p 51):

$\sqrt{r} = b(T - To)$ where,

r = growth rate
b = slope of the regression line
T = temperature (°K)
To = conceptual temperature of no metabolic significance

At low temperatures, two factors govern the point at which growth stops: 1) reaction rates for the individual enzymes in the organism become much slower, and 2) low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms (Mossel and others 1995). At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. While the growth rate increases with increasing temperature, the rate tends to decline rapidly thereafter, until the temperature maximum is reached.

The relationship between temperature and growth rate constant varies significantly across groups of microorganisms. Four major groups of microorganisms have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles, and psychrotrophs. Tables 9 and 10 list the temperature ranges for these four groups (ICMSF 1980) and for pathogens of concern (ICMSF 1996; Doyle and others 2001; Lund and others 2000). The optimum temperature for growth of thermophiles is between 55 to 65 °C (131 to 149 °F) with the maximum as high as 90 °C (194 °F) and a minimum of around 40 °C (104 °F). Mesophiles, which include virtually all human pathogens, have an optimum growth range of between 30 °C (86 °F) and 45 °C (113 °F), and a minimum growth temperature ranging from 5 to 10 °C (41 to 50 °F). Psychrophilic organisms have an optimum growth range of 12 °C (54 °F) to 15 °C (59 °F) with a maximum range of 15 °C (59 °F) to 20 °C (68 °F). There are very few true psychrophilic organisms of consequence to foods. Psychrotrophs such as *L. monocytogenes* and *C. botulinum* type E are capable of growing at low temperatures (minimum of - 0.4 °C [31 °F] and 3.3 °C [38 °F], respectively, to 5 °C [41 °F]), but have a higher growth optimum range (37 °C [99 °F] and 30 °C [86 °F], respectively) than true psychrophiles. Psychrotrophic organisms are much more relevant to food and include spoilage bacteria, spoilage yeast and molds, as well as certain foodborne pathogens.

Growth temperature is known to regulate the expression of virulence genes in certain foodborne pathogens (Montville and Matthews 2001). For example, the expression of proteins governed by the *Yersinia enterocolitica* virulence plasmid is high at 37 °C (99 °F), low at 22 °C (72 °F), and not detectable at 4 °C (39 °F). Growth temperature also impacts an organism's thermal sensitivity. *Listeria monocytogenes*, when held at 48 °C (118 °F) in inoculated sausages, has an increase of 2.4-fold in its D value at 64 °C (147 °F).

It must be emphasized that the lag period and growth rate of a microorganism are influenced not only by temperature but by other intrinsic and extrinsic factors as well. For example, as shown in Table 3-11, the growth rate of *Clostridium perfringens* is significantly lower at pH 5.8 versus pH 7.2 across a wide range of temperatures (ICMSF 1980, p 10). Salmonellae do not grow at temperatures below 5.2 °C (41 °F). The intrinsic factors of the food product, however, have been shown to impact the ability of salmonellae to grow at low temperatures. *Salmonella* Senftenberg,

S. Enteritidis, and *S*. Manhattan were not able to grow in ham salad or custard held at 10 °C (50 °F), but were able to grow in chicken à la king held at 7 °C (45 °F) (ICMSF 1980, p 9).

Staphylococcus aureus has been shown to grow at temperatures as low as 7 °C (45 °F), but the lower limit for enterotoxin production has been shown to be 10 °C (50 °F). In general, toxin production below about 20 °C (68 °F) is slow. For example, in laboratory media at pH 7, the time to produce detectable levels of enterotoxin ranged from 78 - 98 h at 19 °C (66 °F) to 14 - 16 h at 26 °C (79 °F) (ICMSF 1980, p 10). Less favorable conditions, such as reduced pH, slowed enterotoxin production even further.

