7

Preservation by Use of Low Temperatures

Low temperatures are used to retard chemical reactions and action of food enzymes and to slow down or stop the growth and activity of microorganisms in food. The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth; a low enough temperature will prevent the growth of any microorganism.

Any raw plant or animal food may be assumed to contain a variety of bacteria, yeasts, and molds which need only conditions for growth to bring about undesirable changes in the food. Each microorganism present has an optimal, or best, temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Therefore, the cooling of a food from ordinary temperatures has a different effect on the various organisms present. A drop of 10° may stop the growth of some organisms and slow the growth of others but to an extent that would vary with the kind of organism. A further decrease of 10° in temperature would stop the growth of more organisms and make still slower the growth of the others. Low-temperature storage can therefore act as a significant environmental factor influencing the type of spoilage flora to predominate, as illustrated in Table 7.1.

Table 7.1 *Types of Bacteria Causing Spoilage of Chicken Meat*

	Spoilage flora at each temperature, %		
	1C	*10C*	*15C*
Pseudomonas	90	37	15
Acinetobacter	7	26	34
Enterobacteriaceae	3	15	27
Streptococcus		6	8
Aeromonas		4	6
Others		12	10

Source: Tompkin (1973).

The growth and metabolic reactions of microorganisms depend on enzymes, and the rate of enzyme reactions is directly affected by temperature. The most important aspect of this temperature effect is reflected in a decrease in the rate of growth of a microorganism when the temperature is lowered, as can be seen in Table 7.2.

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Table 7.2 *Growth Rate of* Pseudomonas Fragi *at Various Temperatures*

Temperature, C	*Average exponential generation time, min*
0	667
2.5	462
5.0	300
7.5	207
10.0	158
20.0	65

Source: Nickerson and Sinskey (1972).

GROWTH OF MICROORGANISMS AT LOW TEMPERATURES

In general, freezing prevents the growth of most foodborne microorganisms and refrigeration temperatures slow growth rates. Commercial refrigeration temperatures, i.e., lower than 5 to 7.2 C, effectively retard the growth of many foodborne pathogens. One notable exception is *Clostridium botulinum* type E, which has a minimum temperature for growth of about 3.3 C (Table 7.3). *Yersinia enterocolitica* can survive and grow at temperatures as low as 0 to 3 C (Stern and Pierson, 1979). An earlier reference suggested a minimum temperature of 1 to 7 C (Table 7.3). Although concern has been expressed about the low temperature growth limits of *Salmonella* (Table 7.3), Mossel et al. (1981) examined the growth potential of numerous strains and found that only one strain, *S. panama* R IV 1917, could grow at 4 C. Other bacterial foodborne

Table 7.3 *Low Temperature Growth of Some Foodborne Bacterial Pathogens*

Organism	*Minimum temperature for growth, C*	*Reference*
Aeromonas hydrophila	1–5	Eddy (1960)
Bacillus cereus	7	Chung et al. (1976)
Campylobacter jejuni	27	Kreig (1984)
Clostridium botulinum (E)	3.3	Schmidt et al. (1961)
Clostridium perfringens	20 (most strains) 6 (rarely)	Kreig (1984)
Escherichia coli	4	Olsvik and Kapperud (1982)
Listeria monocytogenes	3	Gray and Killinger (1966)
Plesiomonas shigelloides	8	Kreig (1984)
Salmonella	5.2	Matches and Liston (1968)
Staphylococcus aureus	10	Genigeorgis et al. (1969)
Vibrio parahaemolyticus	5	Beuchat (1975)
Yersinia enterocolitica	1–7	Hanna et al. (1977)

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pathogens have a minimum temperature for growth below 7.2 C, and refrigeration therefore may not be depended on to prevent significant growth indefinitely.

