# Water and Ice

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Unnoticed in the darkness of a subterranean cavern, a water droplet trickles slowly down a stalactite, following a path left by countless predecessors, imparting, as did they, a small but almost magical touch of mineral beauty. Pausing at the tip, the droplet grows slowly to full size, then plunges quickly to the cavern floor, as if anxious to perform other tasks or to assume different forms. For water, the possibilities are countless. Some droplets assume roles of quiet beauty — on a child's coat sleeve, where a snowflake of unique design and exquisite perfection lies unnoticed; on a spider's web, where dew drops burst into sudden brilliance at the first touch of the morning sun; in the countryside, where a summer shower brings refreshment; or in the city, where fog gently permeates the night air, subduing harsh sounds with a glaze of tranquility. Others lend themselves to the noise and vigor of a waterfall, to the overwhelming immensity of a glacier, to the ominous nature of an impending storm, or to the persuasiveness of a tear on a woman's cheek. For others the role is less obvious but far more critical. There is life — initiated and sustained by water in a myriad of subtle and poorly understood ways — or death inevitable, catalyzed under special circumstances by a few hostile crystals of ice; or decay at the forest's floor, where water works relentlessly to disassemble the past so life can begin anew. But the form of water most familiar to humans is none of these; rather, it is simple, ordinary, and uninspiring, unworthy of special notice as it flows forth in cool abundance from a household tap. “Humdrum,” galunks a frog in concurrence, or so it seems as he views with stony indifference the watery milieu on which his very life depends. Surely, then, water's most remarkable feature is deception, for it is in reality a substance of infinite complexity, of great and unassessable importance, and one that is endowed with a strangeness and beauty sufficient to excite and challenge anyone making its acquaintance.

2.1 Introduction

On this planet, water is the only substance that occurs abundantly in all three physical states. It is the only common liquid and is the most widely distributed pure solid, being ever present
### TABLE 1 Water Contents of Various Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat</strong></td>
<td></td>
</tr>
<tr>
<td>Pork, raw, composite of lean cuts</td>
<td>53–60</td>
</tr>
<tr>
<td>Beef, raw, retail cuts</td>
<td>50–70</td>
</tr>
<tr>
<td>Chicken, all classes, raw meat without skin</td>
<td>74</td>
</tr>
<tr>
<td>Fish, muscle proteins</td>
<td>65–81</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
</tr>
<tr>
<td>Berries, cherries, pears</td>
<td>80–85</td>
</tr>
<tr>
<td>Apples, peaches, oranges, grapefruit</td>
<td>90–90</td>
</tr>
<tr>
<td>Rhubarb, strawberries, tomatoes</td>
<td>90–95</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
</tr>
<tr>
<td>Avocado, bananas, peas (green)</td>
<td>74–80</td>
</tr>
<tr>
<td>Beets, broccoli, carrots, potatoes</td>
<td>85–90</td>
</tr>
<tr>
<td>Asparagus, beans (green), cabbage, cauliflower, lettuce</td>
<td>90–95</td>
</tr>
</tbody>
</table>

somewhere in the atmosphere as suspended ice particles, or on the earth's surface as various types of snow and ice. It is essential to life: as an important governor of body temperature, as a solvent, as a carrier of nutrients and waste products, as a reactant and reaction medium, as a lubricant and plasticizer, as a stabilizer of biopolymer conformation, as a likely facilitator of the dynamic behavior of macromolecules, including their catalytic (enzymatic) properties, and in other ways yet unknown. It is truly remarkable that organic life should depend so heavily on this small inorganic molecule, and, perhaps even more remarkable, that so few scientists are aware of this fact.

Water is the major component of many foods, each having its own characteristic allotment of this component (Table 1). Water in the proper amount, location, and orientation profoundly influences the structure, appearance, and taste of foods and their susceptibility to spoilage. Because most kinds of fresh foods contain large amounts of water, effective forms of preservation are needed if long-term storage is desired. Removal of water, either by conventional dehydration or by separation locally in the form of pure ice crystals (freezing), greatly alters the native properties of foods and biological matter. Furthermore, all attempts (rehydration, thawing) to return water to its original status are never more than partially successful. Ample justification exists, therefore, to study water and ice with considerable care.

### 2.2 Physical Properties of Water and Ice

As a first step in becoming familiar with water, it is appropriate to consider its physical properties as shown in Table 2. By comparing water's properties with those of molecules of similar molecular weight and atomic composition (CH₄, NH₃, HF, H₂S, H₂Se, H₂Te) it is possible to determine if water behaves in a normal fashion. Based on this comparison, water is found to melt and boil at unusually high temperatures; to exhibit unusually large values for surface tension, permittivity (dielectric constant), heat capacity, and heats of phase transition (heats of fusion, vaporization, and sublimation); to have a moderately low value for density; to exhibit an unusual attribute of expanding upon solidification; and to possess a viscosity that in light of the foregoing oddities, is surprisingly normal.

In addition, the thermal conductivity of water is large compared to those of other liquids, and the thermal conductivity of ice is moderately large compared to those of other nonmetallic
TABLE 2 Physical Properties of Water and Ice

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>18.0153</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>18.0153</td>
</tr>
<tr>
<td>Phase transition properties</td>
<td></td>
</tr>
<tr>
<td>Melting point at 101.3 k Pa (1 atm)</td>
<td>0.000°C</td>
</tr>
<tr>
<td>Boiling point at 101.3 k Pa (1 atm)</td>
<td>100.000°C</td>
</tr>
<tr>
<td>Critical temperature</td>
<td>373.99°C</td>
</tr>
<tr>
<td>Critical pressure</td>
<td>22.064 MPa (218.6 atm)</td>
</tr>
<tr>
<td>Triple point</td>
<td>0.01°C and 611.73 Pa (4.589 mm Hg)</td>
</tr>
<tr>
<td>Enthalpy of fusion at 0°C</td>
<td>6.012 kJ (1.436 kcal)/mol</td>
</tr>
<tr>
<td>Enthalpy of vaporization at 100°C</td>
<td>40.657 kJ (9.711 kcal)/mol</td>
</tr>
<tr>
<td>Enthalpy of sublimination at 0°C</td>
<td>50.91 kJ (12.16 kcal)/mol</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>0.99821</td>
</tr>
<tr>
<td>Viscosity (pa·sec)</td>
<td>1.002×10⁻³</td>
</tr>
<tr>
<td>Surface tension against air (N/m)</td>
<td>72.75×10⁻³</td>
</tr>
<tr>
<td>Vapor pressure (kPa)</td>
<td>2.3388</td>
</tr>
<tr>
<td>Heat capacity (J/g·K)</td>
<td>4.1818</td>
</tr>
<tr>
<td>Thermal conductivity (liquid) (W/m·K)</td>
<td>0.5984</td>
</tr>
<tr>
<td>Thermal diffusivity (m²/s)</td>
<td>1.4×10⁻⁷</td>
</tr>
<tr>
<td>Permittivity (dielectric constant)</td>
<td>80.20</td>
</tr>
</tbody>
</table>

Source: Mainly Ref. 69.

solids. Of greater interest is the fact that the thermal conductivity of ice at 0°C is approximately four times that of water at the same temperature, indicating that ice will conduct heat energy at a much greater rate than will immobilized water (e.g., in tissue). The thermal diffusivities of water and ice are of even greater interest since these values indicate the rate at which the solid and liquid forms of HOH will undergo changes in temperature. Ice has a thermal diffusivity approximately nine times greater than that of water, indicating that ice, in a given environment, will undergo a temperature change at a much greater rate than will water. These sizable differences in thermal conductivity and thermal diffusivity values of water and ice provide a sound basis for explaining why tissues freeze more rapidly than they thaw, when equal but reversed temperature differentials are employed.

2.3 The Water Molecule

Water's unusual properties suggest the existence of strong attractive forces among water molecules, and uncommon structures for water and ice. These features are best explained by considering the nature of first a single water molecule and then small groups of molecules. To form a molecule of water, two hydrogen atoms approach the two $sp^3$ bonding orbitals of oxygen and form two covalent sigma ( ) bonds (40% partial ionic character), each of which has a dissociation energy of 4.6×10² kJ/mol (110 kcal/mol). The localized molecular orbitals remain symmetrically oriented about the original orbital axes, thus retaining an approximate
tetrahedral structure. A schematic orbital model of a water molecule is shown in Figure 1A and the appropriate van der Waals radii are shown in Figure 1B.

The bond angle of the isolated water molecule (vapor state) is 104.5° and this value is near the perfect tetrahedral angle of 109°28'. The O-H internuclear distance is 0.96 Å and the van der Waals radii for oxygen and hydrogen are, respectively, 1.40 and 1.2 Å.

At this point, it is important to emphasize that the picture so far presented is oversimplified. Pure water contains not only ordinary HOH molecules but also many other constituents in trace amounts. In addition to the common isotopes $^{16}$O and $^1$H, also present are $^{17}$O, $^{18}$O, $^2$H.

**FIGURE 1**

Schematic model of a single HOH molecule: (a) sp$^3$ configuration, and (b) van der Waals radii for a HOH molecule in the vapor state.
(deuterium) and $^3$H (tritium), giving rise to 18 isotopic variants of molecular HOH. Water also contains ionic particles such as hydrogen ions (existing as H$_2$O$^+$), hydroxyl ions, and their isotopic variants. Water therefore consists of more than 33 chemical variants of HOH, but the variants occur in only minute amounts.

### 2.4 Association of Water Molecules

The V-like form of an HOH molecule and the polarized nature of the O-H bond result in an unsymmetrical charge distribution and a vapor-state dipole moment of 1.84D for pure water. Polarity of this magnitude produces intermolecular attractive forces, and water molecules therefore associate with considerable tenacity. Water's unusually large intermolecular attractive force cannot, however, be fully accounted for on the basis of its large dipole moment. This is not surprising, since dipole moments give no indication of the degree to which charges are exposed or of the geometry of the molecule, and these aspects, of course, have an important bearing on the intensity of molecular association.

Water's large intermolecular attractive forces can be explained quite adequately in terms of its ability to engage in multiple hydrogen bonding on a three-dimensional basis. Compared to covalent bonds (average bond energy of about 335 kJ/mol), hydrogen bonds are weak (typically 2–40 kJ/mol) and have greater and more variable lengths. The hydrogen bond between oxygen and hydrogen has a dissociation energy of about 13–25 kJ/mol.

Since electrostatic forces make a major contribution to the energy of the hydrogen bond (perhaps the largest contribution), and since an electrostatic model of water is simple and leads to an essentially correct geometric picture of HOH molecules as they are known to exist in ice, further discussion of the geometrical patterns formed by association of water molecules will emphasize electrostatic effects. This simplified approach, while entirely satisfactory for present purposes, must be modified if other behavioral characteristics of water are to be explained satisfactorily.

The highly electronegative oxygen of the water molecule can be visualized as partially drawing away the single electrons from the two covalently bonded hydrogen atoms, thereby leaving each hydrogen atom with a partial positive charge and a minimal electron shield; that is, each hydrogen atom assumes some characteristics of a bare proton. Since the hydrogen—oxygen bonding orbitals are located on two of the axes of an imaginary tetrahedron (Fig. 1a), these two axes can be thought of as representing lines of positive force (hydrogen-bond donor sites). Oxygen's two lone-pair orbitals can be pictured as residing along the remaining two axes of the imaginary tetrahedron, and these then represent lines of negative force (hydrogen-bond acceptor sites). By virtue of these four lines of force, each water molecule is able to hydrogen-bond with a maximum of four others. The resulting tetrahedral arrangement is depicted in Figure 2.

Because each water molecule has an equal number of hydrogen-bond donor and receptor sites, arranged to permit three-dimensional hydrogen bonding, it is found that the attractive forces among water molecules are unusually large, even when compared to those existing among other small molecules that also engage in hydrogen bonding (e.g., NH$_3$, HF). Ammonia, with its tetrahedral arrangement of three hydrogens and one receptor site, and hydrogen fluoride, with its tetrahedral arrangement of one hydrogen and three receptor sites, do not have equal numbers of donor and receptor sites and therefore can form only two-dimensional hydrogen-bonded networks involving less hydrogen bonds per molecule than water.

Conceptualizing the association of a few water molecules becomes considerably more complicated when one considers isotopic variants and hydronium and hydroxyl ions. The
hydronium ion, because of its positive charge, would be expected to exhibit a greater hydrogen-bond donating potential than nonionized water (dashed lines are hydrogen bonds).

![Structure 1](image1)

The hydroxyl ion, because of its negative charge, would be expected to exhibit a greater hydrogen-bond acceptor potential than un-ionized water (XH represents a solute or a water molecule).

![Structure 2](image2)

Water's ability to engage in three-dimensional hydrogen bonding provides a logical explanation for many of its unusual properties; its large values for heat capacity, melting point, boiling point, surface tension, and enthalpies of various phase transitions all are related to the extra energy needed to break intermolecular hydrogen bonds.

The permittivity (dielectric constant) of water is also influenced by hydrogen bonding. Although water is a dipole, this fact alone does not account for the magnitude of its permittivity. Hydrogen-bonded clusters of molecules apparently give rise to multimolecular di-
Hydrogen bonding of water molecules in a tetrahedral configuration. Open circles are oxygen atoms and closed circles are hydrogen atoms. Hydrogen bonds are represented by dashed lines.

poles, which effectively increase the permittivity of water. Water's viscosity is discussed in a later section.

2.5 Structure of Ice

The structure of ice will be considered before the structure of water because the former is far better understood than the latter, and because ice's structure represents a logical extension of the information presented in the previous section.

2.5.1 Pure Ice

Water, with its tetrahedrally directed forces, crystallizes in an open (low density) structure that has been accurately elucidated. The O-O internuclear nearest-neighbor distance in ice is 2.76 Å and the O-O-O bond angle is about 109°, or very close to the perfect tetrahedral angle of 109°28' (Fig. 3). The manner in which each HOH molecule can associate with four others (coordination number of four) is easily visualized in the unit cell of Figure 3 by considering molecule W and its four nearest neighbors 1, 2, 3, and W'.

When several unit cells are combined and viewed from the top (down the c axis) the hexagonal symmetry of ice becomes apparent. This is shown in Figure 4a. The tetrahedral
substructure is evident from molecule W and its four nearest neighbors, with 1, 2, and 3 being visible, and the fourth lying below the plane of the paper directly under molecule W. When Figure 4a is viewed in three dimensions, as in Figure 4b, it is evident that two planes of molecules are involved (open and filled circles). These two planes are parallel, very close together, and they move as a unit during the “slip” or flow of ice under pressure, as in a glacier. Pairs of planes of this type comprise the “basal planes” of ice. By stacking several basal planes, an extended structure of ice is obtained. Three basal planes have been combined to form the structure shown in Figure 5. Viewed down the c axis, the appearance is exactly the same as that shown in Figure 4a, indicating that the basal planes are perfectly aligned. Ice is monorefringent in this direction, whereas it is birefringent in all other directions. The c axis is therefore the optical axis of ice.

With regard to the location of hydrogen atoms in ice, it is generally agreed that:

1. Each line connecting two nearest neighbor oxygen atoms is occupied by one hydrogen atom located 1 ± 0.01 Å from the oxygen to which it is covalently bonded, and 1.76 ± 0.01 Å from the oxygen to which it is hydrogen bonded. This configuration is shown in Figure 6A.

2. If the locations of hydrogen atoms are viewed over a period of time, a somewhat different picture is obtained. A hydrogen atom on a line connecting two nearest neighbor oxygen atoms, X and Y, can situate itself in one of two possible positions—either 1 Å from X or 1 Å from Y. The two positions have an equal probability of being occupied. Expressed in another way, each position will, on the average, be occupied half of the time. This is possible because HOH molecules, except at extremely low temperatures, can cooperatively rotate, and hydrogen atoms can “jump” be-
FIGURE 4
The “basal plane” of ice (combination of two layers of slightly different elevation). Each circle represents the oxygen atom of a water molecule. Open and shaded circles, respectively, represent oxygen atoms in the upper and lower layers of the basal planes. (a) Hexagonal structure viewed down the c axis. Numbered molecules relate to the unit cell in Figure 3. (b) Three-dimensional view of the basal plane. The front edge of view b corresponds to the bottom edge of view a. The crystallographic axes have been positioned in accordance with external (point) symmetry.

tween adjacent oxygen atoms. The resulting mean structure, known also as the half-hydrogen, Pauling, or statistical structure, is shown in Figure 6B.

With respect to crystal symmetry, ordinary ice belongs to the dihexagonal bipyramidal class of the hexagonal system. In addition, ice can exist in nine other crystalline polymorphic structures, and also in an amorphous or vitreous state of rather uncertain but largely noncrystal-
line structure. Of the eleven total structures, only ordinary hexagonal ice is stable under normal pressure at 0°C.

The structure of ice is not as simple as has been indicated. First of all, pure ice contains not only ordinary HOH molecules but also ionic and isotopic variants of HOH. Fortunately, the isotopic variants occur in such small amounts that they can, in most instances, be ignored, leaving for major consideration only HOH, H⁺ (H₂O⁺), and OH⁻.

Second, ice crystals are never perfect, and the defects encountered are usually of the orientational (caused by proton dislocation accompanied by neutralizing orientations) or ionic types (caused by proton dislocation with formation of H₂O⁺ and OH⁻) (see Fig. 7). The presence of these defects provides a means for explaining the mobility of protons in ice and the small decrease in dc electrical conductivity that occurs when water is frozen.

In addition to the atomic mobilities involved in crystal defects, there are other types of activity in ice. Each HOH molecule in ice is believed to vibrate with a root mean amplitude (assuming each molecule vibrates as a unit) of about 0.4 Å at -10°C. Furthermore, HOH molecules that presumably exist in some of the interstitial spaces in ice can apparently diffuse slowly through the lattice.

Ice therefore is far from static or homogeneous, and its characteristics are dependent on temperature. Although the HOH molecules in ice are four-coordinated at all temperatures, it is necessary to lower the temperature to about -180°C or lower to “fix” the hydrogen atoms in one of the many possible configurations. Therefore, only at temperatures near -180°C or lower will
FIGURE 6

Location of hydrogen atoms (●) in the structure of ice. (A) Instantaneous structure. (B) Mean structure [known also as the half-hydrogen (●), Pauling, or statistical structure]. Open circle is oxygen.

FIGURE 7

Schematic representation of proton defects in ice. (A) Formation of orientational defects. (B) Formation of ionic defects. Open and shaded circles represent oxygen and hydrogen atoms, respectively. Solid and dashed lines represent chemical bonds and hydrogen bonds, respectively.
all hydrogen bonds be intact, and as the temperature is raised, the mean number of intact (fixed) hydrogen bonds will decrease gradually.

2.5.2 Ice in the Presence of Solutes

The amount and kind of solutes present can influence the quantity, size, structure, location, and orientation of ice crystals. Consideration here will be given only to the effects of solutes on ice structure. Luyet and co-workers [75,77] studied the nature of ice crystals formed in the presence of various solutes including sucrose, glycerol, gelatin, albumin, and myosin. They devised a classification system based on morphology, elements of symmetry, and the cooling velocity required for development of various types of ice structure. Their four major classes are hexagonal forms, irregular dendrites; coarse spherulites, and evanescent spherulites.

The hexagonal form, which is most highly ordered, is found exclusively in foods, provided extremely rapid freezing is avoided and the solute is of a type and concentration that does not interfere unduly with the mobility of water molecules. Gelatin at high concentrations will, for example, result in more disordered forms of ice crystals.

2.6 Structure of Water

To some, it may seem strange to speak of structure in a liquid when fluidity is the essence of the liquid state. Yet it is an old and well-accepted idea [96] that liquid water has structure, obviously not sufficiently established to produce long-range rigidity, but certainly far more organized than that of molecules in the vapor state, and ample to cause the orientation and mobility of a given water molecule to be influenced by its neighbors.

Evidence for this view is compelling. For example, water is an “open” liquid, being only 60% as dense as would be expected on the basis of close packing that can prevail in nonstructured liquids. Partial retention of the open, hydrogen-bonded, tetrahedral arrangement of ice easily accounts for water's low density. Furthermore, the heat of fusion of ice, while unusually high, is sufficient to break only about 15% of the hydrogen bonds believed to exist in ice. Although this does not necessarily require that 85% of the hydrogen bonds existing in ice be retained in water (for example, more could be broken, but the change in energy could be masked by a simultaneous increase in van der Waals interactions), results of many studies support the notion that many water-water hydrogen bonds do exist.