Table 3-12 illustrates the combined impact of temperature, pH, and a_w on the growth of proteolytic *C. botulinum* type B. This table clearly shows that an interactive effect occurs between these three factors. When measuring the suitability of holding a refrigerated food at room temperature for a period of time, consideration may be given to each factor independently. Doing so, however, ignores the potential to safely hold products for a period of time out of refrigeration based on interaction effects. Consideration of each relevant factor independently may lead to the conclusion that it is not a safe practice to do so, while, in reality, it is actually safe based on the interactive effects. The most appropriate method for evaluating such interactive effects is through a properly designed microbiological challenge study using relevant target microorganisms. Appropriate, validated predictive microbiological models may also be employed for this purpose. The use of challenge studies and/or predictive models can yield scientific data that supports holding a product with a certain formulation for a given time and temperature. It is incumbent upon the producer to have specific knowledge of the food formulation to generate valid scientific data.

Table 3-9. Temperature ranges for prokaryotic microorganisms.			
	Temperature °C (°F)		
Group	**Minimum**	**Optimum**	**Maximum**
Thermophiles	40 - 45 (104 - 113)	55 - 75 (131 - 167)	60 - 90 (140 - 194)
Mesophiles	5 - 15 (41 - 59)	30 - 45 (86 - 113)	35 - 47 (95 - 117)
Psychrophiles	-5 - +5 (23 - 41)	12 - 15 (54 - 59)	15 - 20 (59 - 68)
Psychrotrophs	-5 - +5 (23 - 41)	25 - 30 (77 - 86)	30 - 35 (86 - 95)

Source: Table 1.1 in ICMSF 1980, p 4.

Table 3-10. Approximate minimum, maximum and optimum temperature values in °C (°F) permitting growth of selected pathogens relevant to food.

Organism	Minimum	Optimum	Maximum
Bacillus cereus	5 (41)	28 - 40 (82 - 104)	55 (131)
Campylobacter spp.	32 (90)	42 - 45 (108 - 113)	45 (113)
Clostridium botulinum types A & B*	10 - 12 (50 - 54)	30 - 40 (86 - 104)	50 (122)
Clostridium botulinum type E**	3 - 3.3 (37 - 38)	25 - 37 (77 - 99)	45 (113)
Clostridium perfringens	12 (54)	43 - 47 (109 - 117)	50 (122)
Enterotoxigenic *Escherichia coli*	7 (45)	35 - 40 (95 - 104)	46 (115)
Listeria monocytogenes	0 (32)	30 - 37 (86 - 99)	45 (113)
Salmonella spp.	5 (41)	35 - 37 (95 - 99)	45 - 47 (113 - 117)
Staphylococcus aureus growth	7 (45)	35 - 40 (95 - 104)	48 (118)
Staphylococcus aureus toxin	10 (50)	40 - 45 (104 - 113)	46 (115)
Shigella spp.	7 (45)	37 (99)	45 - 47 (113 - 117)
Vibrio cholerae	10 (50)	37 (99)	43 (109)
Vibrio parahaemolyticus	5 (41)	37 (99)	43 (109)
Vibrio vulnificus	8 (46)	37 (99)	43 (109)
Yersinia enterocolitica	-1 (30)	28 - 30 (82 - 86)	42 (108)

ICMSF 1996; Lund and others 2000; Doyle and others 2001
* proteolytic; ** non-proteolytic

Table 3-11. The relationship of pH and temperature to growth rate of _Clostridium perfringens_ (welchii) F2985/50.

Incubation temperature	Hours to visible turbidity in RCM broth at pH	
	5.8	7.2
15 °C (59 °F)	>700	>700
20 °C (68 °F)	74	48
25 °C (77 °F)	30	24
30 °C (86 °F)	24	8
37 °C (99 °F)	5	5

Source: Table 1.3 in ICMSF 1980, p 10.

Table 3-12. Incubation period, in days, before growth of proteolytic *Clostridium botulinum* type B was observed at various levels of temperature, pH, and a_w.