A few examples, reported by various workers, of low-temperature growth of microorganisms are of interest. Of the molds, *Cladosporium* and *Sporotrichum* have been found growing on foods at –6.7 C and *Penicillium* and *Monilia* at –4 C. Growth by one yeast has taken place at –34 C, and two others grew at –18 C (Michener and Elliott, 1964). Bacteria have been reported growing at temperatures as low as –5 C on meats, –10 C on cured meats, –11 C on fish, –12.2 C on vegetables (peas), and –10 C in ice cream; yeast at –5 C on meats and –17.8 C on oysters; and molds at –7.8 C on meats and vegetables and –6.7 C on berries.

TEMPERATURES EMPLOYED IN LOW-TEMPERATURE STORAGE

Many of the terms used in connection with low-temperature storage are applied rather loosely; e.g., the term "cold storage" may refer to the use of temperatures above or below freezing, although the application of mechanical refrigeration is implied. More often it is considered to be storage above 0 C. The term "frozen storage" is more obvious; the product is stored in a frozen state, but the exact temperature would depend on the product. Most commercial storage freezers are at or below –18 C.

❑ Common, or Cellar, Storage

The temperature in common, or cellar, storage usually is not much below that of the outside air and seldom is lower than 15 C. Root crops, potatoes, cabbage, celery, apples, and similar foods can be stored for limited periods. The deterioration of such fruits and vegetables by their own enzymes and by microorganisms is not prevented but is slower than at atmospheric temperatures. Too low a humidity in the storage cellar results in losses of moisture from the stored food, and too high a humidity favors spoilage by microorganisms. In locations where no refrigeration is available common storage of all foods is the rule.

❑ Chilling, or "Cold Storage"

Chilling storage is at temperatures not far above freezing and usually involves cooling by ice or by mechanical refrigeration. It may be used as the main preservative method for foods or for temporary preservation until some other preservative process is applied. Most perishable foods, including eggs, dairy products, meats, seafood, vegetables, and fruits, may be held in chilling storage for a limited time with little change from their original condition. Enzymatic and microbial changes in the foods are not prevented but are slowed considerably.

Factors to be considered in connection with chilling storage include the temperature of chilling, the relative humidity, air velocity and composition of the atmosphere in the storeroom, and the possible use of ultraviolet rays or other radiations.

Temperature The lower the temperature of storage, the greater the cost. Therefore, although most foods will keep best at a temperature just above their freezing point,

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they are not necessarily stored at this low temperature. Instead, the chilling temperature is selected on the basis of the kind of food and the time and conditions of storage. Certain foods have an optimal storage temperature or range of temperatures well above the freezing point and may be damaged by lower temperatures. A well-known example is the banana, which should not be kept in the refrigerator; it keeps best at about 13.3 to 16.7 C. Some varieties of apples undergo "low-temperature breakdown" at temperatures near freezing, and sweet potatoes keep best at 10 to 12.8 C.

The temperature of a refrigerator is mechanically controlled but varies in different parts, usually between 0 and 10 C.

Relative Humidity The optimal relative humidity of the atmosphere in chilling storage varies with the food stored and with environmental factors such as temperature, composition of the atmosphere, and ray treatments. Too low a relative humidity results in loss of moisture and hence of weight, the wilting and softening of vegetables, and the shrinkage of fruits. Too high a relative humidity favors the growth of spoilage microorganisms. The highest humidity, near saturation, is required for most bacterial growth on the surface of foods; less moisture is needed by yeasts, about 90 to 92 percent, and still less by molds, which can grow in a relative humidity of 85 to 90 percent. Changes in humidity, as well as in temperature, during storage may cause "sweating," or precipitation of moisture, on the food. A moist surface favors microbial spoilage, e.g., slime on the moist surface of sausage.

Examples of how the optimal relative humidity and the temperature for chilling storage vary with the food stored are given in Table 7.4.

Ventilation Ventilation or control of air velocities of the storage room is important in maintaining a uniform relative humidity throughout the room, removing odors, and preventing the development of stale odors and flavors. The rate of air circulation affects the rate of drying of foods. If adequate ventilation is not provided, food in local areas of high humidity may undergo microbial decomposition.