Elucidation of the structure of pure water is an extremely complex problem. Many theories have been set forth, but all are incomplete, overly simple, and subject to weaknesses that are quickly cited by supporters of rival theories. That is, of course, a healthy situation, which will eventually result in an accurate structural picture (or pictures) of water. In the meantime, few statements can be made with any assurance that they will stand essentially unmodified in years to come. Thus, this subject will be dealt with only briefly.

Three general types of models have been proposed: mixture, interstitial, and continuum (also referred to as homogeneous or uniformist models) [5]. Mixture models embody the concept of intermolecular hydrogen bonds being momentarily concentrated in bulky clusters of water molecules that exist in dynamic equilibrium with other more dense species—momentarily meaning ~10^{-11} sec [73].

Continuum models involve the idea that intermolecular hydrogen bonds are distributed uniformly throughout the sample, and that many of the bonds existing in ice simply become distorted rather than broken when ice is melted. It has been suggested that this permits a continuous network of water molecules to exist that is, of course, dynamic in nature [107,120].

The interstitial model involves the concept of water retaining an ice-like or clathrate-type
structure with individual water molecules filling the interstitial spaces of the clathrates. In all three models, the dominant structural feature is the hydrogen-bonded association of liquid water in ephemeral, distorted tetrahedra. All models also permit individual water molecules to frequently alter their bonding arrangements by rapidly terminating one hydrogen bond in exchange for a new one, while maintaining, at constant temperature, a constant degree of hydrogen bonding and structure for the entire system.

The degree of intermolecular hydrogen bonding among water molecules is, of course, temperature dependent. Ice at 0°C has a coordination number (number of nearest neighbors) of 4.0, with nearest neighbors at a distance of 2.76 Å. With input of the latent heat of fusion, melting occurs; that is, some hydrogen bonds are broken (distance between nearest neighbors increases) and others are strained as water molecules assume a fluid state with associations that are, on average, more compact. As the temperature is raised, the coordination number increases from 4.0 in ice at 0°C, to 4.4 in water at 1.50°C, then to 4.9 at 83°C. Simultaneously, the distance between nearest neighbors increases from 2.76 Å in ice at 0°C, to 2.9 Å in water at 1.5°C, then to 3.05 Å at 83°C [7,80].

It is evident, therefore, that the ice-to-water transformation is accompanied by an increase in the distance between nearest neighbors (decreased density) and by an increase in the average number of nearest neighbors (increased density), with the latter factor predominating to yield the familiar net increase in density. Further warming above the melting point causes the density to pass through a maximum at 3.98°C, then gradually decline. It is apparent, then, that the effect of an increase in coordination number predominates at temperatures between 0 and 3.98°C, and that the effect of increasing distance between nearest neighbors (thermal expansion) predominates above 3.98°C.

The low viscosity of water is readily reconcilable with the type of structures that have been described, since the hydrogen-bonded arrangements of water molecules are highly dynamic, allowing individual molecules, within the time frame of nano- to picoseconds, to alter their hydrogen-bonding relationships with neighboring molecules, thereby facilitating mobility and fluidity.

2.7 Water-Solute Interactions

2.7.1 Macroscopic Level (Water Binding, Hydration, and Water Holding Capacity)

Before dealing with water-solute interactions at the molecular level, it is appropriate to discuss water-related phenomena referred to by terms such as water binding, hydration, and water holding capacity.

With respect to foods, the terms “water binding” and “hydration” are often used to convey a general tendency for water to associate with hydrophilic substances, including cellular materials. When used in this manner, the terms pertain to the macroscopic level. Although more specialized terms, such as “water binding potential,” are defined in quantitative terms, they still apply only to the macroscopic level. The degree and tenacity of water binding or hydration depends on a number of factors including the nature of the nonaqueous constituent, salt composition, pH, and temperature.

“Water holding capacity” is a term that is frequently employed to describe the ability of a matrix of molecules, usually macromolecules present at low concentrations, to physically entrap large amounts of water in a manner that inhibits exudation. Familiar food matrices that entrap water in this way include gels of pectin and starch, and cells of tissues, both plant and animal.
Physically entrapped water does not flow from tissue foods even when they are cut or minced. On the other hand, this water behaves almost like pure water during food processing; that is, it is easily removed during drying, is easily converted to ice during freezing, and is available as a solvent. Thus, its bulk flow is severely restricted, but movement of individual molecules is essentially the same as that of water molecules in a dilute salt solution.

Nearly all of the water in tissues and gels can be categorized as physically entrapped, and impairment of the entrapment capability (water holding capacity) of foods has a profound effect on food quality. Examples of quality defects arising from impairment of water holding capacity are syneresis of gels, thaw exudate from previously frozen foods, and inferior performance of animal tissue in sausage resulting from a decline in muscle pH during normal physiological events postmortem.

Gel structures and water holding capacity are discussed more fully in other chapters.

### 2.7.2 Molecular Level: General Comments

Mixing of solutes and water results in altered properties of both constituents. Hydrophilic solutes cause changes in the structure and mobility of adjacent water, and water causes changes in the reactivity, and sometimes structure, of hydrophilic solutes. Hydrophobic groups of added solutes interact only weakly with adjacent water, preferring a nonaqueous environment.

The bonding forces existing between water and various kinds of solutes are of obvious interest, and these are summarized in Table 3.

### 2.7.3 Molecular Level: Bound Water

Bound water is not a homogeneous, easily identifiable entity, and because of this, descriptive terminology is difficult, numerous definitions have been suggested, and there is no consensus about which one is best. This term is controversial, frequently misused, and in general, poorly understood, causing increasing numbers of scientists to suggest that its use be terminated.

<table>
<thead>
<tr>
<th>TABLE 3 Classifications of Types of Water-Solute Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
</tr>
<tr>
<td>Dipole-ion</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Dipole-dipole</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hydrophobic hydration</td>
</tr>
<tr>
<td>Hydrophobic interaction</td>
</tr>
</tbody>
</table>

<sup>a</sup> About 12-25 kJ/mol.

<sup>b</sup> But much weaker than strength of single covalent bond.

<sup>c</sup> R is alkyl group.

<sup>d</sup> Hydrophobic interactions are entropy driven, whereas dipole-ion and dipole-dipole interactions are enthalpy driven.
Although this may be desirable, the term “bound water” is so common in the literature that it must be discussed.

The numerous definitions proposed for “bound water” should indicate why this term has created confusion [3,51]:

1. Bound water is the equilibrium water content of a sample at some appropriate temperature and low relative humidity.
2. Bound water is that which does not contribute significantly to permittivity at high frequencies and therefore has its rotational mobility restricted by the substance with which it is associated.
3. Bound water is that which does not freeze at some arbitrary low temperature (usually -40°C or lower).
4. Bound water is that which is unavailable as a solvent for additional solutes.
5. Bound water is that which produces line broadening in experiments involving proton nuclear magnetic resonance.
6. Bound water is that which moves with a macromolecule in experiments involving sedimentation rates, viscosity, or diffusion.
7. Bound water is that which exists in the vicinity of solutes and other nonaqueous substances and has properties differing significantly from those of “bulk” water in the same system.

All of these definitions are valid, but few will produce the same value when a given sample is analyzed.

From a conceptual standpoint it is useful to think of bound water as “water that exists in the vicinity of solutes and other nonaqueous constituents, and exhibits properties that are significantly altered from those of ‘bulk water’ in the same system.” Bound water should be thought of as having “hindered mobility” as compared to bulk water, not as being “immobilized.” In a typical food of high water content, this type of water comprises only a minute part of the total water present, approximately the first layer of water molecules adjacent to hydrophilic groups. The subject of bound water (hindered water) will be discussed further in the section dealing with molecular mobility (Mm) in frozen systems.

Interactions between water and specific classes of solutes will now be considered.

2.7.4 Interaction of Water with Ions and Ionic Groups

Ions and ionic groups of organic molecules hinder mobility of water molecules to a greater degree than do any other types of solutes. The strength of water-ion bonds is greater than that of water-water hydrogen bonds, but is much less than that of covalent bonds.

The normal structure of pure water (based on a hydrogen-bonded, tetrahedral arrangement) is disrupted by the addition of dissociable solutes. Water and simple inorganic ions undergo dipole-ion interactions. The example in Figure 8 involves hydration of the NaCl ion pair. Only first-layer water molecules in the plane of the paper are illustrated. In a dilute solution of ions in water, second-layer water is believed to exist in a structurally perturbed state because of conflicting structural influences of first-layer water and the more distant, tetrahedrally oriented “bulk-phase” water. In concentrated salt solutions, bulk-phase water would not exist and water structure would be dominated by the ions.

There is abundant evidence indicating that some ions in dilute aqueous solution have a net structure-breaking effect (solution is more fluid than pure water), whereas others have a net structure-forming effect (solution is less fluid than pure water). It should be understood that the term “net structure” refers to all kinds of structures, either normal or new types of water structure. From the standpoint of “normal” water structure, all ions are disruptive.
The ability of a given ion to alter net structure is related closely to its polarizing power (charge divided by radius) or simply the strength of its electric field. Ions that are small and/or multivalent (mostly positive ions, such as Li⁺, Na⁺, H₂O⁺, Ca²⁺, Ba²⁺, Mg²⁺, Al³⁺, F⁻, and OH⁻) have strong electric fields and are net structure formers. The structure imposed by these ions more than compensates for any loss in normal water structure. These ions strongly interact with the four to six first-layer water molecules, causing them to be less mobile and pack more densely than HOH molecules in pure water. Ions that are large and monovalent (most of the negatively charged ions and large positive ions, such as K⁺, Rb⁺, Cs⁺, NH₄⁺, Cl, Br⁻, I⁻, NO₃⁻, BrO₃⁻, IO₃⁻, and ClO₄⁻) have rather weak electric fields and are net structure breakers, although the effect is very slight with K⁺. These ions disrupt the normal structure of water and fail to impose a compensating amount of new structure.

Ions, of course, have effects that extend well beyond their influence on water structure. Through their varying abilities to hydrate (compete for water), alter water structure, influence the permittivity of the aqueous medium, and govern the thickness of the electric double layer around colloids, ions profoundly influence the “degree of hospitality” extended to other nonaqueous solutes and to substances suspended in the medium. Thus, conformation of proteins and stability of colloids (salting-in, salting-out in accord with the Hofmeister or lyotropic series) are greatly influenced by the kinds and amounts of ions present [18,68].

2.7.5 Interaction of Water with Neutral Groups Possessing Hydrogen-Bonding Capabilities (Hydrophilic Solutes)

Interactions between water and nonionic, hydrophilic solutes are weaker than water-ion interactions and about the same strength as those of water-water hydrogen bonds. Depending on the strength of the water-solute hydrogen bonds, first-layer water may or may not exhibit reduced mobility and other altered properties as compared to bulk-phase water.

Solut es capable of hydrogen bonding might be expected to enhance or at least not disrupt the normal structure of pure water. However, in some instances it is found that the distribution and orientation of the solute’s hydrogen-bonding sites are geometrically incompatible with those existing in normal water. Thus, these kinds of solutes frequently have a disruptive influence on the normal structure of water. Urea is a good example of a small hydrogen-bonding solute that for geometric reasons has a marked disruptive effect on the normal structure of water.
It should be noted that the total number of hydrogen bonds per mole of solution may not be significantly altered by addition of a hydrogen-bonding solute that disrupts the normal structure of water. This is possible since disrupted water-water hydrogen bonds may be replaced by water-solute hydrogen bonds. Solutes that behave in this manner have little influence on “net structure” as defined in the previous section.

Hydrogen bonding of water can occur with various potentially eligible groups (e.g., hydroxyl, amino, carbonyl, amide, imino, etc.). This sometimes results in “water bridges” where one water molecule interacts with two eligible hydrogen-bonding sites on one or more solutes. A schematic depiction of water hydrogen bonding (dashed lines) to two kinds of functional groups found in proteins is shown:

\[
\begin{align*}
\text{H} \\
\text{N} \cdots \text{O} \cdots \text{H} \cdots \text{O} = \text{C} \\
\end{align*}
\]

A more elaborate example involving a three-HOH bridge between backbone peptide units in papain is shown in Figure 9.

It has been observed that hydrophilic groups in many crystalline macromolecules are separated by distances identical to the nearest-neighbor oxygen spacing in pure water. If this spacing prevails in hydrated macromolecules this would encourage cooperative hydrogen bonding in first- and second-layer water.
2.7.6 Interaction of Water with Nonpolar Substances

The mixing of water and hydrophobic substances, such as hydrocarbons, rare gases, and the apolar groups of fatty acids, amino acids, and proteins is, not surprisingly, a thermodynamically unfavorable event \( (G > 0) \). The free energy is positive not because \( H \) is positive, which is typically true for low-solubility solutes, but because \( T S \) is negative [30]. This decrease in entropy occurs because of special structures that water forms in the vicinity of these incompatible apolar entities. This process has been referred to as hydrophobic hydration (Table 3 and Fig. 10a).

Because hydrophobic hydration is thermodynamically unfavorable, it is understandable that water would tend to minimize its association with apolar entities that are present. Thus, if two separated apolar groups are present, the incompatible aqueous environment will encourage them to associate, thereby lessening the water-apolar interfacial area—a process that is thermodynamically favorable \( (G < 0) \). This process, which is a partial reversal of hydrophobic hydration, is referred to as “hydrophobic interaction” and in its simplest form can be depicted as

\[
R \text{ (hydrated)} + R \text{ (hydrated)} \rightarrow R_2 \text{ (hydrated)} + H_2O
\]

where \( R \) is an apolar group (Table 3 and Fig. 10b).

Because water and apolar groups exist in an antagonistic relationship, water structures itself to minimize contact with apolar groups. The type of water structure believed to exist in the layer next to apolar groups is depicted in Figure 11. Two aspects of the antagonistic relationship between water and hydrophobic groups are worthy of elaboration: formation of clathrate hydrates, and association of water with hydrophobic groups in proteins.

A clathrate hydrate is an ice-like inclusion compound wherein water, the “host” substance, forms a hydrogen-bonded, cage-like structure that physically entraps a small apolar molecule.

![Figure 10](image)

**FIGURE 10**

Schematic depiction of (a) hydrophobic hydration and (b) hydrophobic association. Open circles are hydrophobic groups. Hatched areas are water. (Adapted from Ref. 28.)
known as the “guest.” These entities are of interest because they represent the most extreme structure-forming response of water to an apolar substance and because microstructures of a similar type may occur naturally in biological matter. Clathrate hydrates are, in fact, crystalline, they can easily be grown to visible size, and some are stable at temperatures above 0°C provided the pressure is sufficient.

The guest molecules of clathrate hydrates are low-molecular-weight compounds with sizes and shapes compatible with the dimensions of host water cages comprised of 20–74 water molecules. Typical guests include low-molecular-weight hydrocarbons and halogenated hydrocarbons; rare gases; short-chain primary, secondary, and tertiary amines; and alkyl ammonium, sulfonium, and phosphonium salts. Interaction between water and guest is slight, usually involving nothing more than weak van der Waals forces. Clathrate hydrates are the extraordinary result of water's attempt to avoid contact with hydrophobic groups.

There is evidence that structures similar to crystalline clathrate hydrates may exist naturally in biological matter, and if so, these structures would be of far greater importance than crystalline hydrates since they would likely influence the conformation, reactivity, and stability of molecules such as proteins. For example, it has been suggested that partial clathrate structures may exist around the exposed hydrophobic groups of proteins. It is also possible that clathrate-like structures of water have a role in the anesthetic action of inert gases such as xenon. For further information on clathrates, the reader is referred to Davidson [15].

Unavoidable association of water with hydrophobic groups of proteins has an important influence on protein functionality [5,124]. The extent of these unavoidable contacts is potentially fairly great because nonpolar side chains exist on about 40% of the amino acids in typical oligomeric food proteins. These nonpolar groups include the methyl group of alanine, the benzyl group of phenylalanine, the isopropyl group of the valine, the mercaptomethyl group of cysteine, and the secondary butyl and isobuty1 groups of the leucines. The nonpolar groups of other compounds such as alcohols, fatty acids, and free amino acids also can participate in hydrophobic interactions, but the consequences of these interactions are undoubtedly less important than those involving proteins.

Because exposure of protein nonpolar groups to water is thermodynamically unfavorable, association of hydrophobic groups or “hydrophobic interaction” is encouraged, and this occurrence is depicted schematically in Figure 12. Hydrophobic interaction provides a major driving
force for protein folding, causing many hydrophobic residues to assume positions in the protein interior. Despite hydrophobic interactions, it is estimated that nonpolar groups in globular proteins typically occupy about 40–50% of the surface area. Hydrophobic interactions also are regarded as being of primary importance in maintaining the tertiary structure of most proteins [19,85,123]. It is therefore of considerable importance that a reduction in temperature causes hydrophobic interactions to become weaker and hydrogen bounds to become stronger.

2.7.7 Details of Water Orientation Adjacent to Organic Molecules

Although determination of the arrangement of water molecules near organic molecules is experimentally difficult, this is an active field of research and useful data have been obtained. The hydrated pyranose sugar ring is shown in Figure 13 and a computer-simulated cross section of hydrated myoglobin is shown in Figure 14. Assuming a separation distance of 2.8 Å between hydration sites and full occupancy of these sites, about 360 HOH molecules would be in the primary hydration shell of myoglobin [71].
2.7.8 Hydration Sequence of a Protein

It is instructive to consider water absorption by a dry food component and the location and properties of water at each stage of the process. A protein is chosen for this exercise because proteins are of major importance in foods, because they contain all of the major types of functional groups that are of interest during hydration, and because good data are available.

Shown in Table 4 are properties of globular proteins (based primarily on lysozyme) and the associated water at various stages of hydration. The corresponding sorption isotherm is shown in Figure 19. The table is self-explanatory except for a few points. Hydration “zones” are referred to in both the table and the figure. These zones are useful aids to discussion but they are unlikely to actually exist (a continuum of water properties is much more likely).

A sample having a water content corresponding to the junction of Zones I and II is said to have a BET monolayer water content (the term “BET” comes from the names of the originators of the concept: Brunauer, Emmett, and Teller [8]). In this instance the BET monolayer water content is about 0.07 g HOH/g dry protein, and this corresponds to a $p/p_0$ value of about 0.2 ($p$ is the partial pressure of water in the food and $p_0$ is the vapor pressure of pure water at the same temperature; $p/p_0$ is more commonly referred to as $a_w$). The BET monolayer value is of special importance because it often provides a good first estimate of the largest moisture content.
a dry product can contain and still exhibit maximum stability. Although the BET value is commonly referred to as a monolayer, this is a faulty concept. For example, in starch the BET value corresponds to about one water molecule per anhydroglucose unit [126].