Temperature	pH	a_w						
		0.997	0.99	0.98	0.97	0.96	0.95	0.94
20 °C (68 °F)	5	--	--	--	--	--	--	--
	6	49	9	--	--	--	--	--
	7	2	2	4	9	--	--	--
	8	2	2	4	14	--	--	--
	9	--	--	--	--	--	--	--
30 °C (86 °F)	5	--	--	--	--	--	--	--
	6	2	2	3	9	--	--	--
	7	1	1	2	3	9	14	--
	8	1	1	2	4	14	--	--
	9	--	--	--	--	--	--	--
40 °C (104 °F)	5	--	--	--	--	--	--	--
	6	1	2	2	3	14	--	--
	7	1	1	1	2	3	9	17
	8	1	1	1	2	9	14	--
	9	--	--	--	--	--	--	--

No growth observed at any pH or a_w level at 10 °C (50 °F).
Source: Table 6 in FDA 1986

3.3. Storage/holding conditions

This discussion of storage conditions will be limited to the storage/holding temperature, and the time/temperature involved in cooling of cooked items, and the relative humidity to which the food or packaging material may be exposed. Other factors that may be included as important considerations for storage, such as the effectiveness of the packaging material at conserving certain characteristics, are discussed in other sections of this chapter.

When considering growth rate of microbial pathogens, time and temperature are integral and must be considered together. As has been stated previously n this chapter, increases in storage and/or display temperature will decrease the shelf life of refrigerated foods since the higher the temperature, the more permissive conditions are for growth. At the same time, those foods that

have been cooked or re-heated and are served or held hot may require appropriate time/temperature control for safety. For example, the primary organism of concern for cooked meat and meat-containing products is *C. perfringens.* Illness symptoms are caused by ingestion of large numbers (greater than 10^8) of vegetative cells. The organism has an optimal growth range of 43 - 47 °C (109-116 °F) and a growth range of 12-50 °C (54 - 122 °F). Generation times as short as 8 min have been reported in certain foods under optimal conditions (ICMSF 1996). Thus time/temperature management is essential for product safety.

The literature is replete with examples of outbreaks of foodborne illness that have resulted from cooling food too slowly, a practice that may permit growth of pathogenic bacteria. Of primary concern in this regard are the spore-forming pathogens that have relatively short lag times and the ability to grow rapidly and/or that may normally be present in large numbers. Organisms that possess such characteristics include *C. perfringens*, and *Bacillus cereus*. As with *C. perfringens*, foodborne illness caused by *B. cereus* is typically associated with consumption of food that has supported growth of the organism to relatively high numbers. The FDA "Bad Bug Book" notes that "The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health" (FDA 2001). In this case, the time and temperature (cooling rate) of certain foods must be addressed to assure rapid cooling for safety.

The effect of the relative humidity of the storage environment on the safety of foods is somewhat more nebulous. The effect may or may not alter the a_w of the food. Such changes are product dependent. The earlier discussion on a_w and its effect on microorganisms in foods provides some background information. In addition, the possibility of surface evaporation or condensation of moisture on a surface should be considered.

Generally, foods that depend on a certain a_w for safety or shelf life considerations will need to be stored such that the environment does not markedly change this characteristic. Foods will eventually come to moisture equilibrium with their surroundings. Thus, processors and distributors need to provide for appropriate storage conditions to account for this fact.

Packaging, as discussed previously in this chapter, will play a major role in the vulnerability of the food to the influence of relative humidity. But even within a sealed container, moisture migration and the phenomenon of environmental temperature fluctuation may play a role. It has been observed that certain foods with low a_w can be subject to moisture condensing on the surface due to wide environmental temperature shifts. This surface water will result in microenvironments favorable to growth of spoilage, and possibly pathogenic, microorganisms. As a general guideline, the product should be held such that environmental moisture, including that within the package, does not have an opportunity to alter the a_w of the product in an unfavorable way.

3.4. Processing steps

The current definition of "potentially hazardous foods" considers the effect of processing in much the same way that it considers pH and a_w: it divides foods into two categories. Low-acid canned foods in a hermetically-sealed container do not require temperature control for safety. This rigid definition fails to address less processed foods, in less robust packaging, which still would not require temperature control for safety. Consider a baked product, such as a pie, with a pH of 5.5 and a_w of 0.96. Since this product is baked to an internal temperature >180 °F (82 °C) to set the product structure of the pie, it will not contain any viable vegetative pathogens. Any pathogenic spores that survive the baking process will be inhibited by the pH and a_w values listed above (ICMSF 1996; see tables 2 and 5). If the product is cooled and packaged under conditions that do not allow recontamination with vegetative pathogens, the product is safe and stable at room temperature until consumed, or until quality considerations (that is, staling) make it unpalatable.