Composition of Storage Atmosphere The amounts and proportions of gases in the storage atmosphere influence preservation by chilling. Usually no attempt

Table 7.4 *Optimal Relative Humidities and Storage Temperatures for Raw Foods*

Product	*Temp., C*	*RH, %*
Apricots	−0.5–0	85–90
Bananas	11.7–15.6	85–90
Beans (snap), peppers	7.2	85–90
Cabbage, lettuce, carrots	0	90–95
Lemons	12.8–14.4	85–90
Melons (cantaloupe)	4.4–10	80–85
Nuts	0–2.2	65–70
Onions	0	70–75
Tomatoes (ripe)	4.4–10	85–90

Source: From USDA Handb. 66.

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is made to control the composition of the atmosphere, although stored plant foods continue to respire, using oxygen and giving off carbon dioxide. In recent years, however, increased attention has been given to "gas storage" of foods, where the composition of the atmosphere has been controlled by the introduction of carbon dioxide, ozone (experimentally) or other gas or the removal of carbon dioxide. Gas storage (see page 206) ordinarily is combined with chilling storage. It has been found that in the presence of optimal concentrations of carbon dioxide or ozone, (1) a food will remain unspoiled for a longer period, (2) a higher relative humidity can be maintained without harm to the keeping quality of certain foods, or (3) a higher storage temperature can be used without shortening the keeping time of the food than is possible with ordinary chilling storage. It is especially advantageous to be able to maintain a high relative humidity without added risk of microbial spoilage, because many foods keep their original qualities better if they lose little moisture.

The optimal concentration of carbon dioxide in the atmosphere varies with the food stored, from the approximately 2.5 percent reported to be best for eggs and 10 percent for chilled beef up to the 100 percent for bacon. For some foods, e.g., apples, the concentration of oxygen as well as that of carbon dioxide is significant, and a definite ratio of these gases is sought. Respiring plant cells may evolve too much carbon dioxide into the storage room for some foods, and then part of it must be removed.

Irradiation The combination of ultraviolet irradiation with chilling storage helps preserve some foods and may permit the use of a higher humidity or storage temperature than is practicable with chilling alone. Ultraviolet lamps have been installed in rooms for the storage of meat and cheese.

❑ Freezing or "Frozen Storage"

The storage of foods in the frozen condition has been an important preservative method for centuries where outdoor freezing temperatures were available. With the development of mechanical refrigeration and the quick-freezing processes, the frozen food industry has expanded rapidly. Even in the home, the freezing of foods has become extensive, now that home deep freezers are readily available. Under the usual conditions of storage of frozen foods, microbial growth is prevented entirely and the action of food enzymes is greatly retarded. The lower the storage temperature, the slower will be any chemical or enzymatic reactions, but most of them will continue slowly at any temperature new used in storage. Therefore, it is a common practice to inactivate enzymes of vegetables by scalding or blanching before freezing when practicable.

Selection and Preparation of Foods for Freezing The quality of the food to be frozen is of prime importance, for the frozen food can be no better than the food was before it was frozen. Fruits and vegetables are selected on the basis of their suitability for freezing and their maturity and are washed, trimmed, cut, or otherwise pretreated as desired. Most vegetables are scalded or blanched, and fruits may be packed in a sirup. Meats and seafood are selected for quality and are handled so as to minimize enzymatic and microbial changes. Most foods are packaged before freezing, but some foods in small pieces, e.g., strawberries, may be frozen before packaging.

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The scalding or blanching of vegetables ordinarily is done with hot water or steam, the extent of the treatment varying with the food. This brief heat treatment is supposed to accomplish the following: (1) inactivation of most of the plant enzymes which otherwise might cause toughness, change in color, mustiness, loss in flavor, softening, and loss in nutritive value, (2) reduction (as high as 99 percent) in the numbers of microorganisms on the food, (3) enhancement of the green color of vegetables such as peas, broccoli, and spinach, (4) wilting of leafy vegetables such as spinach, making them pack better, and (5) displacement of air entrapped in the tissues.