Also note that in Table 4 the term “true monolayer” is used. This term has a meaning quite different from BET monolayer. True monolayer refers to the water content at the junction of zones IIB and III (in this example, a water content of about 0.38 g HOH/g dry protein and a $p/p_0$ of about 0.85). This value corresponds to about 300 mol HOH per mol lysozyme and a moisture content of 27.5 wt%, with one HOH occupying, on average, 20 Å² of protein surface area [103]. This water content is significant because it represents the minimum water content
### Table 4: Water/Protein Properties at Various Stages of Hydration \(^a\)

<table>
<thead>
<tr>
<th>Properties</th>
<th>Constitutional water(^b)</th>
<th>Hydration shell (≤ 3 Å from surface)</th>
<th>Free(^c)</th>
<th>Entrapped(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General description for lysozyme</td>
<td>Constitutional water is assumed to be present in the dry protein at the onset of the hydration process. Water is first absorbed at sites of ionized, carboxylic and amino side chains, with about 40 mol water/mol lysozyme associating in this manner. Further absorption of water results in gradual hydration of less attractive sites, mainly amide carbonyl groups of the protein backbone. Attainment of true monolayer hydration of the protein is achieved at 0.38 g (H_2O/g) dry protein, by water associating with sites that are still less attractive. At this point, there is, on average, 1 HOH/20 Å(^2) of protein surface.</td>
<td></td>
<td>Fully hydrated</td>
<td>Fully hydrated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Approximate water content: g H(_2)O/g dry protein (h) mol H(_2)O/mol dry protein wt% based on lysozyme</th>
<th>&lt;0.01 h</th>
<th>0.01–0.07 h</th>
<th>0.07–0.25 h</th>
<th>0.25–0.58 h</th>
<th>&gt; 0.38 h</th>
<th>&gt; 0.38 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>8–56</td>
<td>56–200</td>
<td>200–304</td>
<td>&gt; 304</td>
<td>&gt; 304</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>1–6.5%</td>
<td>6.5–20%</td>
<td>20–27.5%</td>
<td>&gt; 27.5%</td>
<td>&gt; 27.5%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location on isotherm(^e) Relative vapor pressure (p/p_0) Zone</th>
<th>&lt;0.02(p/p_0)</th>
<th>0.02–0.2(p/p_0)</th>
<th>0.2–0.75(p/p_0)</th>
<th>0.75–0.85(p/p_0)</th>
<th>&gt; 0.85 (p/p_0)</th>
<th>&gt; 0.85 (p/p_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone I, extreme left</td>
<td>Zone I</td>
<td>Zone IIA</td>
<td>Zone IIB</td>
<td>Zone III</td>
<td>Zone III</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water properties</th>
<th>Critical part of native protein structure</th>
<th>Water interacts principally with charged groups (≈2 HOH/group) At 0.07 h: transition in surface water from disordered to ordered and/or from dispersed to clustered</th>
<th>Water interacts principally with polar protein surface groups (≈1 HOH/polar site) Water clusters centered on charged and polar sites Clusters fluctuate in size and/or arrangement</th>
<th>At 0.25 h: start of condensation of water onto weakly interacting unfilled patches of protein surface</th>
<th>At 0.38 h: monolayer of water covers the surface of the protein and water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

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*(table continued on next page)*
<table>
<thead>
<tr>
<th>Properties</th>
<th>Constitutional water&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hydration shell (≤3 Å from surface)</th>
<th>Free&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Entrapped&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>state; associated with completion of charged group hydration</td>
<td>At 0.15 h: long-range connectivity of the surface water is established</td>
<td>phase begins to form, and glass-rubber transition occurs</td>
<td></td>
</tr>
<tr>
<td>Thermodynamic transfer properties&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta G$ (kJ/mol)</td>
<td>$&gt;\cdot6$</td>
<td>-6</td>
<td>-0.8</td>
<td>Close to bulk water</td>
</tr>
<tr>
<td>$\Delta H$ (kJ/mol)</td>
<td>$&gt;\cdot17$</td>
<td>-70</td>
<td>-2.1</td>
<td>Close to bulk water</td>
</tr>
<tr>
<td>Approximate mobility (residence time)</td>
<td>10&lt;sup&gt;-2&lt;/sup&gt; to 10&lt;sup&gt;-8&lt;/sup&gt; sec</td>
<td>&lt;10&lt;sup&gt;-8&lt;/sup&gt; sec</td>
<td>&lt;10&lt;sup&gt;-9&lt;/sup&gt; sec</td>
<td>&lt;10&lt;sup&gt;-9&lt;/sup&gt; to 10&lt;sup&gt;-11&lt;/sup&gt; sec</td>
</tr>
<tr>
<td>Freezability</td>
<td>Unfreezable</td>
<td>Unfreezable</td>
<td>Unfreezable</td>
<td>Unfreezable</td>
</tr>
<tr>
<td>Solvent capability</td>
<td>None</td>
<td>None</td>
<td>None to slight</td>
<td>Slight to moderate</td>
</tr>
<tr>
<td>Protein properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structure</td>
<td>Folded state stable</td>
<td>Water begins to plasticize amorphous regions</td>
<td>Further plasticization of amorphous regions</td>
<td></td>
</tr>
<tr>
<td>Mobility</td>
<td>Enzymatic activity negligible</td>
<td>Enzymatic activity negligible</td>
<td>Internal protein motion (H exchange) increases from 1/1000 at 0.04 h to full solution rate at 0.15 h At 0.1–0.15 h: chymotrypsin and some other enzymes develop activity</td>
<td>At 0.38 h: lysozyme specific activity is 0.1 that in dilute solution</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from Rupley and Careri [103], Otting et al. [86], Lounnas and Pettit [71,72], Franks [30], and other sources. Based largely on lysozyme.

<sup>b</sup>Water molecules that occupy specific locations in the interior of the solute macromolecule.

<sup>c</sup>Macroscopic flow not physically constrained by a macromolecular matrix.

<sup>d</sup>Macroscopic flow physically constrained by a macromolecular matrix.

<sup>e</sup>See Figure 19.

<sup>f</sup>Partial molar values for transfer of water from bulk phase to hydration shell.
needed for “full hydration,” that is, occupancy of all first-layer sites. Further added water will have properties that do not differ significantly from those of bulk water.

2.8 Water Activity and Relative Vapor Pressure

2.8.1 Introduction

It has long been recognized that a relationship, although imperfect, exists between the water content of food and its perishability. Concentration and dehydration processes are conducted primarily for the purpose of decreasing the water content of a food, simultaneously increasing the concentration of solutes and thereby decreasing perishability.

However, it has also been observed that various types of food with the same water content differ significantly in perishability. Thus, water content alone is not a reliable indicator of perishability. This situation is attributable, in part, to differences in the intensity with which water associates with nonaqueous constituents—water engaged in strong associations is less able to support degradative activities, such as growth of microorganisms and hydrolytic chemical reactions, than is weakly associated water. The term “water activity” \( a_w \) was developed to account for the intensity with which water associates with various nonaqueous constituents.

Food stability, safety, and other properties can be predicted far more reliably from \( a_w \) than from water content. Even so, \( a_w \) is not a totally reliable predictor. The reasons for this will be explained in the next section. Despite this lack of perfection, \( a_w \) correlates sufficiently well with rates of microbial growth and many degradative reactions to make it a useful indicator of product stability and microbial safety. The fact that \( a_w \) is specified in some U.S. federal regulations dealing with good manufacturing practices for food attests to its usefulness and credibility [44].

2.8.2 Definition and Measurement

The notion of substance “activity” was rigorously derived from the laws of equilibrium thermodynamics by G. N. Lewis, and its application to foods was pioneered by Scott [108,109]. It is sufficient here to state that

\[
a_w = \frac{f}{f_0} \tag{1}
\]

where \( f \) is the fugacity of the solvent (fugacity being the escaping tendency of a solvent from solution) and \( f_0 \) is the fugacity of the pure solvent. At low pressures (e.g., ambient), the difference between \( f/f_0 \) and \( p/p_0 \) is less than 1%, so defining \( a_w \) in terms of \( p/p_0 \) is clearly justifiable. Thus,

\[
a_w = \frac{p}{p_0} \tag{2}
\]

This equality is based on the assumptions of solution ideality and the existence of thermodynamic equilibrium. With foods, both assumptions are generally violated. Consequently, Equation 2 must be taken as an approximation and the proper expression is

\[
a_w \approx \frac{p}{p_0} \tag{3}
\]

Because \( p/p_0 \) is the measured term and sometimes does not equal \( a_w \), it is more accurate to use the term \( p/p_0 \) rather than \( a_w \). This practice will be followed here. “Relative vapor pressure” (RVP) is the name for \( p/p_0 \), and these terms will be used interchangeably. Despite the scientific soundness of using RVP rather than \( a_w \), the reader should be aware that the term \( a_w \) is in widespread use, appears in other chapters of this book and is not improper provided the user understands its true meaning.

Failure of the \( a_w \)-RVP approach to be a perfect estimator of food stability occurs for two
basic reasons: violation of assumptions underlying Equation 2, and solute-specific effects. Violation of Equation 2 assumptions can, but usually does not, detract unduly from the usefulness of RVP as a technological tool. An exception occurs if dry products are prepared by absorption of water rather than desorption (hysteresis effects), and this will be discussed later. Violation of Equation 2 assumptions does, however, invalidate RVP as a tool for mechanistic interpretations in instances where the theoretical models used are based on these assumptions (often true of models for moisture sorption isotherms).

In a few instances that can be of great importance, solute-specific effects can cause RVP to be a poor indicator of food stability and safety. This can occur even when the assumptions underlying Equation 2 are fully met. In these situations, foods with the same RVP but different solute compositions will exhibit different stabilities and other properties. This is an important point that must not be overlooked by anyone relying on RVP as a tool for judging the safety or stability of food. Figure 15 is provided to reinforce this point. These data clearly indicate that the minimum $p/p_0$ for growth of *Staphylococcus aureus* is dependent on solute type.

RVP is related to percent equilibrium relative humidity (ERH) of the product environment as follows:

$$\text{RVP} = \frac{p}{p_0} = \frac{\%\text{ERH}}{100} \quad (4)$$

Two aspects of this relationship should be noted. First, RVP is an intrinsic property of the sample whereas ERH is a property of the atmosphere in equilibrium with the sample. Second, the Equation 4 relationship is an equality only if equilibrium has been established between the product and its environment. Establishment of equilibrium is a time-consuming process even with very small samples (less than 1 g) and almost impossible in large samples, especially at temperatures below ~50°C.

The RVP of a small sample can be determined by placing it in a closed chamber for a time sufficient to achieve apparent equilibrium (constant weight) and then measuring either pressure or relative humidity in the chamber [33,52,90,119,125]. Various types of instruments are available for measuring pressure (manometers) and relative humidity (electric hygrometers, dew-point instruments). Knowledge of freezing point depression can also be used to determine RVP [26]. Based on collaborative studies, the precision of $a_w$ determinations is about ±0.02.

If one desires to adjust a small sample to a specific RVP, this can be done by placing it in

![Figure 15](image-url)

**FIGURE 15**

Minimum relative vapor pressure (RVP) for growth of *Staphylococcus aureus* as influenced by solute used to produce the RVP. Temperature is close to optimum for growth. PEG is polyethylene glycol. (From Ref. 11.)
a closed chamber at constant temperature, maintaining sample atmosphere at constant relative humidity by means of an appropriate saturated salt solution [69,119], and storing it until constant sample weight is achieved.

2.8.3 Temperature Dependence

Relative vapor pressure is temperature dependent, and the Clausius-Clapeyron equation in modified form provides a means for estimating this temperature dependence. This equation, although based on $a_w$, is applicable to RVP and has the following form [128]:

$$\frac{d \ln a_w}{d(1/T)} = \frac{-\Delta H}{R}$$  \hspace{1cm} (5)

where $T$ is absolute temperature, $R$ is the gas constant, and $\Delta H$ is the isosteric net heat of sorption at the water content of the sample. By rearrangement, this equation can be made to conform to the generalized equation for a straight line. It then becomes evident that a plot of $\ln a_w$ versus $1/T$ (at constant water content) should be linear and the same should be true for $\ln p/p_o$ versus $1/T$.

Linear plots of $\ln p/p_o$ versus $1/T$ for native potato starch at various moisture contents are shown in Figure 16. It is apparent that the degree of temperature dependence is a function of moisture content. At a starting $p/p_o$ of 0.5, the temperature coefficient is 0.0034°C$^{-1}$ over the temperature range 2–40°C. Based on the work of several investigators, temperature coefficients for $p/p_o$ (temperature range 5–50°C; starting $p/p_o$ 0.5) range from 0.003 to 0.02°C$^{-1}$ for high-carbohydrate or high-protein foods [128]. Thus, depending on the product, a 10°C change

![Figure 16](image.png)

**FIGURE 16**
Relationship between relative water vapor pressure and temperature for native potato starch of different water contents. Water content values following each line are g HOH/g dry starch. (From Ref. 128.)
in temperature can cause a 0.03–0.2 change in \( p/p_0 \). This behavior can be important for a packaged food because it will undergo a change in RVP with a change in temperature, causing the temperature dependence of its stability to be greater than that of the same product unpackaged.

Plots of \( \ln(p/p_0) \) versus \( 1/T \) are not always linear over broad temperature ranges, and they generally exhibit sharp breaks with the onset of ice formation. Before showing data at subfreezing temperatures, it is appropriate to consider the definition of RVP as it applies to subfreezing temperatures. This is necessary because a question arises as to whether the denominator term \( (p_0) \) should be equated to the vapor pressure of supercooled water or to the vapor pressure of ice. The vapor pressure of supercooled water turns out to be the proper choice because (a) values of RVP at subfreezing temperatures can then, and only then, be accurately compared to RVP values at above-freezing temperatures and (b) choice of the vapor pressure of ice as \( p_0 \) would result, for samples containing ice, in a meaningless situation whereby RVP would be unity at all subfreezing temperatures. The second point results because the partial pressure of water in a frozen food is equal to the vapor pressure of ice at the same temperature [25,121].

Because the vapor pressure of supercooled water has been measured down to -15°C, and the vapor pressure of ice has been measured to much lower temperatures, it is possible to accurately calculate RVP values for frozen foods. This is clearly apparent when one considers the following relationship:

\[
\alpha_W = \frac{p_{\text{ff}}}{p_{\text{0(SCW)}}} = \frac{p_{\text{ice}}}{p_{\text{0(SCW)}}} 
\]

Where \( p_{\text{ff}} \) is the partial pressure of water in partially frozen food, \( p_{\text{0(SCW)}} \) is the vapor pressure of pure supercooled water, and \( p_{\text{ice}} \) is the vapor pressure of pure ice.

Presented in Table 5 are RVP values calculated from the vapor pressures of ice and supercooled water, and these values are identical to those of frozen foods at the same temperatures. Figure 17 is a plot of \( \log(p/p_0) \) versus \( 1/T \), illustrating that (a) the relationship is linear at

| TABLE 5 Vapor Pressures and Vapor Pressure Ratios of Water and Ice |
|------------------|---------------|---------------|---------------|
| Temperature (°C) | Liquid water\(^a\) (Pa) (torr) | Ice\(^b\) or food containing ice (Pa) (torr) | \( p_{\text{ice}} / p_{\text{water}} \) |
| 0                | 611\(^b\) 4.58 | 611 4.58 | 1.00 |
| -5               | 421 3.16 | 402 3.02 | 0.95 |
| -10              | 287 2.15 | 260 1.95 | 0.91 |
| -15              | 191 1.43 | 165 1.24 | 0.86 |
| -20              | 125 0.94 | 103 0.77 | 0.82 |
| -25              | 80.7 0.61 | 63 0.47 | 0.78 |
| -30              | 50.9 0.38 | 38 0.29 | 0.75 |
| -40              | 18.9 0.14 | 13 0.098 | 0.69 |
| -50              | 6.4 0.05 | 3.9 0.029 | 0.61 |

\(^a\)Supercooled at all temperatures except 0°C. Observed data above -15°C, calculated below -15°C [79].

\(^b\)Observed data from Ref. 69.
subfreezing temperatures, (b) the influence of temperature on RVP is typically far greater at subfreezing temperatures than at above-freezing temperatures, and (c) a sharp break occurs in the plot at the freezing point of the sample.

Two important distinctions should be noted when comparing RVP values at above- and below-freezing temperatures. First, at above-freezing temperatures, RVP is a function of sample composition and temperature, with the former factor predominating. At subfreezing temperatures, RVP becomes independent of sample composition and depends solely on temperature; that is, in the presence of an ice phase RVP values are not influenced by the kind or ratio of solutes present. As a consequence, any subfreezing event that is influenced by the kind of solute present (e.g., diffusion-controlled processes, catalyzed reactions, and reactions that are affected by the absence or presence of cryoprotective agents, by antimicrobial agents, and/or by chemicals that alter pH and oxidation-reduction potential) cannot be accurately forecast based on the RVP value [25]. Consequently, RVP values at subfreezing temperatures are far less valuable indicators of physical and chemical events than are RVP values at above-freezing temperatures. It follows that knowledge of RVP at a subfreezing temperature cannot be used to predict RVP at an above-freezing temperature.

Second, as the temperature is changed sufficiently to form or melt ice, the meaning of RVP, in terms of food stability, also changes. For example, in a product at -15°C ($p/p_0=0.86$), microorganisms will not grow and chemical reactions will occur slowly. However, at 20°C and
$p/p_0$ 0.86, some chemical reactions will occur rapidly and some microorganisms will grow at moderate rates.

### 2.9 Moisture Sorption Isotherms

#### 2.9.1 Definition and Zones

A plot of water content (expressed as mass of water per unit mass of dry material) of a food versus $p/p_0$ at constant temperature is known as a moisture sorption isotherm (MSI). Information derived from MSIs are useful (a) for concentration and dehydration processes, because the ease or difficulty of water removal is related to RVP, (b) for formulating food mixtures so as to avoid moisture transfer among the ingredients, (c) to determine the moisture barrier properties needed in a packaging material, (d) to determine what moisture content will curtail growth of microorganisms of interest, and (e) to predict the chemical and physical stability of food as a function of water content (see next section).

Shown in Figure 18 is a schematic MSI for a high-moisture food plotted to include the full range of water content from normal to dry. This kind of plot is not very useful because the data of greatest interest—those in the low-moisture region—are not shown in sufficient detail. Omission of the high-moisture region and expansion of the low-moisture region, as is usually done, yields an MSI that is much more useful (Fig. 19).

Several substances that have MSIs of markedly different shapes are shown in Figure 20. These are resorption (or adsorption) isotherms prepared by adding water to previously dried samples. Desorption isotherms are also common. Isotherms with a sigmoidal shape are characteristic of most foods. Foods such as fruits, confections, and coffee extract that contain large amounts of sugar and other small, soluble molecules and are not rich in polymeric materials exhibit a J-type isotherm shown as curve 1 in Figure 20. The shape and position of the isotherm are determined by several factors including sample composition, physical structure of the sample (e.g., crystalline or amorphous), sample pretreatments, temperature, and methodology.

Many attempts have been made to model MSIs, but success in achieving good conform-
ance of a model to the full range of actual data for an MSI has been difficult. The oldest and best known model is that of Brunauer, Emmett, and Teller [8]. One of the best models is that developed by Guggenheim [36], Anderson [2], and DeBoer [16], and this is referred to as the GAB model.

As an aid to understanding the meaning and usefulness of sorption isotherms it is sometimes appropriate to divide them into zones as indicated in Figure 19. As water is added (resorption), sample composition moves from Zone I (dry) to Zone III (high moisture) and the properties of water associated with each zone differ significantly. These properties are described next and are summarized in Table 4.

Water present in Zone I of the isotherm is most strongly sorbed and least mobile. This water associates with accessible polar sites by water-ion or water-dipole interactions, is unfreezable at -40°C, has no ability to dissolve solutes, and is not present in sufficient amount to have a plasticizing effect on the solid. It behaves simply as part of the solid.

The high-moisture end of Zone I (boundary of Zones I and II) corresponds to the “BET monolayer” moisture content of the food. The BET monolayer value should be thought of as approximating the amount of water needed to form a monolayer over accessible, highly polar groups of the dry matter. In the case of starch, this amounts to one HOH per anhydroglucose unit [126]. Zone I water constitutes a tiny fraction of the total water in a high-moisture food material.

Water added in Zone II occupies first-layer sites that are still available. This water associates with neighboring water molecules and solute molecules primarily by hydrogen bonding, is slightly less mobile than bulk water, and most of it is unfreezable at -40°C. As water is added in the vicinity of the low-moisture end of Zone II, it exerts a significant plasticizing action on solutes, lowers their glass transition temperatures, and causes incipient swelling of the solid matrix. This action, coupled with the beginning of solution processes, leads to an
acceleration in the rate of most reactions. Water in Zones I and Zone II usually constitutes less than 5% of the water in a high-moisture food material.

In the vicinity of the junction of Zones II and III, water is sufficient to complete a true monolayer hydration shell for macromolecules such as globular proteins, and is sufficient to lower the glass transition temperature of macromolecules so that sample temperature and \( T_g \) are equal. Further addition of water (Zone III) causes a glass-rubber transition in samples containing glassy regions, a very large decrease in viscosity, a very large increase in molecular mobility, and commensurate increases in the rates of many reactions. This water is freezable, available as a solvent, and readily supports the growth of microorganism. Zone III water is referred to as bulk-phase water (Table 4). Additional water will have the properties of bulk-phase water and will not alter properties of existing solutes.