Scientifically sound criteria for determining whether foods require time/temperature control for safety should consider 1) processes that destroy vegetative cells but not spores (when product formulation is capable of inhibiting spore germination); 2) post-process handling and packaging conditions that prevent reintroduction of vegetative pathogens onto or into the product before packaging; and 3) the use of packaging materials that while they do not provide a hermetic seal, do prevent reintroduction of vegetative pathogens into the product.

4. Other factors

4.1. Intended end-use of product

In addition to carefully assessing how the product is produced and distributed, it is important to consider how the food will ultimately be prepared, handled, and/or stored by the end user. A food product that does not require time/temperature control for safety at one point in the food production or distribution chain may require time/temperature control at another point, depending on its intended use. For example, a thermally processed food that is hot-filled into its final packaging may not require refrigeration if spore-forming pathogens are not capable of outgrowth. However, once the food item is taken out of its original packaging, it may require time/temperature control for safety if the product is likely to be recontaminated during its intended use.

4.2. Product history and traditional use

The panel struggled with the concept of product history and traditional use as a means to determine the need for time/temperature control for safety. For example, there are foods which have a long history of safe storage use at ambient temperatures, yet have formulations, pH, and a_w that would designate them as "temperature controlled for safety" (TCS) foods. Paramount among them is white bread, but products such as intact fruits and vegetables, other breads, bottled waters, and some processed cheeses have a history of being stored and used at ambient temperatures with no public health impact. In addition, moisture protein ratios (MPR) for shelf-

stable fermented sausages were developed to ensure process control values for these sausages that also have a traditional history of safety as a non-TCS food. Moreover, an evaluation of the food characteristics provides a scientific explanation for the products to be safely stored at ambient temperatures. For example, baking of bread controls the growth of pathogens in the interior, and the low a_w precludes the growth of pathogens on the outer surface, so that it can be stored safely at ambient temperatures. Clearly these products' traditional uses and histories provide a valid justification for a decision to be made based on history. Care must be observed, however, as this traditional history can be influenced by the intrinsic and extrinsic factors and any changes in product end- use, processes, formulation, physical structure, processing, distribution, and/or storage. Changes in any of these parameters may invalidate the sole use of history as a basis for decisions on whether a food needs temperature control for safety.

The panel recognizes that the use of history as a factor to decide whether a product needs time/temperature control for safety can be subjective. As a guidance, one should determine whether the food in question or any of its ingredients have been previously implicated as a common vehicle of foodborne disease as a result of abuse or storage at ambient temperature. Of particular importance are the microbiological agents that may be of concern based on food formulation, or that may be responsible for illnesses associated with the food and the reported contributing factors that have led to documented illnesses. Has adequate temperature control been clearly documented as a factor that can prevent or reduce the risk of illness associated with the food? As intrinsic or extrinsic factors change (for example, MAP or greatly extended shelf life), historical evidence alone may not be appropriate in determining potential risk. Therefore, for a product to be identified as non-TCS based on history and traditional use, the intrinsic and extrinsic factors affecting microbial growth need to have remained constant. Lastly, product history alone should not be used as the sole factor in determining whether a food needs time/temperature control for safety. This decision requires a valid scientific rationale such as that provided above for white bread.

4.3. Interactions of factors

Traditional food preservation techniques have used combinations of pH, a_w, atmosphere, numerous preservatives, and other inhibitory factors. Microbiologists have often referred to this phenomenon as the "hurdle effect". For example, certain processed meat products and pickles may use the salt-to-moisture ratio (brine ratio) to control pathogens. USDA recognizes this strategy in designating as shelf-stable semi-dry sausages with a moisture-protein ratio of less than or equal to 3.1:1 and pH less than or equal to 5.0.