Freezing of Foods The rate of freezing of foods depends on a number of factors, such as the method employed, the temperature, circulation of air or refrigerant, size and shape of package, and kind of food. **Sharp freezing** usually refers to freezing in air with only natural air circulation or at best with electric fans. The temperature is usually −23.3 C or lower but may vary from −15 to −29 C, and freezing may take from 3 to 72 hr. This sometimes is termed **slow freezing** to contrast it to **quick freezing,** in which the food is frozen in a relatively short time. Quick freezing is variously defined but in general implies a freezing time of 30 min or less and usually the freezing of small packages or units of food. Quick freezing is accomplished by one of three general methods: (1) direct immersion of the food or the packaged food in a refrigerant, as in the freezing of fish in brine or of berries in special sirups, (2) indirect contact with the refrigerant, where the food or package is in contact with the passage through which the refrigerant at −17.8 to −45.6 C flows, or (3) air-blast freezing, where frigid air at −17.8 to −34.4 C is blown across the materials being frozen.

A method for the overseas shipment of frozen, packaged foods involves nitrogen freezing of the cartoned foods in a special aluminum case and ordinary storage on the ship. The original low temperature and the insulation guarantee that the food will remain in the frozen condition for the desired period. Certain fruits and vegetables, fish, shrimp, and mushrooms now are being frozen by means of liquid nitrogen. For **dehydrofreezing,** fruits and vegetables have about half their moisture removed before freezing.

The advantages claimed for quick freezing over slow freezing are that (1) smaller ice crystals are formed, hence there is less mechanical destruction of intact cells of the food, (2) there is a shorter period of solidification and therefore less time for diffusion of soluble materials and separation of ice, (3) there is more prompt prevention of microbial growth, and (4) there is more rapid slowing of enzyme action. Quick-frozen foods, therefore, are supposed to thaw to a condition more like that of the original food than slow-frozen foods. This seems to be true for some foods, e.g., vegetables, but not necessarily for all foods. Research on fish, for example, has indicated little advantage for quick freezing over slow freezing.

Changes during Preparation for Freezing The rate and kind of deterioration of foods before freezing will depend on the condition of the food at harvesting or slaughter and the methods of handling thereafter. The temperatures at which the food is held and other environmental conditions will determine the kinds of microorganisms to grow and the changes to be produced. The condition of the food at the time of freezing will determine the potential quality of the frozen food.

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Changes during Freezing The quick-freezing process rapidly slows chemical and enzymatic reactions in the foods and stops microbial growth. A similar effect is produced by sharp or slow freezing, but with less rapidity. The physical effects of freezing are of great importance. There is an expansion in volume of the frozen food, and ice crystals form and grow in size. These crystals usually are larger with slow freezing, and more ice accumulates between tissue cells than with quick freezing and may crush cells. Water is drawn from the cells to form such ice, with a resultant increase in the concentration of solutes in the unfrozen liquor, which in this way has a constantly dropping freezing point until a stable condition is reached. It is claimed that the ice crystals rupture tissue cells or even microorganisms, but some workers minimize the importance of such an effect. The increased concentration of solutes in the cells hastens the salting out, dehydration, and denaturation of proteins and causes irreversible changes in colloidal systems, such as the syneresis of hydrophilic colloids. Furthermore, it is thought to be responsible for killing microorganisms.

Changes during Storage During storage of the food in the frozen condition chemical and enzymatic reactions proceed slowly. Meat, poultry, and fish proteins may become irreversibly dehydrated, the red myoglobin of meat may be oxidized—especially at surfaces—to brown metmyoglobin, and fats of meat and fish may become oxidized and hydrolyzed. The unfrozen, concentrated solution of sugars, salts, etc., may ooze from packages of fruits or concentrates during storage as a viscous material called the **metacryotic liquid.** Fluctuation in the storage temperature results in growth in the size of ice crystals and in physical damage to the food. Desiccation of the food is likely to take place at its surface during storage. When ice crystals evaporate from an area at the surface, a defect called **freezer burn** is produced on fruits, vegetables, meat, poultry, and fish. The spot usually appears dry, grainy, and brownish; in this area the chemical changes mentioned above take place, and the tissues become dry and tough.