In gels or cellular systems, bulk-phase water is physically entrapped so that macroscopic flow is impeded. In all other respects this water has properties similar to that of water in a dilute salt solution. This is reasonable, since a typical water molecule added in Zone III is “insulated” from the effects of solutes molecules by several layers of Zone I and Zone II water molecules. The bulk-phase water of Zone III, either entrapped or free, usually constitutes more than 95% of the total water in a high-moisture food, a fact that is not evident from Figure 19.

As mentioned earlier, the zone boundaries indicated in Figure 19 are simply an aid to discussion rather than a reality. It is believed that water molecules can interchange rapidly within and between “zones” and that the concept of a continuum of water properties existing through Zones I–III is conceptually sounder than the notion of distinctly different properties existing in each zone. It is also of interest that addition of water to a dry material containing only a few water molecules will increase the mobility and lessen the residence time of these original water
molecules [103]. However, addition of water to materials already having complete or near complete hydration shells is unlikely to have a significant effect on the properties of water originally present.

The important effects that these solute-induced differences in water properties have on stability of foods will be discussed in a later section. At this point, it will suffice to say that the most mobile fraction of water existing in any food sample governs stability.

### 2.9.2 Temperature Dependence

As mentioned earlier, RVP is temperature dependent; thus MSIs must also be temperature dependent. An example involving potato slices is shown in Figure 21. At any given moisture content, food $p/p_0$ increasing with increasing temperature, in conformity with the Clausius-Clapeyron equation.

### 2.9.3 Hysteresis

An additional complication is that an MSI prepared by addition of water (resorption) to a dry sample will not necessarily be superimposable on an isotherm prepared by desorption. This lack
of superimposability is referred to as “hysteresis,” and a schematic example is shown in Figure 22. Typically, at any given $p/p_0$, the water content of the sample will be greater during desorption than during resorption. MSIs of polymers, glasses of low-molecular-weight compounds, and many foods exhibit hysteresis [46,47].

The magnitude of hysteresis, the shape of the curves, and the inception and termination points of the hysteresis loop can vary considerably depending on factors such as nature of the food, the physical changes it undergoes when water is removed or added, temperature, the rate of desorption, and the degree of water removal during desorption [45]. The effect of temperature is pronounced; hysteresis is often not detectable at high temperatures (~80°C) and generally becomes increasingly evident as the temperature is lowered [126].

Several largely qualitative theories have been advanced to explain sorption hysteresis [46,47]. These theories involve factors such as swelling phenomena, metastable local domains, chemisorption, phase transitions, capillary phenomena, and the fact that nonequilibrium states become increasingly persistent as the temperature is lowered. A definitive explanation (or explanations) of sorption hysteresis has yet to be formulated.

Sorption hysteresis is more than a laboratory curiosity. Labuza et al. [55] have conclusively established that lipid oxidation in strained meat from chicken and pork, at $p/p_0$ values in the range of 0.75–0.84, proceeds much more rapidly if the samples are adjusted to the desired $p/p_0$ value by desorption rather than resorption. The desorption samples, as already noted, would contain more water at a given $p/p_0$ than the resorption samples. This would cause the high-moisture sample to have a lower viscosity, which in turn would cause greater catalyst mobility, greater exposure of catalytic sites because of the swollen matrix, and somewhat greater oxygen diffusivity than in the lower moisture (resorption) sample.

In another study, Labuza et al. [56] found that the $p/p_0$ needed to stop the growth of several microorganisms is significantly lower if the product is prepared by desorption rather than resorption.

By now it should be abundantly clear that MSIs are highly product specific, that the MSI for a given product can be changed significantly by the manner in which the product is prepared, and that these points are of practical importance.
2.10 Relative Vapor Pressure and Food Stability

Food stability and \( p/p_0 \) are closely related in many situations. The data in Figure 23 and Table 6 provide examples of these relationships. Shown in Table 6 are various common microorganisms and the range of RVP permitting their growth. Also shown in this table are common foods categorized according to their RVP.

Data in Figure 23 are typical qualitative relationships between reaction rate and \( p/p_0 \) in the

![Figure 23](image)

FIGURE 23
Relationships among relative water vapor pressure, food stability and sorption isotherms. (A) Microbial growth versus \( p/p_0 \). (B) Enzymic hydrolysis versus \( p/p_0 \). (C) Oxidation (nonenzymic) versus \( p/p_0 \). (D) Maillard browning versus \( p/p_0 \). (E) Miscellaneous reaction rates versus \( p/p_0 \). (F) Water content versus \( p/p_0 \). All ordinates are “relative rate” except for F. Data from various sources.
<table>
<thead>
<tr>
<th>Range of ( p/p_0 )</th>
<th>Microorganisms generally inhibited by lowest ( p/p_0 ) in this range</th>
<th>Foods generally within this range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00–0.95</td>
<td><em>Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens</em> yeasts</td>
<td>Highly perishable (fresh) foods and canned fruits, vegetables, meat, fish, and milk; cooked sausages and breads; foods containing up to approximately 40% (w/w) sucrose or 7% sodium chloride</td>
</tr>
<tr>
<td>0.95–0.91</td>
<td><em>Salmonella, Vibrio parahaemolyticus, C. botulinum, Serratia, Lactobacillus, Pediococcus</em> some molds, yeasts (<em>Rhodotorula, Pichia</em>)</td>
<td>Some cheeses (Cheddar, Swiss, Muenster, Provolone), cured meat (ham), some fruit juice concentrates; foods containing up to 55% (w/w) sucrose or 12% sodium chloride</td>
</tr>
<tr>
<td>0.91–0.87</td>
<td>Many yeasts (<em>Candida, Torulopsis, Hansenula</em>), <em>Micrococcus</em></td>
<td>Fermented sausage (salami), sponge cakes, dry cheeses, margarine; foods containing up to 65% (w/w) sucrose (saturated) or 15% sodium chloride</td>
</tr>
<tr>
<td>0.87–0.80</td>
<td>Most molds (<em>mycotoxigenic penicillia</em>), <em>Staphylococcus aureus</em>, <em>most Saccharomyces</em> (bailiipp., <em>Debaryomyces</em>)</td>
<td>Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrups; flour, rice, pulses containing 15–17% moisture; fruit cake; country-style ham, fondants, high-ratio cakes</td>
</tr>
<tr>
<td>0.80–0.75</td>
<td>Most halophilic bacteria, <em>mycotoxigenic aspergilli</em></td>
<td>Jam, marmalade, marzipan, glacé fruits, some marshmallows</td>
</tr>
<tr>
<td>0.75–0.65</td>
<td><em>Xerophilic molds</em> (<em>Aspergillus chevalieri, A. candidus, Wallemia sebi</em>), <em>Saccharomyces bisporus</em></td>
<td>Rolled oats containing approximately 10% moisture; grained nougats, fudge, marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts</td>
</tr>
<tr>
<td>0.65–0.60</td>
<td><em>Osmophilic yeasts</em> (<em>Saccharomyces rouxii</em>) <em>few molds</em> (<em>Aspergillus echinulatus, Monascus bisporus</em>)</td>
<td>Dried fruits containing 15–20% moisture; some toffees and caramels; honey</td>
</tr>
<tr>
<td>0.50</td>
<td>No microbial proliferation</td>
<td>Pasta containing approximately 12% moisture; spices containing approximately 10% moisture</td>
</tr>
<tr>
<td>0.40</td>
<td>No microbial proliferation</td>
<td>Whole egg powder containing approximately 5% moisture</td>
</tr>
<tr>
<td>0.30</td>
<td>No microbial proliferation</td>
<td>Cookies, crackers, bread crusts, etc. containing 3–5% moisture</td>
</tr>
<tr>
<td>0.20</td>
<td>No microbial proliferation</td>
<td>Whole milk powder containing 2–3% moisture; dried vegetables containing approximately 5% moisture; corn flakes containing approximately 5% moisture; country style cookies, crackers</td>
</tr>
</tbody>
</table>

*Source: Ref. 6*
temperature range 25–45°C. For comparative purposes a typical isotherm, Figure 23F, is also shown. It is important to remember that the exact reaction rates and the positions and shapes of the curves (Fig. 23A-E) can be altered by sample composition, physical state and structure of the sample, composition of the atmosphere (especially oxygen), temperature, and by hysteresis effects.

For all chemical reactions in Figure 23, minimum reaction rates during desorption are typically first encountered at the boundary of Zones I and II of the isotherm \( (p/p_0) \) \( 0.20–0.30 \), and all but oxidative reactions remain at this minimum as \( p/p_0 \) is further reduced. During desorption, the water content at the first-encountered rate minimum is the “BET monolayer” water content.

The unusual relationship between rate of lipid oxidation and \( p/p_0 \) at very low values of \( p/p_0 \) deserves comment (Fig. 23C). Starting at the extreme left of the isotherm, added water decreases the rate of oxidation until the BET monolayer value is attained. Clearly, overdrying of samples subject to oxidation will result in less than optimum stability. Karel and Yong [48] have offered the following interpretative suggestions regarding this behavior. The first water added to a very dry sample is believed to bind hydroperoxides, interfering with their decomposition and thereby hindering the progress of oxidation. In addition, this water hydrates metal ions that catalyze oxidation, apparently reducing their effectiveness.

Addition of water beyond the boundary of Zones I and II (Fig. 23C and Fig. 23F) results in increased rates of oxidation. Karel and Yong [48] suggested that water added in this region of the isotherm accelerates oxidation by increasing the solubility of oxygen and by allowing macromolecules to swell, thereby exposing more catalytic sites. At still greater \( p/p_0 \) values (> ~ 0.80) the added water may retard rates of oxidation, and the suggested explanation is that dilution of catalysts reduces their effectiveness.

It should be noted that curves for the Maillard reaction, vitamin B\(_1\) degradation, and microbial growth all exhibit rate maxima at intermediate to high \( p/p_0 \) values (Fig. 23A, D, E). Two possibilities have been advanced to account for the decline in reaction rate that sometimes accompanies increases in RVP in foods having moderate to high moisture contents [20,54].

1. For those reactions in which water is a product, an increase in water content can result in product inhibition.

2. When the water content of the sample is such that solubility, accessibility (surfaces of macromolecules), and mobility of rate-enhancing constituents are no longer rate-limiting, then further addition of water will dilute rate-enhancing constituents and decrease the reaction rate.

Since the BET monolayer value of a food provides a good first estimate of the water content providing maximum stability of a dry product, knowledge of this value is of considerable practical importance. Determining the BET monolayer value for a specific food can be done with moderate ease if data for the low-moisture end of the MSI are available. One can then use the BET equation developed by Brunauer et al. [8] to compute the monolayer value

\[
\frac{a_w}{m(1 - a_w)} = \frac{1}{m_1c} + \frac{C - 1}{m_1c} a_w
\]

where \( a_w \) is water activity, \( m \) is water content (g H\(_2\)O/g dry matter), \( m_1 \) is the BET monolayer value, and \( C \) is a constant. In practice, \( p/p_0 \) values are used in Equation 8 rather than \( a_w \) values.

From this equation, it is apparent that a plot of \( a_w/m(1-a_w) \) versus \( a_w \), known as a BET plot, should yield a straight line. An example for native potato starch, with \( a_w \) replaced by \( p/p_0 \), is shown in Figure 24. The linear relationship, as is generally acknowledged, begins to deteriorate at \( p/p_0 \) values greater than about 0.35.
The BET monolayer value can be calculated as follows:

\[
\text{Monolayer value} = m_1 = \frac{1}{(y \text{ intercept}) + \text{(slope)}}
\]

From Figure 24, the \(y\) intercept is 0.6. Calculation of the slope from Figure 24 yields a value of 10.7. Thus,

\[
m_1 = \frac{1}{0.6 + 10.7} = 0.088 \text{ g H}_2\text{O/g dry matter}
\]

In this particular instance the BET monolayer value corresponds to a \(p/p_0\) of 0.2. The GAB equation yields a similar monolayer value [81].

In addition to chemical reactions and microbial growth, \(p/p_0\) also influences the texture of dry and semidry foods. For example, suitably low RVPs are necessary if crispness of crackers, popped corn, and potato chips is to be retained; if caking of granulated sugar, dry milk, and instant coffee is to be avoided; and if stickiness of hard candy is to be prevented [53]. The maximum \(p/p_0\) that can be tolerated in dry materials without incurring loss of desirable properties ranges from 0.35 to 0.5, depending on the product. Furthermore, suitably high water activities of soft-textured foods are needed to avoid undesirable hardness.

### 2.11 Molecular Mobility (Mm) and Food Stability

#### 2.11.1 Introduction

Even though the RVP approach has served the food industry well, this should not preclude consideration of other approaches that can supplement or partially replace RVP as a tool for predicting and controlling food stability and processability. In recent years, evidence has become increasingly compelling that molecular mobility (Mm; translational or rotational motion) may
be an attribute of foods that deserves attention because it is related causally to many important diffusion-limited properties of food.

Luyet and associated in the United States and Rey in France were apparently the first to draw attention to the relevance of Mm (glassy states, recrystallization, collapse temperatures during freeze drying) to properties of biological materials [74, 76, 78, 94]. John D. Ferry, a professor of chemistry at the University of Wisconsin, and his associates formulated many of the basic concepts pertaining to Mm in nonequilibrium systems consisting of synthetic, amorphous polymers [27,130]. In 1966, White and Cakebread [129] described the important role of glassy and supersaturated states in various sugar-containing foods and suggested that the existence of these states has an important influence on the stability and processability of many foods. Duckworth et al. [17] demonstrated the relevance of Mm to rates of nonenzymic browning and ascorbic acid oxidation, and thereby provided further evidence that the relationship between Mm and food stability is one of considerable importance.

Widespread recent interest in the relationship between Mm and food properties was created primarily by Felix Franks [29] and the team of Louise Slade and Harry Levine [60–67, 112–118]. They showed that important basic principles underlying the behavior of synthetic, amorphous polymers, as developed by Ferry's group and others, apply to the behavior of glass-forming foods. Slade and Levine used the phrase “food polymer science approach” to describe the interrelationships just mentioned; however, the term “molecular mobility” (Mm) seems preferable because this term is simple and emphasizes the underlying aspect of importance. The work of Slade and Levine has been relied on heavily during preparation of this section on Mm.

The importance of the Mm concept, which lay unappreciated by food scientists for many years, lends support to an important principle: For those in applied sciences who aspire to engage in pioneering work, much more can be gained from scientific literature that underlies or is peripheral to their primary field of endeavor than from literature that is central to it. Evidence now suggests that Mm is causally related to diffusion-limited properties of foods that contain, besides water, substantial amounts of amorphous, primarily hydrophilic molecules, ranging in size from monomers to polymers. The key constituents with respect to Mm are water and the dominant solute or solutes (solute or solutes comprising the major fraction of the solute portion). Foods of this type include starch-containing foods, such as pasta, boiled confections, protein-based foods, intermediate-moisture foods, and dried, frozen, or freeze-dried foods. Some properties and behavioral characteristics of food that are dependent on Mm are shown in Table 7.

When a food is cooled and/or reduced in moisture content so that all or part of it is converted to a glassy state, Mm is greatly reduced and diffusion-limited properties become stable. It is important to note the qualifying term “diffusion-limited.” Most physical properties/changes are diffusion-limited, but some chemical properties/reactions are controlled more by chemical reactivity than diffusion. Approaches to predicting whether rates of chemical reactions are limited by diffusion or chemical reactivity are available but calculations are not simple. This matter will be discussed further in Section 2.11.3.2. Even if future work establishes that the Mm approach applies to virtually all physical properties of foods but only to some chemical properties, the importance of this approach remains sufficient to justify careful study.

It is appropriate at this point to suggest that the RVP and Mm approaches to food stability are, for the most part, complementary rather than competitive. Although the RVP approach focuses on the “availability” of water in foods, such as the degree to which it can function as a solvent, and the Mm approach focuses on microviscosity and diffusibility of chemicals in foods, the latter, of course, is dependent on water and its properties [83].

Because several terms used in the following discussion will be unfamiliar to many readers of this book and/or have been poorly defined in other works, a glossary appears at the end of this chapter. Many readers will find it useful to study the glossary before proceeding further.
TABLE 7 Some Properties and Behavioral Characteristics of Foods That Are Governed by Molecular Mobility
(Diffusion-Limited Changes in Products Containing Amorphous Regions)

<table>
<thead>
<tr>
<th>Dry or semidry foods</th>
<th>Frozen foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow properties and stickiness</td>
<td>Moisture migration (ice crystallization, formation of in-package ice)</td>
</tr>
<tr>
<td>Crystallization and recrystallization</td>
<td></td>
</tr>
<tr>
<td>Sugar bloom in chocolate</td>
<td>Lactose crystallization (&quot;sandiness&quot; in frozen desserts)</td>
</tr>
<tr>
<td>Cracking of foods during drying</td>
<td></td>
</tr>
<tr>
<td>Texture of dry and intermediate moisture foods</td>
<td>Enzymatic activity</td>
</tr>
<tr>
<td>Collapse of structure during secondary (desorption) phase of freeze-drying</td>
<td>Structural collapse of amorphous phase during sublimation (primary) phase of freeze-drying</td>
</tr>
<tr>
<td>Escape of volatiles encapsulated in a solid, amorphous matrix</td>
<td>Shrinkage (partial collapse of foam-like frozen desserts)</td>
</tr>
<tr>
<td>Enzymatic activity</td>
<td></td>
</tr>
<tr>
<td>Maillard reaction</td>
<td></td>
</tr>
<tr>
<td>Gelatinization of starch</td>
<td></td>
</tr>
<tr>
<td>Staling of bakery products caused by retrogradation of starch</td>
<td></td>
</tr>
<tr>
<td>Cracking of baked goods during cooling</td>
<td></td>
</tr>
<tr>
<td>Thermal inactivation of microbial spores</td>
<td></td>
</tr>
</tbody>
</table>

*Source:* Adapted from Ref. 114.

2.11.2 State Diagrams

Consideration of “state” diagrams is highly pertinent to the discussion of Mm and stability of foods that are frozen or have reduced moisture contents. State diagrams are much more suitable for this purpose than conventional phase diagrams. Phase diagrams pertain solely to equilibrium conditions. State diagrams contain equilibrium information as well as information on conditions of nonequilibrium and metastable equilibrium “states.” State diagrams are, therefore, supplemented phase diagrams, and they are appropriate because foods that are dried, partially dried, or frozen do not exist in a state of thermodynamic equilibrium.

A simplified temperature-composition state diagram for a binary system is shown in Figure 25. Important additions to the standard phase diagram are the glass transition curve $T_g$ and the line extending from $T_g$ to $T_g'$, with both lines representing metastable conditions. Samples located above the glass transition curve and not on any line exist, with few exceptions, in a state of nonequilibrium, as will be discussed subsequently. State diagrams of this format will be used several times during the following discussion of Mm in foods.

When using these diagrams, it is assumed that pressure is constant and the time dependency of the metastable states, although real, is of little or no commercial importance (not true of nonequilibrium states). It also should be recognized that each simple system will have its own characteristic state diagram that differs quantitatively, but not qualitatively, from that in Figure 25, and that most foods are so complex that they cannot be accurately or easily represented on a state diagram. For all complex foods, both dry and frozen, accurate determination of a glass transition curve (or more properly zone, as will be discussed later) is difficult, but estimates are essential if the Mm approach to food stability is to be used effectively. Although estimates of $T_g$ for complex foods are not easy to obtain, this can be done with accuracy sufficient for commercial use.
State diagram of a binary system. Assumptions: maximal freeze concentration, no solute crystallization, constant pressure, no time dependence. $T_m^l$ is the melting point curve, $T_{E}$ is the eutectic point, $T_m^s$ is the solubility curve, $T_g$ is the glass transition curve, and $T_g'$ is the solute-specific glass transition temperature of a maximally freeze concentrated solution. Heavy dashed lines represent conditions of metastable equilibrium. All other lines represent conditions of equilibrium.

Establishing the equilibrium curves ($T_m^l$ and $T_m^s$; Fig. 25) for complex foods also can be difficult. For dry or semidry foods the $T_m^s$ curve, the major equilibrium curve of importance, usually cannot be accurately depicted as a single line. A common approach is to base the state diagram on water and a food solute of dominating importance to the properties of the complex food, then deduce properties of the complex food from this diagram. For example, a state diagram for sucrose-water is useful for predicting the properties and behavior of cookies during baking and storage [67]. Determining curves for dry or semidry complex foods that do not contain a dominating solute is a difficult matter that has not yet been satisfactorily resolved.