In salad dressings and mayonnaise-type products, the acid-to-moisture ratio along with pH is the governing factor for pathogen control. An acid:moisture ratio > 0.70 in combination with a pH < 4.1 is often used as the pathogen-control target level for these products. Usually, these ratios are combined with other factors such as pH or added antimicrobials to effect pathogen control (Mossel and others 1995). It is the interaction of these factors that controls the ability of pathogens to proliferate in foods.

Despite this long-standing recognition of the concept of hurdle technology (the possible synergistic effect of combining different inhibitory factors), the current definition of potentially

hazardous foods only considers pH and a_w independently, and does not address their interaction. The panel believes that these interactions have to be taken into consideration.

Scientific advances in predictive food microbiology over the last two decades have repeatedly shown that different inhibitory factors that might not prevent pathogen growth when considered singly will prevent pathogen growth when used in concert. Table 3-13 summarizes a series of predictions from the USDA Pathogen Modeling Program ver. 5.1. It should be noted that this model was developed in broth with salt and pH combinations and that growth of bacteria in food systems will likely differ. Also, the salt used to control the a_w results in additional microbial inhibitory effects that may be lacking if other compounds are used. The values are the time in hours needed for a 3 log increase in *S. aureus* (see Chapter 6, section 9) concentration as a function of the pH and a_w values shown.

It is clear from the numerical values shown that even though a food might have a pH of 5.0 and an a_w of 0.92 (for example), after 72 h at room temperature, it may show a minimal increase in *S. aureus* concentration, and thus not constitute a significant risk to public health.

Table 3-13. USDA pathogen modeling program predictions for time in hours needed for a 3 log increase in *Staphylococcus aureus* concentration as a function of the pH and water activity at 25 °C (77 °F)[1]					
		Critical pH values			
		4.2	**4.6**	**5.0**	**5.5**
Critical a_w values	**0.85**	Outside	Outside	Outside	Outside
	0.90	Outside	Outside	Outside	Outside
	0.92	Outside	171.3	113.1	80.7
	0.93	Outside	143.0	93.0	65.5
	0.94	Outside	120.6	77.3	53.6
	0.95	Outside	101.4	63.9	43.6
	0.96	Outside	86.3	53.4	35.9

[1]Conditions labeled "outside" are outside the range of the current model

Models that address the interaction of other factors (for example, atmosphere, preservatives) have been published, but are not nearly as numerous as models using pH and a_w. Individual companies have shown, however, that in-house models incorporating preservative effects can be useful tools in reducing the need for extensive challenge testing and assessing risk. However, a general model for foods to cover all interactions of atmospheric gases and/or preservative combinations with pH and a_w does not currently exist.

Scientifically sound criteria for determining whether foods require time/temperature control for safety could consider the interaction of only pH and a_w factors using data from microbial growth models such as those shown in the table above. In order to design effective combinations of factors, an understanding of the pathogen (vegetative or spore-forming) and of the mechanisms by which individual factors exert their impact are necessary.

References

Banwart GJ. 1979. Basic Food Microbiology. Westport, Conn.: AVI. Chapter 4, Factors that affect microbial growth in food; p 115 (table 4.6).

JA, Showalter RK. 1981. Infiltration of tomatoes by aqueous bacterial suspensions. Phytopathology 71(5):515-8.

KC, Goepfert JM. 1970. Growth of *Salmonella* at low pH. J Food Sci 35:326-8. CE, Board RG. 1991. Growth of *Salmonella enteritidis* in artificially contaminated hens' shell eggs. Epidemiol Infect 106:271-81.

Davidson PM, Branen AL, editors. 1993. Antimicrobials in foods. 2nd ed. New York: Marcel Dekker. 647 p. (Food Science, 10).

Rosario BA, Beuchat LR. 1995. Survival and growth of enterohemorrhagic *Escherichia coli* 0157:H7 in cantaloupe and watermelon. J Food Prot 58:105-7.

MP, Beuchat LR, Montville TJ, editors. 2001. Food microbiology: fundamentals and frontiers. 2nd ed. Washington (DC): American Society for Microbiology.

Farber JM. 1991. Microbiological aspects of modified atmosphere packaging technology--a review. J Food Prot 54:58-70.