At freezing temperatures vegetative cells of microorganisms that are unable to multiply will, in time, die. There is a slow but continuous decrease in numbers of viable microorganisms as storage continues, with some species dying more rapidly than others but with representatives of most species surviving for months or even years.

Changes during Thawing Most of the changes that seem to appear during thawing are the result of freezing and storage but do not become evident earlier. When the ice crystals melt, the liquid either is absorbed back into the tissue cells or leaks out from the food. Slow, well-controlled thawing usually results in better return of moisture to the cells than does rapid thawing and results in a food more like the original food that was frozen. The pink or reddish liquid that comes from meat on thawing is called **drip,** or **bleeding,** and the liquid oozing from fruits or vegetables on thawing is termed **leakage.** The wilting or flabbiness of vegetables and the mushiness of fruits on thawing are chiefly the result of physical damage during freezing. During thawing the rate of action of enzymes in the food will increase, but the time for action will be comparatively short if the food is utilized promptly.

If thawing is reasonably rapid and the food is used promptly, there should be little trouble with growth of microorganisms because the temperatures will be too

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low for any appreciable amount of growth. Only when the thawing is very slow or when the thawed food is allowed to stand at room temperature is there opportunity for any considerable amount of growth and activity of microorganisms. The kinds of organisms growing depend on the temperature of thawing and the time the food was allowed to stand after thawing.

Disposal of Thawed Foods Sometimes power failures lead to partial or complete thawing of foods in freezers. Thawed fruits may be refrozen. Flesh foods and vegetables may be refrozen if the packages still contain some ice. Refrozen foods will contain large ice crystals and may show leakage of liquid (syneresis) and mushiness. If thawed, flesh foods can be used if their temperature is below 3.3 C. In case of doubt, the food should be discarded.

Precooked Frozen Foods Precooked frozen foods include such a variety of types of foods and food products that they can be most conveniently discussed together. Most such foods are meat, fish, or poultry products, e.g., soups, creamed products, stews, pies, fried fish or poultry, chow mein, barbecued meat, meat loaf, and chicken à la king. Some bakery products, fruits, and vegetables may be cooked and then frozen, however. The precooking process usually is enough to kill any pathogens in the raw material and greatly reduce the total number of microorganisms present. Most samples of precooked frozen foods examined by various workers suggest that such items can be prepared commercially with total counts of less that 50,000 per gram. The cooking process would not destroy any performed staphylococcus toxin in the food. Enterococci survive freezing and persist longer during storage than do coliform bacteria and therefore are recommended as "indicator bacteria" for possible fecal contamination.

It is especially important to prevent contamination of the food after the precooking, for any pathogenic or spoilage organism then introduced would find competition from other organisms greatly reduced and the cooked food probably a better culture medium than the original raw material if opportunity for growth were given. Therefore it also is important that cooling and freezing be done promptly after cooking so as to give no opportunity for such growth.

If these precooked frozen foods are kept at warm temperatures too long after thawing, there may be growth and toxin production by food-poisoning organisms, although no such occurrence has ever been reported. The final cooking of "warming up" of these products in the home or restaurant is not always enough of a heat treatment to greatly reduce numbers of organisms present or guarantee that any pathogens present will be killed or toxins destroyed.

EFFECT OF SUBFREEZING AND FREEZING TEMPERATURES ON MICROORGANISMS

It is difficult to discuss the consequences of freezing on microorganisms because of the numerous variables and observed effects. An obvious problem with such observations is that it is impossible to study effects of freezing on cells without observing effects of cooling (decrease to 0 C) and effects of thawing. Marth (1973)

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has summarized the freezing of microorganisms as involving (1) cooling of the cells to 0 C, (2) further cooling with extra- and possibly intracellular ice-crystal formation, (3) concentration of extra- and intracellular solutes, (4) storage of cells in a frozen state, and (5) thawing of the cells and substrate.

Freezing usually results in a considerable reduction in the number of viable organisms in a food. This observed decrease in recoverable numbers can be the result of lethal or sublethal effects.