For frozen foods, the situation is somewhat better because the melting point curve ($T_m^l$), the major equilibrium curve of importance, is often known or easily determined. Thus, it is possible, with accuracy sufficient for commercial purposes, to prepare a state diagram for a complex frozen food.

For binary systems, the effect of different solutes on the glass transition curve is shown schematically in Figure 26. Note that the left end of the $T_g$ curve is always fixed at -135°C, the $T_g$ of water. Thus, differences in location of the curve depend on $T_g'$ and on $T_g$ of the dry solute.
State diagram of a binary system showing the influence of solute type on the position of the glass transition curve. The extreme left position of the $T_g$ curve is always fixed at the vitrification temperature of pure water (-135°C), the midpoint at the solute's $T_v'$, and the extreme right position at the $T_g$ of the pure solute; a and b are curves for different solutes. Assumptions stated in Figure 25 apply here.

2.11.3 Nine Key Concepts Underlying the Molecular Mobility Approach to Food Stability

2.11.3.1 Concept 1. Many Foods Contain Amorphous Components and Exist in a State of Metastable Equilibrium or Nonequilibrium

Complex foods frequently contain amorphous (noncrystalline solid or supersaturated liquid) regions. Biopolymers are typically amorphous or partly amorphous. Examples include proteins such as gelatin, elastin, and gluten, and carbohydrates such as amylopectin and amylose. Many small molecules such as sugars also can exist in an amorphous state, and all dried, partially dried, frozen, and freeze-dried foods contain amorphous regions.

Amorphous regions exist in metastable equilibrium or nonequilibrium. Attainment of thermodynamic equilibrium (minimum free energy) is not a goal during food processing, though this condition results in maximum stability. Thermodynamic equilibrium is incompatible with life, including that of fruits and vegetables postharvest, and is incompatible with satisfactory quality in foods. Thus, a major goal of food scientists/technologists, although they rarely view their duties in this manner, is to maximize the number of desirable food attributes that depend on metastable equilibrium states, and to achieve acceptable stability for those desirable attributes that must unavoidably depend on nonequilibrium states. Hard candy (amorphous solid) is a
common example of a metastable food, and emulsions, small ice crystals, and unsaturated lipids are examples of food components that exist in a state of unstable nonequilibrium. Metastable states of food components often can be achieved by drying or freezing.

2.11.3.2 Concept 2. The Rates of Most Physical Events and Some Chemical Events Are Governed by Molecular Mobility (Mm)

Because most foods exist in metastable or nonequilibrium states, kinetic rather than thermodynamic approaches are often more appropriate for understanding, predicting, and controlling their properties. Molecular mobility (Mm) is a kinetic approach considered appropriate for this purpose because it is causatively related to rates of diffusion-limited events in foods. The WLF (Williams-Landel-Ferry) equation (see Section 2.11.3.5) provides the means for estimating Mm at temperatures above the glass transition temperature and below \( T_{\text{gel}} \) or \( T_{\text{m}} \). State diagrams indicate conditions of temperature and composition that permit metastable and nonequilibrium states to exist.

The utility of the Mm approach for predicting many kinds of physical changes has been reasonably well established. However, situations do exist where the Mm approach is of questionable value or is clearly unsuitable. Some examples are (a) chemical reactions whose rates are not strongly influenced by diffusion, (b) desirable or undesirable effects achieved through the action of specific chemicals (e.g., alteration of pH or oxygen tension), (c) situations in which sample Mm is estimated on the basis of a polymeric component (\( T_g \) of polymer) and where Mm of small molecules that can penetrate the polymer matrix is a primary determinant of the product attribute of interest \[110,111\], and (d) growth of vegetative cells of microorganisms \[p/p_0\] is a more reliable estimator than Mm) \[12,13\]. Point (a) deserves further attention because most authors of papers on Mm and its relevance to food stability are strangely silent on this important topic. Some useful references are Rice \[95\], Connors \[14\], Kopelman and Koo \[50\], Bell and Hageman \[2a\], and Haynes \[40\].

It is appropriate to first consider chemical reactions in a solution at ambient temperature. In this temperature range, some reactions are diffusion-limited but many are not. At constant temperature and pressure, three primary factors govern the rate at which a chemical reaction will occur: a diffusion factor, \( D \) (to sustain a reaction, reactants must first encounter each other), a frequency-of-collision factor, \( A \) (number of collisions per unit time following an encounter), and a chemical activation-energy factor, \( E_a \) (once a collision occurs between properly oriented reactants the energy available must be sufficient to cause a reaction, that is, the activation energy for the reaction must be exceeded). The latter two terms are incorporated in the Arrhenius relationship depicting the temperature dependence of the reaction rate constant. For a reaction to be diffusion-limited, it is clear that factors \( A \) and \( E_a \) must not be rate-limiting; that is, properly oriented reactants must collide with great frequency and the activation energy must be sufficiently low that collisions have a high probability of resulting in reaction. Diffusion-limited reactions typically have low activation energies (8–25 kJ/mol). In addition, most “fast reactions” (small \( A \) and large \( E_a \)) are diffusion-limited. Examples of diffusion-limited reactions are proton transfer reactions, radical recombination reactions, acid-base reactions involving transport of \( \text{H}^+ \) and \( \text{OH}^- \), many enzyme-catalyzed reactions, protein folding reactions, polymer chain growth, and oxygenation/deoxygenation of hemoglobin and myoglobin \[89,95\]. These reactions may involve a variety of chemical entities including molecules, atoms, ions, and radicals. At room temperature, diffusion-limited reactions occur with bimolecular rate constants of about \( 10^{10} \) to \( 10^{11} \, \text{M}^{-1}\text{s}^{-1} \); therefore a rate constant of this magnitude is regarded as presumptive evidence of a diffusion-limited reaction. It is also important to note that reactions in solution can go no faster
than the diffusion-limited rate; that is, the diffusion-limited rate is the maximum rate possible (conventional reaction mechanisms are assumed). Thus, reactions occurring at rates significantly slower than the diffusion-limited maximum are limited by $A$ or $E_a$ or a combination of these two.

The theory describing diffusion-limited reactions was developed by Smoluchowski in 1917. For spherical, uncharged particles, the second-order diffusion-limited rate constant is

$$\kappa_{\text{diff}} = \frac{4\pi N_A}{1000} \left( \frac{D_1 + D_2}{r} \right)$$

where $\Lambda$ is Avogadro's number, $D_1$ and $D_2$ are the diffusion constants for particles 1 and 2, respectively, and $r$ is the sum of the radii of particles 1 and 2, that is, the distance of closest approach. This equation was subsequently modified by Debye to accommodate charged particles and by others to accommodate additional characteristics of real systems. Nonetheless, the Smoluchowski equation provides an order-of-magnitude estimate of $k_{\text{diff}}$.

Of considerable pertinence to the present discussion is the viscosity and temperature dependence of the diffusion constant. This relationship is represented by the Stokes-Einstein equation:

$$D = \frac{k}{\mu r_s}$$

where $k$ is the Boltzmann constant, $T$ is absolute temperature, $\mu$ is a numerical constant (about 6), $\eta$ is viscosity, and $r_s$ is the hydrodynamic radius of the diffusing species. This dependence of $D$ (and therefore $k_{\text{diff}}$) on viscosity is a point of special interest because viscosity increases dramatically as temperature is reduced in the WLF region.

It appears likely that rates of some reactions in high moisture foods at ambient conditions are diffusion-limited and rates of others are not. Those that are would be expected to conform reasonably well to WLF kinetics as temperature is lowered or water content is reduced. Among those that are not diffusion-limited at ambient conditions (probably uncatalyzed, slow reactions), many probably become so as temperature is lowered below freezing or moisture is reduced to the point where solute saturation/supersaturation becomes common. This is likely because a temperature decrease would reduce the thermal energy available for activation and would increase viscosity dramatically, and/or because a reduction in water content would cause a pronounced increase in viscosity. Because the frequency-of-collision factor, $A$, is not strongly viscosity dependent, it probably is not an important determinant of the reaction-limiting mechanism under the circumstances described [9]. This likely conversion of some chemical reactions from non-diffusion-limited to diffusion-limited as temperature is lowered or water content is reduced should result, for those reactions behaving in this manner, in poor conformance to WLF kinetics in the upper part of the WLF region and much better conformance in the lower part of the WLF region.

### 2.11.3.3 Concept 3. Free Volume Is Mechanistically Related to $M_m$

As temperature is lowered, free volume decreases, making translational and rotational motion ($M_m$) more difficult. This has a direct bearing on movement of polymer segments, and hence on local viscosity in foods. Upon cooling to $T_g$, free volume becomes sufficiently small to stop translational motion of polymer segments. Thus, at temperatures $< T_g$, the stability of diffusion-limited properties of food is generally good. Increases in free volume (usually undesirable) can be achieved by addition of a low-molecular-weight solvent, such as water, or by increasing temperature, with both actions increasing translational motion of molecules. The free volume
The concept is useful in that it provides a mechanistic basis for the $M_m$ versus stability relationship, but as yet, it has not been successfully applied as a quantitative tool for predicting food stability.

### 2.11.3.4 Concept 4. Most Foods Exhibit a Glass Transition Temperature ($T_g$ or $T_g'$) or Range

The $T_g$ has an important relationship to the stability of diffusion-limited properties of food. Foods that normally contain amorphous regions or develop them during cooling or drying will exhibit a $T_g$ or $T_g'$ range. In biological systems, solutes seldom crystallize during cooling or drying so amorphous regions and glass transitions are common. The stability of diffusion-limited properties of substances of this type often can be estimated from the relationship between $M_m$ and $T_g$. $M_m$, and thus all diffusion-limited events, including many quality-deteriorating reactions, usually becomes severely restricted below $T_g$.

Unfortunately, many foods are stored at $T > T_g$, resulting in much greater $M_m$ and much poorer stability than that attainable at $T < T_g$.

To determine $T_g$ with reasonable accuracy it must be measured. In simple systems this can be accomplished using a differential scanning calorimeter (DSC) equipped with a derivative plotting accessory, but great care must be exercised to achieve accurate results. In complex systems (most foods), accurate determination of $T_g$ by DSC is very difficult, and dynamic mechanical analysis (DMA) and dynamic mechanical thermal analysis (DMTA) have been used as alternate techniques [77a]. All of these approaches are costly, and none is suited to in-plant measurements.

The observed $T_g$ depends on the type of instrument used, the experimental conditions employed, and the interpretative skill of the operator [1,10,39,43,82,92,101,111]. A particularly good example concerns values for $T_g'$ of frozen samples determined under conditions where maximal freeze concentration is presumably achieved. For a given substance, $T_g'$ values reported after about 1990 tend to be lower than values determined earlier. These discrepancies are caused by differences in experimental procedures, clearly demonstrating the inappropriateness of reporting $T_g'$ values to an accuracy of greater than ±1°C. Whether the recent values more accurately reflect conditions prevailing in commercial foods is still a matter of controversy, and it should be noted that $T_g'$ values reported by Slade and Levine pertain to samples with initial water contents of 80%. This is fairly typical of natural foods, whereas values reported more recently are based on initial water contents that are very low. Several criteria for ascertaining whether a glass transition has been accurately determined by DSC are given by Wolanczyk [131].

Various equations are available for calculating $T_g$ of samples containing only a few components, and this approach can provide a useful estimate when sample composition and $T_g$ values of the components are known. The oldest and simplest equation, as applied here to a binary system, is that of Gordon and Taylor [34].

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2}$$

where $T_{g1}$ and $T_{g2}$ are the glass transition values (in K) of components 1 (water) and 2 of the sample, respectively; $w_1$ and $w_2$ are the weight fractions of components 1 and 2 of the sample; and $k$ is an empirical constant. Refinements to this equation are available in the literature [38].

### 2.11.3.5 Concept 5. Molecular Mobility and Diffusion-Limited Food Stability Are Unusually Temperature Dependent Between $M_m$ and $T_g$ (Note: $M_m$ Here Is Taken to Mean Either $T_m^l$ or $T_m^s$, Whichever Is Appropriate)

Within this temperature range, which for foods may be as large as 100°C or as small as about 10°C, the vast array of products having amorphous regions will exhibit a temperature depend-
ency for Mm and viscoelastic properties that is unusually large. The Mm is quite intense at Tw and is very subdued for most molecules at or below Tg. This temperature range encompasses product consistencies termed “rubbery” and “glassy” (although the “rubbery” term applies only when large polymers are present). Qualitative relationships between substance properties and substance temperature, including the range m– Tg, are depicted in Figure 27.

The temperature dependence of Mm, and that of food properties that depend strongly on Mm (most physical properties and some chemical properties), is far greater in zone m– Tg than it is at temperatures above or below this zone. Thus, one usually finds a change in slope (change in activation energy) of Arrhenius plots upon passage from temperatures outside zone m– Tg to temperatures inside. Estimating the temperature dependence of reaction rates in zone m– Tg has been the subject of considerable research, and an equation that applies accurately to all types of reactions and conditions has not been devised. In zone m– Tg, rates of many physical events conform more closely to the WLF equation [27,130], and to similar equations, than they do to the Arrhenius equation. Because the dependency of chemical reactions on Mm can vary greatly depending on reactant type, neither WLF nor the Arrhenius equation applies to all chemical reactions in zone m– Tg. Conformance of physical and chemical events to WLF or Arrhenius

![Figure 27](image_url)

**Figure 27**
Schematic interrelations among temperature, appropriate type of kinetics, viscosity, molecular mobility, free volume, and relative rates of diffusion-dependent events. WLF kinetics based on mean constants. Other terms are defined in legend of Figure 25.
relationships is much less satisfactory in the presence of ice than in its absence because the concentrative effects of ice formation
are not accommodated by either approach.

Because the WLF equation is a useful tool for estimating rates of physical events in zone \( T_m - T_g \), it deserves further discussion. The WLF equation expressed in terms of viscosity is

\[
\log(\eta/\eta_0) = -\frac{C_1(T - T_g)}{C_2 + (T - T_g)}
\]

where \( \eta \) is viscosity at product temperature \( T \) (Note: \( \eta \) can be replaced by \( 1/Mm \), or any other diffusion-limited relaxation process), \( \eta_0 \) is viscosity at product temperature \( T_g \), and \( C_1 \) (dimensionless) and \( C_2 \) (K) are constants [27,130]. The \( T_g \) is generally accepted as the reference temperature in the WLF equation when ice is not present. In the presence of ice, disagreement still exists as to whether \( T_g \) or \( T_g' \) is more appropriate [93,111]. This point will be discussed further in the section dealing with technological aspects of freezing preservation.

The terms \( C_1 \) and \( C_2 \) are substance-specific constants (i.e., not temperature dependent). They have mean (sometimes called “universal”) values of 17.44 and 51.6, respectively, for many synthetic, totally amorphous polymers that are pure (no diluent). The numerical values of these constants vary substantially with water content and substance type, so values appropriate for foods often differ appreciably from the mean values. Constants specific for the food under study must be used if reasonable conformance of data to the equation is desired [49,111].

The WLF equation specifies a very large temperature dependence of substance properties in zone \( T_m - T_g \). If one assumes conformance to WLF kinetics (mean constants), the absence of ice, and an initial substance temperature \( T_g \), then warming will result in the following sequence of changes in viscosity (or \( 1/Mm \)) [115,117]:

1. An approximate \( 10^3 \)-fold decrease in viscosity and increase in \( Mm \) during an isotemperature conversion from a glass to a supersaturated liquid.

2. An approximate \( 10^5 \)-fold decrease in viscosity and increase in \( Mm \) with warming through the 20°C interval immediately above \( T_g \). (In the unfrozen fraction of frozen samples, the change in these properties per °C is even greater.)

3. An approximate \( 10^{12} \)-fold decrease in viscosity and increase in \( Mm \) with warming through the entire range from \( T_g \) to \( T_m \).

With respect to diffusion-limited food stability in the WLF region \( T_m - T_g \), two terms are of key importance: \( T_g \) (or \( T_g' \)) and \( Mm/g \), where \( T \) is product temperature, defines the location of the food in the WLF region. The log(\( Mm/g \)) (or any other viscosity-related property, such as \( Mm \)) varies curvilinearly with \( T_g \). The term \( Mm/g \) (calculated on the basis of K) provides a rough estimate of product viscosity (inverse relationship with \( Mm \)) at \( T_g \). Knowledge of product viscosity at \( T_g \) is important because viscosity at \( T_g \) is the reference value in the WLF equation, and this value can vary considerably with changes in product composition.

Valuable concepts pertaining to \( T_m - T_g \), \( Mm/g \), and \( Mm/g \) have been developed based largely on diffusion-limited properties of carbohydrates [62,112-114,117]:

1. The magnitude of the \( T_m - T_g \) region can vary from about 10 to about 100°C, depending on product composition.

2. Product stability depends on product temperature, \( T_g \), in the zone \( T_m - T_g \), that is, it is inversely related to \( Mm/g \).

3. At a given value of \( T_g \) and constant solids content, \( Mm/g \) varies inversely with \( Mm \). Thus, \( Mm/g \) is directly related to both diffusion-limited product stability and product rigidity (viscosity) at both \( T_g \) and temperatures > \( T_g \) in the WLF region. For example, at any given in the WLF zone, substances with small \( Mm/g \) values (e.g., fructose)
result in larger values of Mm and greater rates of diffusion-limited events than do substances with large \( m \/ \epsilon \) values (e.g., glycerol [113]). Small differences in the value of \( m \/ \epsilon \) result in very large differences in both Mm and product stability [113].

4. \( m \/ \epsilon \) is highly dependent on solute type (Table 8).

5. For equal \( m \/ \epsilon \) at a given product temperature, an increase in solids content results in decreased Mm and increased product stability.

At this point it is appropriate to mention two approaches that have been used to study the interrelations between Mm, \( T_g \), and food properties/stability. One approach, as just discussed, involves testing whether physical and chemical changes in foods over the temperature range \( m \sim T_g \) conform to WLF kinetics. Some studies of this kind have resulted in good conformity (physical properties) to WLF kinetics and others poor (some chemical reactions) (Fig. 28).

A second approach is simply to determine whether food stability differs markedly above and below \( T_g \) (or \( T_g' \), with little attention given to characterization of kinetics. With this approach, the expectation is not so demanding and the results are often much more favorable; that is, it is found that desirable food properties, especially physical properties, are typically retained much better below \( T_g \) than they are above. \( T_g \) appears to be much less reliable for predicting stability of chemical properties than it is for physical properties. The temperature below which oxidation of ascorbic acid exhibits greatly reduced temperature dependency (practical termination temperature) appears to correspond fairly well to \( T_g' \), at least under the conditions used in Figure 29. However, the same is not true for non enzymatic browning. In Figure 30, the practical termination temperature for non enzymatic browning is well above sample \( T_g \), whereas in Figure 31 the termination temperature is well below sample \( T_g \). Differences in sample composition probably account for the dissimilar results in the two browning studies.

In closing this section, it is appropriate to note that an algebraic equation is available to interrelate food texture, temperature, and moisture content in the vicinity of \( T_g \) (or other critical temperatures) [88]. Although the equation cannot be used to predict textural changes, it can be used to create quantitative, three-dimensional graphs that effectively display these interrelationships near \( T_g \). Large differences exist among products with respect to dependency of a given textural property on temperature and moisture, and these differences are clearly evident on graphs of this kind.

2.11.3.6 Concept 6. Water is a Plasticizer of Great Effectiveness and it Greatly Affects \( T_g \)

This is especially true with regard to polymeric, oligomeric, and monomeric food substances that are hydrophilic and contain amorphous regions. This plasticizing action results in enhanced Mm, both above and below \( T_g \). As water increases, \( T_g \) decreases and free volume increases (Fig. 32). This occurs because the average molecular weight of the mixture decreases. In general, \( T_g \) decreases about 5–10°C per wt% water added [118]. One should be aware, however, that the presence of water does not assure that plasticization has occurred; water must be absorbed in amorphous regions to be effective.