U.S. Food and Drug Administration. 1986 May 9. Retail food protection program information manual, part 6 - Inspection, chapter 01 - code interpretations, section 04 - interpretations by code section. Washington (DC): FDA, Center for Food Safety and Applied Nutrition, Retail Food Protection Branch. Table 6, p 11-12.

Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. The "Bad Bug Book"[1] [Foodborne pathogenic microorganisms and natural toxins handbook]. . Accessed 2001 Dec 10.

Ferreira MASS, Lund BM. 1987. The influence of pH and temperature on initiation of growth of *Salmonella* spp. Lett Appl Microbiol 5:67-70.

Golden DA, Rhodehamel EJ, Kautter DA. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J Food Prot 56:194-6.

Huss HH, Schaeffer I, Rye Peterson E, Cann DC. 1979. Toxin production by *Clostridium botulinum* type E in fresh herring in relation to the measured oxidation-reduction potential (Eh). Nord Veterinaermed 31:81-6.

[ICMSF] International Commission on Microbiological Specification for Foods. 1980. Microbial ecology of foods. Volume 1, Factors affecting life and death of microorganisms. Orlando: Academic Pr. p 311.

[ICMSF] International Commission on Microbiological Specification for Foods. 1996. Microorganisms in foods. Roberts TA, Baird-Parker AC, Tompkin RB, editors. Volume 5, Characteristics of microbial pathogens. London: Blackie Academic & Professional. p 513.

Jay JM. 2000. Modern food microbiology. 6th ed. Gaithersburg (MD): Aspen. p 679.

Leistner L. 1995. Principles and applications of hurdle technology. In: Gould GW, editor. New methods of food preservation. London: Blackie Academic & Professional. p 1-21.

Lin CM, Wei CI. 1997. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. J Food Prot 60(7):858-63.

Loss CR, Hotchkiss JH. 2002. Inhibition of microbial growth by low-pressure and ambient pressure gasses. In: Juneja VK, Sofos JN, editors. Control of foodborne microorganisms. New York: Marcel Dekker. p 245-79. Forthcoming.

Luck E, Jager M. 1997. Antimicrobial food additives: characteristics, uses, effects. Springer: Berlin. 260 p.

Lund BM, Baird-Parker TC, Gould GW, editors. 2000. The microbiological safety and quality of foods. Volume 1 & 2. Gaithersburg (MD): Aspen.

Montville TJ, Matthews KR. 2001. Chapter 2: Principles which influence microbial growth, survival, and death in foods. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington (DC): ASM Pr. p 13-32.

Morris JG. 2000. The effect of redox potential. In: Lund BL, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Volume 1. Gaithersburg (MD): Aspen. p 235-50.

Mossel DAA, Thomas G. 1988. Securite microbioligique des plats prepares refrigeres: recommandations en matiere d'analyse des risques, conception et surveillance du processus de fabrication. Microbiologie--Aliements--Nutrition 6:289-309.

Mossel DAA, Corry JEL, Struijk CB, Baird RM. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley and Sons. 699 p.

[NIST] National Institute of Standards and Technology. 2000. Uniform laws and regulations in the areas of legal metrology and engine fuel quality [as adopted by the 84th National Conference on Weights and Measures 1999]. 2000 ed. Gaithersburg (MD): U.S. Dept. of Commerce, Technology Administration, National Institute of Standards and Technology. Uniform open dating regulation; p 117-22. (NIST Handbook 130). B. 1996. Fundamental food microbiology. Boca Raton (FL): CRC Press. 516 p.

Smelt JPPM, Raatjes JGM, Crowther JC, Verrips CT. 1982. Growth and toxin formation by *Clostridium botulinum* at low pH values. J Appl Bacteriol 52:75-82.

Tanaka N, Traisman E, Plantong P, Finn L, Flom W, Meskey L, Guggisberg J. 1986. Evaluation of factors involved in antibotulinal properties of pasteurized process cheese spreads. J Food Prot 49(7):526-31.

[USDA] U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Laboratory. USDA Pathogen Modeling Program Version 5.1.