❑ Lethal Effects

Many cells are killed by freezing, but this is not a sterilization procedure. One of the most widely used techniques for the *preservation* of cultures is by freezing and frozen storage, usually in liquid nitrogen. Lethal effects are thought to be the result of denaturation or flocculation of essential cell proteins or enzymes possibly as a result of the increased concentration of solutes in the unfrozen water or perhaps in part because of physical damage by ice crystals. Rapid cooling of cells from an optimal temperature to 0 C can also result in death. This observation is referred to as **cold shock** and is thought to be related to alterations of lipids in the membrane with damage to the permeability of the cell or to the release of repair enzyme inhibitors, e.g., a ribonuclease inhibitor.

❑ Sublethal Effects

During normal microbiological enumeration on frozen foods a reduction in numbers may not always represent true death of the population. Actually, some of the cells may be in an injured or damaged state which prevents their recovery for enumeration. Cells in this state have been referred to as freeze-injured, frost-injured, or metabolically injured. Freezing of microorganisms in a food may therefore result in cryoinjury. Since these cells can be recovered if repair time is permitted or additional nutritional factors are added to the enumeration media, they are not really dead.

The attention given to cryoinjury of microorganisms in frozen foods is related to a concern over the significance of bacteriological standards on such items, the adequacy of enumeration procedures, the pathogenicity of injured pathogens, and interpretation of viable numbers of microorganisms in frozen foods.

❑ Response of Microorganisms to Freezing

The following variables or factors occur during freezing and perhaps dictate why some microorganisms die, some are injured, and some are not damaged.

1. ***The kind of microorganism and its state.*** Resistance to freezing varies with the kind of microorganism, its phase of growth, and whether it is a vegetative cell or a spore. Christophersen (1973) has classified microorganisms on the basis of sensitivity to freezing as (*a*) susceptible, (*b*) moderately resistant, and (*c*) insensitive organisms. The vegetative cells of yeast and mold and many gram-negative bacteria would be in the first group. Gram-positive organisms including staphylococci and enterococci would be in the second group. The third group would be predominantly sporeformers, since the spores of bacilli and clostridia are very

resistant to freezing. Bacteria in the logarithmic phase of growth would be more easily killed than those in other phases.

2. ***The freezing rate.*** There appears to be a critical range of temperatures (see below) which result in lethal effects; therefore, faster freezing rates would tend to be less destructive since the critical range would be passed through faster.

3. ***The freezing temperature.*** High freezing temperatures are more lethal. More organisms are inactivated at –4 to –10 C than at –15 to –30 C.

4. ***The time of frozen storage.*** The initial killing rate during freezing is rapid, but it is followed by a gradual reduction of microorganisms and is referred to as **storage death.** The number of viable organisms decreases with lengthened time of storage. Storage of frozen foods in the critical range of temperatures would result in a more rapid reduction than at higher or lower freezing temperatures.

5. ***The kind of food.*** The composition of the food influences the rate of death of organisms during freezing and storage. Sugar, salt, proteins, colloids, fat, and other substances may be protective, whereas high moisture and low pH may hasten killing.

6. ***Influence of defrosting.*** The response of microorganisms to the rate of defrosting varies. Rapid warming has been found to be harmful to some bacteria.

7. ***Alternate freezing and thawing.*** Alternate freezing and thawing is reported to hasten the killing of microorganisms but apparently does not always do so.

8. ***Possible events during freezing of the cell.*** As the temperature is lowered, more and more water freezes. The remaining or unfrozen free water at each temperature therefore becomes more and more concentrated with solutes (salts, proteins, nucleic acids, etc.). This can change the pH of cellular matter, concentrate electrolytes, alter colloidal states, denature proteins, and increase viscosity. Ice crystals can form outside the cell ("extracellular ice") and draw water out of the cell with a resulting dehydration or concentration effect. Intracellular crystals may form and grow or crystallize right through the cell, resulting in altered permeability or "holes" in the membrane and cell wall. Intracellular ice is thought to be more harmful to cells than are extracellular ice crystals.

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