Water, because of its small molecular mass, can remain surprisingly mobile within a glassy matrix. This mobility no doubt accounts, as previously noted, for the ability of some chemical reactions involving small molecules to continue at measurable rates somewhat below the \( T_g \) of a polymer matrix, and for water to be desorbable during the secondary phase of freeze drying at temperatures < \( T_g \).
Comparison of the temperature dependence (\(T - T_g\)) of viscosity (log \(1 / h_{Tg}\); inverse of molecular mobility) as estimated from the WLF equation using mean constants, with the temperature dependencies of the rates at which various foods deteriorate. Note: Curves for rate of deterioration of foods have been adjusted vertically to avoid overlap; thus the values shown are relative values and meaning should be attached only to the slopes of these curves. Curve a is WLF viscosity, which is usually assumed to be inversely proportional to the rates of diffusion-dependent reactions. Curve b is the pseudo-first-order rate constant for loss of ascorbic acid in frozen peas. Curve c is the rate of enzyme-catalyzed hydrolysis of disodium p-nitrophenyl phosphate in aqueous maltodextrin. Curve d is the rate constant for the decrease in protein solubility in frozen cod. Curve e is the rate constant for the increase in “Instron peak force” for frozen cod. Curve f is the mean rate of increase in the apparent viscosity of egg yolk (during cooling, the early stages of freezing account for the steep slope at the right end of the curve). Curve g is the “kinetic constant” for the growth of ice crystals in frozen beef. (Compiled from various sources by Simatos and Blond [110].)

2.11.3.7 Concept 7. Solute Type Greatly Affects \(T_g\) and \(T'_g\)

These relationships are important for predicting the behavior of compounds, but they are not simple. Thus, the discussion here is far from complete. For further information the reader is referred to Slade and Levine [113]. \(T_g\) is strongly dependent on both solute type and moisture content, except for observed \(T'_g\), which depends primarily on solute type and only slightly on water content (initial).

Attention will first be given to the relationship between solute molecular weight (MW) and \(T'_g\). Figure 33 is a plot of \(T'_g\) versus MW for sugars, glycosides, and polyols with maximum MW of about 1200. \(T'_g\) (and \(T_g\)) increase proportionately with increases in solute MW.
Loss of ascorbic acid with time at various temperatures. Initial samples contained ascorbic acid (40 mg/100 ml) and 10% w/w maltodextrin (M100) in degassed acetate buffer, pH 5.8. $T_g$ is -10°C. Upper three lines are data for temperatures of -11.5, -14.3, and -17.7°C, is -8.0°C, and is -5.6°C. (From Ref. 70.)

Over the range of MW shown. This is an expected relationship because translational mobility of molecules decreases with increasing size, so that a large molecule requires a higher temperature for movement than does a smaller one. However, with MW greater than 3000 (dextrose equivalent, DE, of $\sim$6 for starch hydrolysis products) $\varepsilon$ becomes independent of MW, as is shown in Figure 34. An exception occurs when time and large-molecule concentration are sufficient to allow “entanglement networks” (see later section) to form. In this instance, $\varepsilon$ continues to rise somewhat with increasing MW. A noteworthy aspect of Figure 34 is the relationship shown between functional properties of solutes and their DE (or MW). Compounds on the small-MW portion (vertical leg) of the curve serve, for example, as sweeteners.

Rate of nonenzymatic browning in cabbage and potato as a function of product water content and $T - T_g$ (From Ref. 49.)
FIGURE 31
Rate of nonenzymatic browning in a model system as a function of $T - T_g$. Maltodextrin (DE 10), L-lysine, and D-xylose were used at ratios of 13:1:1. Storage temperature was held constant at each of the temperatures indicated and $T_g$ was altered by changing water content of the sample.
(From Ref. 99.)

FIGURE 32
$T_g$ of wheat gluten as a function of water content.
(From Ref. 42.)
**FIGURE 33**

$T_g'$ as influenced by solute molecular weight. $T_g'$ values were determined from 20 wt% solutions of sugars (O), glycosides (x), and polyols (*) that were maximally freeze concentrated.

(From Ref. 61.)

**FIGURE 34**

$T_g'$ as influenced by number-average molecular weight and dextrose equivalent (DE) of commercial starch hydrolysis products. $T_g'$ values were determined from maximally freeze-concentrated solutions that initially contained 80 wt% water. (Adapted from Ref. 60.)
humectants, participants in the Maillard reaction, and cryoprotectants. Compounds on the plateau region of the curve have entirely different functions, as indicated.

Plots of MW versus $\gamma$ that are linear and provide greater insight to the relationship between MW and $\gamma$ have been developed for solutes with MW of less than about 3000 [113]. For dry solutes, the appropriate plot is $T'_{\gamma}$ versus $-1/MW_{w}$ of the solute. For samples containing significant amounts of water, the appropriate plot is $T'_{\gamma}$ versus $-1/MW_{w}$ of the water-solute solution that exists at $T'_{\gamma}$ of the sample. It should be noted that the good linearity of these plots deteriorates significantly when members of nonhomologous series are incorporated on the same plot. This indicates that solute attributes other than MW have an influence on $T'_{\gamma}$ and $T'_{\gamma'}$. Thus, one cannot expect to obtain the same product properties (stability, processability) when chemicals of the same MW (or same DE) are interchanged. This failure to achieve uniform performance among compounds of the same MW is especially true when the interchange involves different chemical families. For example, for different sugars of equal MW, the difference in $T'_{\gamma}$ can be as much as 10°C. Different performance can also occur between molecules of the same type if fractions of different configurations are present [114]. $\gamma$ and $T'_{\gamma}$ values for selected pure carbohydrates, along with associated values for molecular weight and several other properties of importance, are listed in Table 8.

A final point should be mentioned before closing this section. Most, perhaps all, bio-polymers of high MW have very similar glass curves and exhibit $T'_{\gamma}$ values near -10°C. Those with $T'_{\gamma}$ values in this range include polysaccharides such as starch, maltoolxtrin, cellulose, hemicellulose, carboxymethylcellulose, dextran, and xanthan; and proteins such as gluten, glutenin, gliadin, zein, collagen, gelatin, elastin, keratin, albumins, globulins, and casein [117].

2.11.3.8 Concept 8. Solute Type Has a Profound Effect on $W'_{\gamma}$

The term $W_{\gamma}$ is the water content of the sample at $\gamma$, and $W'_{\gamma}$ is the unfrozen water content at $T'_{\gamma}$. The terms $C_{\gamma}$ and $C'_{\gamma}$ are the solids content of the sample at $\gamma$ and $T'_{\gamma}$ respectively ($C'_{\gamma} = 100 - W'_{\gamma}$, and the same relationship applies to $C_{\gamma}$ and $W_{\gamma}$ at a given temperature). The following important relationships were derived for $W'_{\gamma}$ of carbohydrates but are believed to apply to $W_{\gamma}$ and to substances other than carbohydrates:

1. The value of $W'_{\gamma}$ varies directly with Mm and inversely with product stability at $T'_{\gamma}$. Increases in product water content above $W'_{\gamma}$ or $W_{\gamma}$ cause product stability to decrease and Mm to increase.

2. The $W'_{\gamma}$ values vary greatly with solute type as is apparent from data in Table 8. The value of $W'_{\gamma}$ generally varies inversely with $T'_{\gamma}$ and MW, but these are qualitative relationships and are not suitable for predictive purposes, except for members of a homologous series of compounds. Generally, the most reliable values of solute or product $W'_{\gamma}$ are obtained by measurement. However, the experimental procedure chosen can greatly affect the value obtained, and the procedure most likely to give values of greatest relevance to food is still a matter of controversy [114].

Based on what has been said, one should not expect the same performance within the WLF zone when compounds are interchanged on an equal weight or equal $p/p_0$ basis, because these compounds may have different values for $W'_{\gamma}$ and $T'_{\gamma}$ (or $W_{\gamma}$ and $\gamma$). Compare, for example, the $W'_{\gamma}$ values of glucose versus fructose and lactose versus trehalose (Table 8). Although each pair member has the same molecular weight, their effect on product stability usually is quite different, and this is at least partly attributable to the difference in $W'_{\gamma}$. 
2.11.3.9 Concept 9. Molecular Entanglement Can Greatly Affect the Properties of Food

Macromolecule entanglement (see glossary) can lead to the formation of entanglement networks (EN) when solute molecular size is sufficient (>~3,000 MW, <DE of ~6 for carbohydrates), when solute concentration exceeds a critical value, and sufficient time elapses. Besides carbohydrates, proteins can form EN, examples being gluten in wheat flour dough and sodium caseinate in imitation mozzarella cheese.

ENs have a profound effect on the properties of food. For example, it has been suggested, based on some supporting evidence, that ENs can slow rates of crystallization in frozen foods, retard moisture migration in baked goods, help retain crispness of breakfast cereals, help reduce sogginess of pastries and pie crusts, and facilitate drying, gel formation, and encapsulation processes [114]. Once conditions result in an EN, further increases in MW will not only cause further increases in \( T_g' \), but will also result in firmer networks [64].

2.11.4 Technological Aspects: Freezing

Although freezing is regarded as the best method of long-term preservation for most kinds of foods, the benefits of this preservation technique derive primarily from low temperature as such, not from ice formation. The formation of ice in cellular foods and food gels has two important adverse consequences: (a) nonaqueous constituents become concentrated in the unfrozen phase (an unfrozen phase exists in foods at all storage temperatures used commercially), and (b) all water converted to ice increases 9% in volume. Both occurrences deserve further comment.

During freezing of aqueous solutions, cellular suspensions, or tissues, water from solution is transferred into ice crystals of variable but high degree of purity. Nearly all of the nonaqueous constituents are therefore concentrated in a diminishing quantity of unfrozen water. The net effect is similar to conventional dehydration except in this instance the temperature is lower and the separated water is deposited locally as ice. The degree of concentration is influenced mainly by the final temperature, and to a lesser degree by agitation, rate of cooling, and formation of eutectics (crystallization of solutes—uncommon).

Because of the freeze-concentration effect, the unfrozen phase changes significantly in properties such as pH, titratable acidity, ionic strength, viscosity, freezing point (and all other colligative properties), surface and interfacial tension, and oxidation-reduction potential. In addition, solutes sometimes crystallize, supersaturated oxygen and carbon dioxide may be expelled from solution, water structure and water-solute interactions may be drastically altered, and macromolecules will be forced closer together, making interactions more probable. These changes in concentration-related properties often favor increases in reaction rates. Thus, freezing can have two opposing effects on reaction rate: lowering temperature, as such, will always decrease reaction rates, and freeze-concentration, as such, will sometimes increase reaction rates. It should not be surprising, therefore, that reaction rates at subfreezing temperatures do not conform well to either Arrhenius or WLF kinetics, and that these deviations sometimes can be very large. In fact, it is not uncommon to find reactions that accelerate during freezing [22,23].

With this background, specific examples of freezing and the importance of Mm to the stability of frozen foods can be presented. Slow freezing of a complex food will be considered first. Very slow freezing results in close conformance to solid-liquid equilibrium and maximal freeze-concentration. Starting at A in Figure 35, removal of sensible heat moves the product to B, the initial freezing point of the sample. Because nucleation is difficult, further removal of heat results in undercooling and nucleation begins at point C. Nucleation is immediately followed by crystal growth, release of latent heat of crystallization and a rise in temperature to D. Further
removal of heat causes additional ice formation, concentration of the unfrozen phase, depression of its freezing point, and alteration of its composition in conformance with path D to \( T \). The \( T \) in the complex food being considered represents \( T_{\text{Emax}} \) for the solute with the highest eutectic point (temperature at which saturation of the least soluble solute is achieved). Solutes in complex frozen foods seldom crystallize at or below their eutectic points. An occasional exception of commercial importance is formation of a lactose eutectic in frozen desserts. This results in a textural defect known as “sandiness.”

Assuming eutectics do not form, further ice formation leads to metastable supersaturation of many solutes (an amorphous liquid phase) and compositional changes of the unfrozen fraction in accord with path to \( T \). Point \( T \) is the recommended storage temperature (-20°C) for most frozen foods. Unfortunately, point \( T \) is above the glass transition temperature of most foods, indicating that \( M_m \) will be moderately intense and the food’s diffusion-limited physical and chemical properties will be relatively unstable and highly temperature-dependent. Exact conformance with WLF kinetics should not be expected because freeze-concentration effects during cooling, and melt-dilution effects during warming, are not accounted for in the WLF equation.

If cooling is continued below point \( T \), additional ice formation and freeze concentration occur, causing composition of the unfrozen fraction to change from that at \( T \) to that at \( T' \) \cite{77a,97,102,111}. At \( T' \), most of the supersaturated unfrozen phase converts to a glass, encompassing the ice crystals. The \( T' \) is a quasi-invariant \( T_g \) that applies only to the maximally freeze-concentrated unfrozen phase. The observed \( T_g' \) depends primarily on solute composition of the sample and secondarily on initial water content of the sample (\( T_g \) is strongly dependent on both solute composition and water content). Observed \( T_g' \) is not totally invari-
TABLE 9 Glass Transition \( T_{g}' \) and DE Values for Commercial Starch Hydrolysis Products (SHP)

<table>
<thead>
<tr>
<th>SHP</th>
<th>Manufacturer</th>
<th>Starch source</th>
<th>( T_{g}' )</th>
<th>DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staley 300</td>
<td>Staley(^a)</td>
<td>Corn</td>
<td>-24</td>
<td>35</td>
</tr>
<tr>
<td>Maltrin M250</td>
<td>GPC(^b) (1982)</td>
<td>Dent corn</td>
<td>-18</td>
<td>25</td>
</tr>
<tr>
<td>Maltrin M150</td>
<td>GPC</td>
<td>Dent corn</td>
<td>-14</td>
<td>15</td>
</tr>
<tr>
<td>Paselli SA-10</td>
<td>Avebe(^c)</td>
<td>Potato (Ap)</td>
<td>-10</td>
<td>10</td>
</tr>
<tr>
<td>Crystal gum</td>
<td>National(^d)</td>
<td>Tapioca</td>
<td>-6</td>
<td>5</td>
</tr>
<tr>
<td>Stadex 9</td>
<td>Staley</td>
<td>Dent corn</td>
<td>-5</td>
<td>3.4</td>
</tr>
<tr>
<td>AB 7436</td>
<td>Anheuser-Busch</td>
<td>Waxy maize</td>
<td>-4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^a\)A. E. Staley Manufacturing Co.
\(^b\)Grain Processing Corp.
\(^c\)Avebe America.
\(^d\)National Starch and Chemical.

Source: From Ref. 114.

ant because maximum ice formation is seldom obtained during procedures typically used for its determination.

Further cooling causes no further freeze-concentration, simply removal of sensible heat and alteration of product temperature in the direction of point F. Below \( T_{g}' \), \( M_m \) is greatly reduced and diffusion-limited properties usually exhibit excellent stability.

Some \( T_{g}' \) values for starch hydrolysis products, amino acids, proteins, and foods are listed in Tables 9–11. These values should be regarded as “observed” or “apparent” in \( T_{g}' \) values because maximum ice formation would be almost impossible under the measurement circumstances employed. These observed \( T_{g}' \) values are, however, probably of greater relevance to practical situations than the true (somewhat lower) \( T_{g}' \) values. The range of \( T_{g}' \) values for foods, and the variation of \( T_{g}' \) with location in a tissue, should be noted. More than one \( T_{g}' \) can occur in a product when, for example, the product contains a major chemical constituent that exists in two conformational forms, or when different domains in the product contain different ratios of macromolecules to small-solute molecules. In this instance the highest \( T_{g}' \) is usually considered most important.

Because most fruits have very low \( T_{g}' \) values, and storage temperature is typically > \( T_{g}' \), texture stability during frozen storage is often poor. One would expect that vegetables, with \( T_{g}' \) values that are typically quite high, would exhibit storage lives that are longer than those of fruits. This is sometimes but not always true. The quality attribute that limits the storage life of vegetables (or any kind of food) can differ from one vegetable to another, and it is likely that some of these attributes are influenced less by \( M_m \) than others.
values for fish (cod, mackerel) and beef in Table 11 were determined in 1996 and they differ markedly from earlier data (cod, -77°C [84]; beef, -60°C [91]). The earlier data are probably in error because the dominance of large protein polymers in muscle should result in \( T_g' \) values similar to those of other proteins (Table 10). Based on the muscle \( T_g' \) values in Table 11, one would be expect (as is generally observed) that all physical changes and all chemical changes that are diffusion limited would be effectively retarded during typical commercial frozen storage. Because storage lipids exist in domains separate from that of myofibrillar proteins, they probably are not protected by a glassy matrix during frozen storage and typically exhibit instability.

Values of \( W_g' \) for several solutes are shown in Tables 8, 10, and 11, but these values are subject to some uncertainty. \( W_g' \) values determined recently using altered techniques tend to be smaller than earlier values (mainly those of Slade and Levine). Consensus on what type of
<table>
<thead>
<tr>
<th>Substance</th>
<th>MW</th>
<th>pH (20wt%)</th>
<th>$T_g'$ (°C)</th>
<th>$W_g'$ (wt%)</th>
<th>$W_{UFW}$ (g UFW/g dry AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>75.1</td>
<td>9.1</td>
<td>-58</td>
<td>63</td>
<td>1.7</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>89.1</td>
<td>6.2</td>
<td>-51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Threonine</td>
<td>119.1</td>
<td>6.0</td>
<td>-41</td>
<td>51</td>
<td>1.0</td>
</tr>
<tr>
<td>DL-Aspartic acid</td>
<td>133.1</td>
<td>9.9</td>
<td>-50</td>
<td>66</td>
<td>2.0</td>
</tr>
<tr>
<td>DL-Glutamic acid · H$_2$O</td>
<td>147.1</td>
<td>8.4</td>
<td>-48</td>
<td>61</td>
<td>1.6</td>
</tr>
<tr>
<td>DL-Lysine · HCl</td>
<td>182.7</td>
<td>5.5</td>
<td>-48</td>
<td>55</td>
<td>1.2</td>
</tr>
<tr>
<td>DL-Arginine · HCl</td>
<td>210.7</td>
<td>6.1</td>
<td>-44</td>
<td>43</td>
<td>0.7</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>-13</td>
<td>25-31</td>
<td>0.33-0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>-13</td>
<td>38</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen (bovine, Sigma C9879)$^f$</td>
<td>-6.6 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caseinate, sodium</td>
<td>-10</td>
<td>39</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin (175 bloom, pig skin)</td>
<td>-12</td>
<td>34</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin (300 bloom, pig skin)</td>
<td>-10</td>
<td>40</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluten (Sigma, wheat)</td>
<td>-7</td>
<td>28</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluten (&quot;vital wheat gluten,&quot; commercial sample)</td>
<td>-5 to -10</td>
<td>7-29</td>
<td>0.07-0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^aW_g'$ is unfrozen water existing in sample at $T_g'$.

$^bUFW$ is unfrozen water.

$^c$Solubilized with NaOH.

$^d$"As is" pH.

$^e$Undergoes solute crystallization.

$^f$From N. Brake and O. Fennema (unpublished). Determined by DSC; scanned from -60°C to 25°C at 5°C/min after tempering the sample for 1 hr at -10°C.

Mean of 2 replicates ± SD.

Source: Ref. 118.
measurement yields $W_g'$ values most relevant to food stability has not yet been achieved [39,92,97]. It is important to note, however, that the Slade and Levine values reported here were determined using initial sample compositions close to those of high-moisture foods, and this has an important influence on the value obtained.

Two points need to be made about the term “unfrozen” as used in the definition of $W_g'$. First, unfrozen refers to a practical time scale. The unfrozen fraction will decrease somewhat over very long periods of time because water is not totally immobile at $T_g'$ and equilibrium between the unfrozen phase and the glass phase is a metastable equilibrium, not a global one (not lowest free energy). Second, the term “unfrozen” has often been regarded as synonymous with “bound” water; however, bound water has been defined in so many other ways that the term has fallen into disrepute. A significant amount of $W_g'$ water is engaged in interactions, mainly hydrogen bonds, that do not differ significantly in strength from water-water hydrogen bonds.

### Table 11 Glass Transition ($T_g'$) Values of Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>$T_g'$ (°C)</th>
<th>$W_g'$ (wt%)</th>
<th>Food</th>
<th>$T_g'$ (°C)</th>
<th>$W_g'$ (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruit juices</strong></td>
<td></td>
<td></td>
<td><strong>Frozen desserts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange (various</td>
<td>-37.5 ± 1.0</td>
<td></td>
<td>Ice cream, vanilla</td>
<td>-31 to -33</td>
<td>32-37</td>
</tr>
<tr>
<td>samples</td>
<td></td>
<td></td>
<td>Ice milk, vanilla</td>
<td>-30 to -31</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>-37</td>
<td></td>
<td>Three commercial</td>
<td>-31 to -33</td>
<td>32-37</td>
</tr>
<tr>
<td>Pear</td>
<td>-40</td>
<td></td>
<td>Soft serve</td>
<td>-28 to -34</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>-40</td>
<td></td>
<td>Cheddar</td>
<td>-24</td>
<td></td>
</tr>
<tr>
<td>Prune</td>
<td>-41</td>
<td></td>
<td>Provolone</td>
<td>-13</td>
<td></td>
</tr>
<tr>
<td>White grape</td>
<td>-42</td>
<td></td>
<td>Cheese</td>
<td>-24</td>
<td></td>
</tr>
<tr>
<td>Lemon (various</td>
<td>-43 ± 1.5</td>
<td></td>
<td>Cod muscle$^{b,c}$</td>
<td>-11.7 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>samples)</td>
<td></td>
<td></td>
<td>Cod muscle, water</td>
<td>-6.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Insoluble fraction$^{b,d}$</td>
<td>-12.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Vegetables, fresh or frozen</strong></td>
<td></td>
<td></td>
<td>Mackerel muscle$^{b,c}$</td>
<td>-7.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Strawberry</td>
<td>-41</td>
<td></td>
<td>Beef muscle$^{b,c}$</td>
<td>-12.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Sparkleberry, center</td>
<td>-41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sparkleberry, whole</td>
<td>-39 and -33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sparkleberry, intermediate</td>
<td>33 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other cultivars</td>
<td>-33 and -41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberry</td>
<td>[18-24]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flash</td>
<td>-41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>-41 and -32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach</td>
<td>-36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>-35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>-42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Delicious</td>
<td>-42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granny Smith</td>
<td>-41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato, fresh, flesh</td>
<td>-41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet corn</td>
<td>-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garden fresh, endosperm</td>
<td>-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$From Levine and Slade [65, 66], unless otherwise indicated. $W_g'$ is unfrozen water existing in sample at $T_g'$.$^b$From N. Blake and O. Fennema (unpublished). Determined by DSC, scanned from -60°C to 25°C at 5°C/min after annealing for 1 hr at -15°C.$^c$Means of 4 replicates ± SD.$^d$Means of 2 replicates ± SD.
This water is unfrozen simply because local viscosity in the glass state is sufficiently great to preclude, over a practical time span, the translational and rotation motions required for further ice and solute crystallization (formation of eutectics). Thus, most of the \( W' \) water should be regarded as metastable and severely “hindered” in mobility.

Even though foods are frozen commercially at relatively slow rates (a few minutes to about 1 hr to attain -20°C) compared to typical rates for small samples of biological materials, maximal freeze concentration is unlikely. Increased rates of freezing affect the temperature-composition relationship, as shown schematically in Figure 36. This leads to the obvious question as to what is the appropriate reference temperature for foods frozen under commercial conditions—\( T_g \) or \( T'_g \)? This is another area of disagreement. Slade and Levine [114] argue that \( T'_g \) is the appropriate value. However, choice of this value is open to question because initial \( T_g \) (immediately following freezing) will always be \( < T'_g \) and the approach of \( T_g \) to \( T'_g \) during frozen storage (caused by additional ice formation) will be slow and probably incomplete.

The choice of initial \( T_g \) as the reference \( T_g \), as some have suggested, is also open to question because (a) initial \( T_g \) is influenced not only by product type but also by freezing rate [43], and (b) initial \( T_g \) does not remain constant with time of frozen storage, but rather it increases at a commercially important rate at storage temperatures in the \( T_m - T_g \) zone, and more slowly, but at a significant rate, at storage temperatures \( < T_g \) [10,93,101]. The same considerations apply to \( W_g \) and \( W'_g \).

Unfortunately, the important matter of selecting an appropriate reference \( T_g \) for frozen foods cannot be resolved unambiguously because appropriate data are not available. In the meantime, the best that can be done is to suggest that \( T'_g \) be regarded as a temperature zone rather than as a specific temperature. The lower boundary of the zone will depend on freezing rate and time/temperature of storage, but in commercially important situations it is reasonable

![Figure 36](image.png)

FIGURE 36

State diagram of a binary system showing the effects of increasing rates of freezing (rate a < b < c < d) on \( T_m - T_g \). The \( T_m - T_g \) curve is the only one where maximal freeze-concentration occurs. Assumptions stated in Figure 25 apply here.
to suggest that this boundary (initial $T_g'$) probably will not extend below $T_g' - 10^\circ C$. The mean $T_g'$ for a food product marketed through retail channels, because of the relatively high mean storage temperature, is likely to be closer to $T_g'$ than to initial $T_g'$ [43,101]. The term $T_g'$ will continue to be used here with the understanding that it should be regarded as a temperature zone.

Several additional points regarding rates of diffusion-limited events in frozen foods deserve mentioning:

1. Product stability (diffusion-limited) can be increased by (a) lowering the storage temperature closer to, or preferably below, $T_g'$, and/or (b) raising $T_g'$ by incorporating high-molecular weight-solutes in the product. The latter is beneficial because it increases the probability that the storage temperature will be below $T_g'$, and it reduces $M_m$ at any given product temperature above $T_g'$.

2. The rate of recrystallization is explicitly related to $T_g'$ (recrystallization refers to an increase in the mean size of ice crystals and a simultaneous decrease in their number). Provided maximum ice crystallization has occurred, the critical temperature of recrystallization ($T_r$) is the highest temperature at which ice recrystallization can be avoided (often $T_g'$; see Fig. 37). Rates of recrystallization in zone $T_g'$ to $T_g'$ sometimes conform reasonably well to WLF kinetics (Fig. 37). If ice crystallization is not maximal, then the highest temperature at which ice recrystallization can be avoided is approximately equal to $T_g'$. Generally, $T_g'$ must be somewhat greater than $T_g'$ or $T_g'$ for rates to become of practical significance.

3. In general, high $T_g'$ (achieved by adding water-soluble macromolecules) and low $W_g'$ values are associated with firm frozen texture and good storage stability at a given

![FIGURE 37](image)

Rate of ice recrystallization in ice cream as a function of storage temperature ($T_s$), type of sweetener, and presence or absence of stabilizer. The solid curve is plotted from the WLF equation based on a nominal recrystallization rate of 3 mm/day at $T_s=25^\circ C$. $T_g'$ is "apparent" $T_g'$. HFCS is high-fructose corn syrup, SUC is sucrose, CS is corn syrup, DE is dextrose equivalent, $d$ is days, and numerals in the body of the figure refer to dextrose equivalent. When single sweeteners were used they are indicated by arrows. Symbols without arrows represent ice creams made from 1:1 mixtures of two sweeteners selected from the sweeteners shown and 42DECS. Stabilizer, when used, was a 20–80 blend of carrageenan and locust bean gum at a combined concentration of 0.1 wt%. (From Ref. 37.)
subfreezing storage temperature. Conversely, low \( T_{g'} \) and high \( W_{g'} \) values (achieved by adding monomeric substances) are associated with soft frozen texture and relatively poor storage stability [65].

### 2.11.5 Technological Aspects: Air Drying

The paths (temperature-composition) of a product during air dehydration at a constant air temperature can also be followed on Figure 35. Indicated temperatures are lower than those used commercially simply to allow entry of data on the standardized state diagram used here. The example chosen for discussion is a complex food, and its \( T_{n_{eff}} \) curve is based on a food component that has a dominating influence on the location of this curve. Starting at point A, air drying will elevate product temperature and remove moisture, causing the product soon to acquire properties commensurate with point H (wet bulb temperature of air). Further moisture removal causes the product to arrive at and pass through I, the saturation point of the dominating solute (DS) present, with little or no solute crystallization (none is assumed). This sequence results in creation of major regions of liquid amorphous DS, in addition to smaller regions of liquid amorphous substances that may already have been present because of minor solutes with saturation temperatures higher than that of the DS. As drying continues to point J, product temperature approaches the dry bulb temperature of the air. If drying is terminated at point J and the product is cooled to point K then the product will be above the glass transition curve, Mm will be comparatively intense, and stability of diffusion-limited properties will be relatively poor and strongly temperature-dependent (WLF kinetics). Alternatively, if drying is continued from point J to L and the product is then cooled to G, it will be below the \( T_g \) curve, Mm will be greatly subdued, and diffusion-limited properties will be stable and only weakly temperature-dependent.

### 2.11.6 Technological Aspects: Vacuum Freeze-Drying (Lyophilization)

Product paths during vacuum freeze-drying can also be followed on Figure 35. The first stage of freeze-drying coincides fairly closely with the path for slow freezing, ABCDE. If product temperature is not allowed to go below temperature E during ice sublimation (primary freeze drying), path EG would be a typical path. The early part of EG would involve ice sublimation (primary drying), during which collapse of the product cannot occur because of the presence of ice crystals. However, at some point along path E to G, ice sublimation is completed and the desorption period (secondary phase) ensues. This can (and often does) occur before the product passes the glass transition curve. Collapse during this phase of freeze drying is likely, not only for products that were initially fluid, but also to a lesser degree for food tissues. Collapse is likely because no ice is present to provide structural support and product \( T_c > T_{g} \), so Mm is sufficient to preclude rigidity. This scenario is not uncommon during freeze-drying of food tissues and it results in less than optimum product quality. Product collapse results in decreased product porosity (slower drying) and poorer rehydration characteristics. To prevent collapse, path ABCDEFG must be followed.

Provided maximum ice crystallization has occurred, the critical temperature for structural collapse \( (T_c) \) is the highest temperature at which collapse can be avoided during the primary stage of freeze drying \( (T_{c} \sim T_{g'}) \). The \( T_c \) values for some carbohydrates are shown in Table 8 [49,87,102]. If ice crystallization is not maximal then the highest temperature at which collapse can be avoided during primary freeze drying is approximately equal to \( g \). Generally, \( T_{g'} \) must be somewhat greater than \( T_c \) or \( g \) for rates to become of practical significance.
If a product’s composition can be altered, it is desirable to raise $T_g'$ (or $\mu / \epsilon$) and lower $W_g'$ as much as possible. This can be accomplished by the addition of high-molecular-weight polymers and will enable higher freeze-drying temperatures to be used (less energy, greater rate of drying) without danger of product collapse [31].

### 2.11.7 Technological Aspects: Other Applications of the Mm Approach (Partial Listing)

1. Crystallization. Knowledge of $\epsilon$ and $\mu / \epsilon$ ratios allows accurate predictions of whether nucleation of solutes will occur, and if it does, the rate at which subsequent crystal growth will occur over the zone of $m$ to $\epsilon$ (or $T_g'$) [97,102,104,114].

2. Enzyme activity [114]. The rates of many enzyme-catalyzed interactions are diffusion-limited, and in these instances, reaction rates are greatly slowed at $< \epsilon$ or $< T_g'$.  

3. Thermal resistance of bacterial spores. Thermal inactivation of spores of Bacillus stearothermophilus occurs in accord with WLF kinetics, and higher $\epsilon$ values are associated with greater resistance [105].

4. Color of gluten. For heat-treated gluten, plots of $\epsilon$ versus moisture content (0–15%) and “critical temperature for color change” versus moisture content are virtually identical [32].

5. Other miscellaneous properties. Valuable information about gelation [97,114], caking [87], sticky point [87,97], and texture, including texture of bakery goods [67,82,114], can be obtained from knowledge of $\epsilon$ and state diagrams.

### 2.11.8 Technological Aspects: Estimation of Relative Shelf Life

This topic will be dealt with in only a cursory manner. Shown on the temperature-composition state diagram in Figure 38 are zones of differing product stability. The reference line is derived from the $\epsilon$ curve in the absence of ice and the $T_g'$ zone in the presence of ice. Below this line (zone) physical properties are generally quite stable, and the same is true of those chemical properties for which stability is diffusion-limited. Above this line (zone) and below the intersecting curves for $T_m^{-1}$ and $T_m^s$, physical changes often conform to WLF kinetics. Product stability declines greatly as product conditions move upward or to the left in the WLF zone. This occurs with increases in product temperature and/or increases in moisture content. Above the $m$ curves, attributes that depend on diffusion (Mm) are relatively unstable and become more so with movement to the upper left corner of the graph.

It should be reemphasized that storage below $\epsilon$ or $T_g'$ is highly desirable because this will stabilize food properties that are diffusion-limited. However, this is not an all-or-none situation. When storage temperatures $< \epsilon$ or $< T_g'$ are not feasible, minimizing temperature deviations above $\epsilon$ or $T_g'$ will greatly help.

### 2.11.9 Technological Aspects: Relationship of $\epsilon$ and Mm to Relative Vapor Pressure ($p/p_0$) and Moisture Sorption Isotherms

It should come as no surprise that a product-specific, consistent relationship exists between $\epsilon$ and $p/p_0$ (RVP). This arises logically from two other well-established relationships: that of water content versus $\epsilon$, and water content versus $p/p_0$ (moisture sorption isotherm, MSI). Plots of $\epsilon$ versus $p/p_0$ exhibit a large central zone of linearity and curvilinear tails. Examples for several maltodextrins are shown in Figure 39 [97].
FIGURE 38
State diagram of a binary system showing stabilities of diffusion-dependent properties of food. It is assumed that the "Tg zone" is the appropriate Tg for frozen foods, but this is still a matter of some disagreement. Other assumptions stated in Figure 25 apply here.

FIGURE 39
Relationship between calculated glass transition temperature and p/p0 (25°C) for several carbohydrates of differing molecular weight. M is maltodextrin and numerals following M are molecular weight. Tg values in the linear range are calculated from the equation of Fox and Flora: Tg = Tg(∞) - 1/M, where Tg(∞) is the Tg value of highest molecular weight substance. (From Ref. 100.)
Of greater interest is an isotemperature comparison between the $p/p_0$ value required to produce $T_{g}$ (RVP$_g$) and the $p/p_0$ at the BET monolayer value (RVP$_{mono}$). Some data relating RVP$_g$ and RVP$_{mono}$, both at 25°C, are shown in Figure 40 (note: maltodextrins were prepared using ultrapure water). In these examples, RVP$_g$ and RVP$_{mono}$ are never the same (note, however, that values for $T_{g}$ and monolayer moisture are zones rather than precise values). Assuming that future studies establish the general applicability of these results, three conclusions can be suggested: (a) RVP$_g$ tends to be greater than RVP$_{mono}$ in samples containing primarily water and macromolecules, whereas the reverse tends to be true in samples containing water and solutes with molecular weights that are small and/or of mixed size; (b) because RVP$_{mono}$ is often a good predictor of the RVP at which many chemical reactions attain a practical minimum rate during drying (see Fig. 23) and because RVP$_g$ differs from the monolayer RVP, it follows that $T_{g}$ is not a reliable indicator of this practical rate minimum for many chemical reactions (e.g., see Fig. 31); and (c) because $T_{g}$ is often a good indicator of the point at which rates of diffusion-limited events become highly sensitive to temperature during sample warming or hydration, and because RVP$_g$ and RVP$_{mono}$ often differ, it follows that RVP$_{mono}$ is not a reliable indicator of the critical point for diffusion-limited events.

2.11.10 Summary Statements Regarding the Mm Approach to Food Stability

1. Molecular mobility, Mm, as reflected by $T_{g}$, the magnitude of $T_m - T_{g}$, and the product location in the WLF zone $m - T_{g}$ (i.e., deviations in product $T_{g}$ above $T_{g}$, and $W$ above $W_{g}$), provides a promising and potentially powerful tool for assessing the stability of important properties of food that are diffusion-dependent.

2. In general, the original diffusion-dependent properties of food are well retained during storage at $T_{g}$ (or $T'_{g}$).

![Figure 40](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAIgAAADqCAIAAADk...)

**Figure 40** Comparisons of RVPs at 25°C required to produce a $T_{g}$ of 25°C or a BET monolayer moisture value. M-DE is maltodextrin-dextrose equivalent. (Data from Ref. 98.)
3. The two key constituents with respect to Mm are water and the dominant solute or solutes (solute or solutes comprising the major fraction of the solute portion).

4. A solute's ability to reduce Mm usually varies inversely with (a) molecular weight (macromolecules effectively decrease Mm and raise \( T_g \); water has the reverse effect) and (b) the amount of water, \( W_g \), associated with the solute at \( T_g \) (or \( W_g \) at \( v \)).

5. As compared to the RVP approach, the Mm approach appears (a) to be significantly more useful for estimating rates of occurrences that are diffusion-limited, such as the physical properties of frozen foods (RVP is not useful for predicting either physical or chemical properties of frozen foods), optimum conditions for freeze drying, and physical changes involving crystallization, recrystallization, gelatinization and starch retrogradation, (b) to be about equally useful for estimating conditions causing caking, stickiness, and crispness in products stored at near-ambient temperatures, and (c) to be significantly less useful or unreliable, in products free of ice, for estimating growth of vegetative microorganisms [12,13] and for estimating rates of chemical reactions that are not diffusion-limited (inherently slow reactions, e.g., those with high activation energies, and those occurring in a relatively low-viscosity medium such as high-moisture foods).

6. The Mm approach will not be brought to a level of usefulness that equals or exceeds that of the \( p/p_0 \) approach until methods that are rapid, accurate, and economical are developed for determining Mm and glass transitions in foods.

2.12 Combined Methods Approach to Food Stability

At this point, it is hoped that the reader will have realized that RVP and Mm are useful estimators of food stability but that neither is totally sufficient by itself. It has been mentioned several times that factors not accommodated by either of these approaches can have important influences on food stability and safety. The “combined methods approach” to controlling microbial growth in foods was developed specifically to deal with this reality. This approach is mentioned in a book on food chemistry because it demonstrates convincingly that (a) the RVP approach to controlling microbial growth is often inadequate when used alone, (b) the RVP approach, because it is based on a single parameter, is not a totally reliable predictor of chemical stability, and (c) the Mm approach, because it too is based on a single parameter, is unlikely to be a totally reliable predictor of chemical stability.

The combined methods approach (originally called “hurdle approach”) was developed by Professor L. Leistner and others to determine conditions needed to limit the growth of microorganisms in nonsterile foods [57,58,59]. This approach involves manipulating various growth-controlling parameters in a manner such that growth will not occur; each parameter is a “hurdle” to microbial growth. The approach is best illustrated by several examples in Figure 41. The dashed, undulating line is meant to represent the progress of a microorganism in its attempt to overcome the inhibitory hurdles, with growth occurring only after all hurdles have been overcome. Size of the hurdle indicates relative inhibitory effectiveness. Obviously, a microorganism in real life would confront all hurdles simultaneously rather than in sequence as is shown, and some of the factors would act synergistically.

In example 1, six hurdles are present and growth is satisfactorily controlled because the microorganisms are unable to overcome all hurdles. Example 2 is a more realistic one in which a typical microbial population is present and the various hurdles differ in their inhibitory effectiveness, with RVP and preservatives being the most potent ones. This example also results...
The combined methods approach to controlling growth of microorganisms in nonsterilized food. F is heating, t is chilling, RVP is relative vapor pressure, pH is acidification, \(E_h\) is redox potential, pres. is chemical preservative, and N is nutrients. (Adapted from Ref. 58.)

in satisfactory control of microbial growth. Example 3 represents the same product and the same hurdles with a small starting population of microorganisms that result from good sanitary practices. In this example, fewer hurdles suffice. Example 4 represents the same product and the same hurdles with a large starting population of microorganisms that result from poor sanitary practices. Here, the hurdles are insufficient to provide satisfactory control of microbial growth. Example 5 represents the same hurdles and population of organisms as in Example 2, but in this instance the sample is rich in nutrients. Because of the nutrients, the hurdles that were adequate in Example 2 now are inadequate. In example 6, the product and hurdles remain unchanged but the microorganisms are given a substerilizing treatment before storage. The surviving but damaged organisms are less able to overcome the hurdles and fewer hurdles suffice.

The lesson to be learned is this: Although RVP and Mm are powerful tools for predicting and controlling the properties and stability of food, there are many occasions when neither is
sufficient alone and other factors, such as chemical properties of the solute, pH, and oxidation—reduction potential, must be also considered.

2.13 Concluding Comments About Water

Water is typically the most abundant constituent in food, is of critical importance to the desirable qualities of food, is the cause of food's perishable nature, is a rate determinant of many chemical reactions, both desirable and undesirable, is a strong causative agent of undesirable side effects during freezing, is associated with nonaqueous food constituents in ways so complex that once these associations are disturbed by drying or freezing they can never again be completely restored, and, above all else, is frustratingly complex in behavior, inadequately studied, and poorly understood.

Glossary: Molecular Mobility and Food Stability

Amorphous This refers to a nonequilibrium, noncrystalline state of a substance. When saturation conditions prevail and a solute remains noncrystalline the supersaturated solution can be regarded as amorphous. An amorphous solid is generally called a glass and is characterized by a viscosity of greater than about $10^{12}$ Pa sec.

Collapse This refers to both visibly evident collapse, such as the collapse of foods during freeze drying, and to collapse at the molecular level that consists of conversion from a nonequilibrium state to a lower state of free energy (relaxation). The kinetics of collapse is governed by molecular mobility, $M_m$, of the system.

Eutectic temperature ($T_E$) An invariant point on a temperature—composition phase diagram of a binary solution where solution can exist in equilibrium with both crystalline solute and crystalline solvent. Under equilibrium conditions, cooling at $T_E$ results in simultaneous crystallization of solvent and solute in constant proportion and at constant temperature until maximum solidification has occurred. The $T_E$ is, therefore, the highest temperature at which maximum crystallization can (but usually does not) occur.

Free volume Free volume is space not occupied by molecules. A useful mental image can be created by imagining a container filled with the maximum number of bees that can be accommodated in hovering flight. “Filled” means no more bees can be accommodated in a hovering state, not that all space is occupied. The analogous space in liquids is called “free volume” and it accounts for the fact that liquids can be compressed if the pressure is great enough. Free volume can also be thought of as the “elbow room” molecules require to undergo vibrational, rotational and translational motions [41]. Both free volume and molecular mobility increase with increasing temperature. Temperature dependence of free volume is small below $T_g$, and large between $T_m$ and $T_g$.

Glass state (glassy) A substance existing as an amorphous (noncrystalline) solid is said to be in a glassy state. The Stokes viscosity (local viscosity, not bulk viscosity) is appropriate for characterizing glasses, and this value, at the temperature of incipient glass formation ($T_g$), ranges from $10^{10}$ to $10^{14}$ Pa sec, depending on the solute. This viscosity is sufficient to reduce translational and rotational mobility of most large molecules to a point of practical insignificance. In complex, polymer-dominated systems, very small molecules, most notably water, retain significant translational and rotational mobility at temperatures well below sample $T_g$. Vibrational mobility, of course, does not cease until the temperature is reduced to absolute zero.
Glass transition temperature (\(T_g\)). The glass transition temperature, \(T_g\), is the temperature at which a supersaturated solution (amorphous liquid) converts to a glass. This is a second-order transition involving a step change in specific heat at the transition temperature, allowing this transition to be measured by differential scanning calorimetry (first-order transitions involve changes in physical state among gases, liquids, and crystalline solids). \(T_g\) values are observed in substances that contain sizeable regions that are amorphous or partially amorphous (all food tissues and many other foods), regardless of whether they contain ice. For partially crystalline polymeric substances, only the amorphous regions exhibit a glass transition. The \(T_g\) is dependent on solute type and water content. \(T_g'\) is a special \(T_g\) that applies only to samples containing ice, and only when ice has been formed so maximum freeze-concentration occurs (very slow cooling). For a given solute, the observed \(T_g'\) is a quasi-invariant point on a temperature-composition state diagram (not invariant because maximum ice formation is extremely difficult to obtain during typical measurement techniques, thus causing the observed \(T_g'\) to drift slightly lower with extended storage time). Below \(T_g\) or \(T_g'\) of a complex sample, all but small molecules lose their translational mobility while retaining limited rotational and vibrational mobility.

Macromolecular entanglement. This refers to the interaction of large polymers in a random fashion without chemical bonding and with or without hydrogen bonding. When entanglement of macromolecules is sufficiently extensive (requires a minimum critical concentration of the macromolecule and time), a viscoelastic entanglement network forms. This type of amorphous network can be dispersed by dilution, and can exist in conjunction with microcrystalline gels, which cannot be dispersed by dilution.

Metastable state. This refers to a state of pseudo-equilibrium or apparent equilibrium that is stable over practical times. A metastable state is not, however, the most stable equilibrium because it possesses free energy that is greater than that of the global equilibrium state under the same conditions of pressure, temperature and composition. A metastable state can exist—that is, conversion to a more stable equilibrium state of lower free energy will not occur—if the activation energy is sufficiently high to prevent conversion to an equilibrium state of lower free energy during the period of interest.

Molecular mobility (Mm). This refers to either translational or rotational motion of molecules (vibrational mobility is not of concern in the context of food stability).

**Molecular weight, number average (MW\(_n\))**

\[
MW_n = \frac{\sum n_i M_i}{\sum n_i}
\]

where \(n\) is the number of molecules of a given molecular species and \(M\) is the molecular weight of the same species, with \(i\) kinds of molecules present.

**Molecular weight, weight average (MW\(_w\))**

\[
MW_w = \frac{\sum n_i M_i^2}{\sum n_i M_i}
\]

Plasticizer. A substance incorporated into a polymeric material to increase its deformability. A true solvent is always a plasticizer, but a plasticizer is not always a true solvent [106,117]. A plasticizer decreases the \(T_g\) of a polymer. Water is a highly effective plasticizer of hydrophilic, amorphous polymers. Its low molecular weight leads to increased free volume, decreased local viscosity, and increased Mm.
Relaxation Relaxation refers to passage from a nonequilibrium state to a more stable (lower free energy) state. This term is also used to indicate the decay of a stress.

Rubbery state A term used to describe the viscoelastic nature of large polymers in the temperature range \( T_m \) to \( T_g \), that is, when the substance or a part of the substance is between the glassy and liquid states. Small polymers and solutes of low molecular weight that exist in this temperature zone are highly viscous but not elastic, and are not called rubbery [27].

State diagram A phase diagram supplemented with lines depicting boundaries of various nonequilibrium and metastable states. Such a diagram is sometimes called a “supplemented phase diagram.”

Temperature of solute crystallization/dissolution (\( T_{m}^{n} \)) for simple and complex aqueous samples \( T_{m}^{n} \) is the highest temperature at which a crystalline solute can exist in equilibrium with an aqueous solution of a given composition. The highest temperature at which this can occur for a given sample is the sample's initial crystallization temperature. The \( T_{m}^{n} \) also can be thought of as the incipient saturation temperature during cooling, or the temperature at which the last of a crystalline solute, present in a saturated solution, melts or dissolves upon warming. On a temperature—composition phase diagram for a binary aqueous solution, \( T_{m}^{n} \) values for samples with differing temperatures constitute the \( T_{m}^{n} \) curve.

Temperature of ice melting/crystallization (\( T_{m}^{l} \)) for simple and complex aqueous samples This is the temperature at which ice can exist in equilibrium with an aqueous solution of a given composition. The highest temperature at which this can occur is the initial freezing point. On a temperature—composition phase diagram for a binary aqueous solution, \( T_{m}^{l} \) values for samples with differing initial ratios of solute to water constitute the \( T_{m}^{l} \) curve.

Vitrification Solidification of an entire sample as a glass, that is, no crystallization of solvents or solutes.

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Abbreviations and Symbols

- \( C_1, C_2 \) Constants for the WLF equation
- \( C_g \) Solute concentration (wt%) existing in sample at \( T_g \)
- \( C_g' \) Solute concentration (wt%) existing in sample at \( T_g' \)
- DE Dextrose equivalent
- DS Dominating solute
- ERH Percent equilibrium relative humidity
- Mm Molecular mobility
- MSI Moisture sorption isotherm
- \( MW_n \) Number-average molecular weight (see glossary)
$MW_w$ Weight-average molecular weight (see glossary)

Viscosity of sample at temperature
Viscosity of sample at $\varepsilon$ (or $T_{\varepsilon}'$)

$p$

Vapor pressure of the sample

$p_0$

Vapor pressure of pure water

Sample temperature

$e$

Collapse temperature

$e$

Eutectic temperature

$g$

Glass transition temperature of the sample

$T_{\varepsilon}'$

Glass transition temperature of a maximally freeze-concentrated sample

$m$

Either $T_m^1$ or $T_m^s$

$T_m^1$

Melting or freezing temperature of water in a solution

$T_m^s$

Crystallization or melting (dissolution) temperature of solute in a solution

$r$

Recrystallization temperature

$W$

Water content of sample (wt%)

$W_g$

Water (wt%) existing in sample at $\varepsilon$

$W_g'$

Unfrozen water (wt%) existing in sample at $T_{\varepsilon}'$

$W_m$ (or $m$)

“Monolayer” water content

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9
Minerals

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9.6. Summary
9.1 Introduction

Ninety chemical elements occur naturally in the earth’s crust. About 25 of them are known to be essential to life and thus are present in living cells (Fig. 1). Since our food is ultimately derived from living plants or animals, we can expect to find these 25 elements in foods as well. Foods also contain other elements, since living systems can accumulate nonessential elements from their environment. Moreover, elements may enter foods as contaminants during harvest, processing, and storage, or they may be present in intentional food additives.

While there is no universally accepted definition of “mineral” as it applies to food and nutrition, the term usually refers to elements other than C, H, O, and N that are present in foods. These four nonmineral elements are present primarily in organic molecules and water, and constitute about 99% of the total number of atoms in living systems [10]. Thus mineral elements are present in relatively low concentrations in foods. Nevertheless, they play key functional roles in both living systems and foods.

Historically, minerals have been classified as either major or trace, depending on their concentrations in plants and animals. This classification arose at a time when analytical methods were not capable of measuring small concentrations of elements with much precision. Thus the term “trace” was used to indicate the presence of an element that could not be measured accurately. Today, modern methods and instruments allow for extremely precise and accurate measurement of virtually all of the elements in the periodic table (Fig. 1) [28]. Nevertheless, the terms major and trace continue to be used to describe mineral elements in biological systems. Major minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride. Trace elements include iron, iodine, zinc, selenium, chromium, copper, fluorine, lead, and tin.

9.2 Principles of Mineral Chemistry

Many different chemical forms of mineral elements are present in foods. These forms are commonly referred to as species and include compounds, complexes, and free ions [44]. Given the diversity of chemical properties among the mineral elements, the number and diversity of nonmineral compounds in foods that can bind mineral elements, and the chemical changes that occur in foods during processing and storage, it is not surprising that the number of different mineral species in foods is large indeed. Moreover, since foods are so complex and since many mineral species are unstable, it is very difficult to isolate and characterize mineral species in

![Periodic table of the naturally occurring elements. Shaded elements are believed to be essential nutrients for animals and humans.](image-url)
foods. Thus, our understanding of the exact chemical forms of minerals in foods remains limited. Fortunately, principles and concepts from the vast literature in inorganic, organic, and biochemistry can be very useful in guiding predictions about the behavior of mineral elements in foods.

9.2.1 Solubility of Minerals in Aqueous Systems

All biological systems contain water, and most nutrients are delivered to and metabolized by organisms in an aqueous environment. Thus the availabilities and reactivities of minerals depend, in large part, on solubility in water. This excludes the elemental form of nearly all elements (dioxygen and nitrogen are exceptions) from physiological activity in living systems since these forms, such as elemental iron, are insoluble in water and therefore are unavailable for incorporation into organisms or biological molecules.

The species (forms) of elements present in food vary considerably depending on the chemical properties of the element. Elements in groups IA and VIIA (Fig. 1) exist in foods predominantly as free ionic species (Na\(^+\), K\(^+\), Cl\(^-\), and F\(^-\)). These ions are highly water soluble and have low affinities for most ligands; thus they exist primarily as free ions in aqueous systems. Most other minerals are present as complexes, chelates, or oxygen-containing anions (see later discussion of complexes and chelates).

The solubilities of mineral complexes and chelates may be very different from that of inorganic salts. For example, if ferric chloride is dissolved in water, the iron will soon precipitate as ferric hydroxide. On the other hand, ferric iron chelated with citrate is quite soluble. Conversely, calcium as calcium chloride is quite soluble, while calcium chelated with oxalate ion is insoluble.

9.2.2 Minerals and Acid/Base Chemistry

Much of the chemistry of the mineral elements can be understood by applying the concepts of acid/base chemistry. Moreover, acids and bases may profoundly influence functional properties and stabilities of other foods components by altering the pH of the food. Thus acid/base chemistry is of critical importance to food scientists. A brief review of acid/base chemistry follows. For a more complete treatment of this topic, see Shriver et al. [38] or other textbooks on inorganic chemistry.

9.2.2.1 Bronsted Theory of Acids and Bases

A Bronsted acid is any substance capable of donating a proton.

A Bronsted base is any substance capable of accepting a proton.

Many acids and bases occur naturally in foods, and they may be used as food additives or processing aids. Common organic acids include acetic, lactic, and citric acids. Phosphoric acid is an example of a mineral acid present in foods. It is used as an acidulant and flavoring agent in some carbonated soft drinks. It is a tribasic acid (contains three available protons).

\[
\begin{align*}
\text{H}_3\text{PO}_4 & \leftrightarrow \text{H}_2\text{PO}_4^- + \text{H}^+ & pK_1 = 2.12 \\
\text{H}_2\text{PO}_4^- & \leftrightarrow \text{HPO}_4^{2-} + \text{H}^+ & pK_2 = 7.1 \\
\text{HPO}_4^{2-} & \leftrightarrow \text{PO}_4^{3-} + \text{H}^+ & pK_3 = 12.4
\end{align*}
\]

Other common mineral acids include HCl and H\(_2\)SO\(_4\). They are not added to foods directly, although they may be generated in foods during processing or cooking. For example,
H₂SO₄ is produced when the common leavening acid sodium aluminum sulfate is heated in the presence of water:

\[
\text{Na}_2\text{SO}_4 \cdot \text{Al}_2(\text{SO}_4)_3 + 6\text{H}_2\text{O} \rightarrow \text{Na}_2\text{SO}_4 + 2\text{Al}^3+ + 3\text{H}_2\text{SO}_4
\]

Some of the intermediate steps in this reaction are:

\[
\text{Al}_2(\text{SO}_4)_3 \rightleftharpoons 2\text{Al}^{3+} + 2\text{SO}_4^{2-}
\]

Aluminum ions may then form complexes with water to produce hydrated aluminum ions:

\[
\text{Al}^{3+} + 6\text{H}_2\text{O} \rightleftharpoons \text{Al(H}_2\text{O)}_6^{3+}
\]

The Bronsted acidity of the coordinated water molecules is increased as a result of the electron-withdrawing capabilities of the metal ion:

\[
\text{Al(H}_2\text{O)}_6^{3+} + \text{H}_2\text{O} \rightarrow \text{Al(H}_2\text{O)}_5(\text{OH})^{2+} + \text{H}_3\text{O}^+
\]

Acid strength varies as a function of the electronegativity of the metal ion. The acid strength of a hydrated aluminum ion is about the same as that of acetic acid; \(pK_a\) values of \((\text{H}_2\text{O})_6^{3+}\) and acetic acid are 5.0 and 4.8, respectively.

9.2.2.2 Lewis Theory of Acids and Bases

An alternative, and more general, definition of an acid and a base was developed by G. N. Lewis in the 1930s [38]:

A Lewis acid is an electron pair acceptor.

A Lewis base is an electron pair donor.

By convention, Lewis acids are often represented as “A” and Lewis bases as “B.” The reaction between a Lewis acid and a Lewis base then becomes:

\[
\text{A} + :\text{B} \rightarrow \text{A-B}
\]

It is important to remember that this reaction does not involve a change in the oxidation state of either A or B; that is, it is not a redox reaction. Thus, A must possess a vacant low-energy orbital and B must possess an unshared pair of electrons. The bonding results from the interaction of orbitals from the acid and the base to form new molecular orbitals. The stability of the complex depends in large part on the reduction of electronic energy that occurs when orbitals from A and :B interact to form bonding molecular orbitals. The electronic structures of these complexes are very intricate since multiple atomic orbitals may be involved. The \(d^-\)metals for example, can contribute up to nine atomic orbitals (1., 3\(p\), and 5\(d\) orbitals) to the formation of molecular orbitals. See Shriver et al. [38] for an excellent discussion of molecular orbital theory. The product of the reaction between a Lewis acid and a Lewis base is commonly referred to as a complex where A and :B are bonded together through the sharing of the electron pair donated by :B.

The Lewis acid/base concept is key to understanding the chemistry of minerals in foods because metal cations present are Lewis acids and they are bound to Lewis bases. The complexes resulting from reactions between metal cations and food molecules range from metal hydrates, to metal-containing pigments such as hemoglobin and chlorophyll, to metalloenzymes.

The number of Lewis base molecules that may bind to a metal ion is more or less independent of the charge on the metal ion. This number, usually referred to as the coordination number, may range from 1 to 12 but is most commonly 6. For example, Fe\(^{3+}\) binds six water molecules to form hexaaquo iron, which takes on an octahedral geometry (Fig. 2).
FIGURE 2
Ferric iron with six coordinated water molecules. This is the predominant form of Fe$^{3+}$ in acidic (pH < 1) aqueous solutions.

The electron-donating species in these complexes are commonly referred to as ligands. The principal electron donating atoms in ligands are oxygen, nitrogen, and sulfur. Thus many food molecules including proteins, carbohydrates, phospholipids, and organic acids are ligands for mineral ions. Ligands may be classified according to the number of bonds they can form with a metal ion. Those that form one bond are monodentate ligands; those that form two bonds are bidentate; and so on. Ligands that form two or more bonds are referred to collectively as multidentate ligands. Some common examples of ligands are shown in Figure 3.

Stabilities of metal complexes may be expressed as the equilibrium constant for the reaction representing the formation of the complex. The terms "stability constant," $K$, and "formation constant" are often used interchangeably. The generalized reaction for formation of a complex of a metal ion (M) and a ligand (L) is [38]:

$M + L \rightleftharpoons ML \quad K_1 = \frac{[ML]}{[M][L]}$

$ML + L \rightleftharpoons ML_2 \quad K_2 = \frac{[ML_2]}{[ML][L]}$

FIGURE 3
Examples of ligands coordinated with a metal ion (M$^+$)
TABLE 1 Stability Constants (log K) for Selected Metal Complexes and Chelates

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Cu^{2+}</th>
<th>Fe^{3+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH⁻</td>
<td>6.3</td>
<td>11.8</td>
</tr>
<tr>
<td>Oxalate</td>
<td>4.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>10.3</td>
<td>10.0</td>
</tr>
<tr>
<td>EDTA</td>
<td>18.7</td>
<td>25.1</td>
</tr>
</tbody>
</table>

*Note:* Values are corrected to a constant ionic strength.

*Source:* Adapted from Ref. 10.

When more than one ligand is bound to one metal ion, the overall formation constant may be expressed as:

\[ K_n = \frac{[ML_n]}{[ML_{n-1}][L]} \]

where \( K_n = K_1 K_2 \ldots K_n \).

The brackets indicate the equilibrium activities of the bracketed species. Thus \( K \) (or \( \beta_n \)) is an expression of the affinity of ligands for metal ions. Ligands forming complexes with large stability constants will displace metals from complexes with smaller stability constants. Most stability constants are very large, so values for \( K \) are often expressed as the logarithms of the actual values. Table 1 shows some stability constants for Cu^{2+} and Fe^{3+}.

9.2.2.3 Activity Versus Concentration

The behavior of ions in solution is influenced by ionic strength. At high ionic strength the “activity” of an ion with respect to its participation in complex formation or other chemical reactions will be different than it would be at the same concentration in a similar solution of lower ionic strength. Recall that the ionic strength of a solution is given by

\[ \mu = \sqrt[2]{m_1 Z_1^2 + m_2 Z_2^2 + m_3 Z_3^2 + \ldots} \]

where \( m_1, m_2, \) and \( m_3 \) are the molar concentrations of various ions in solution and \( Z \) is the ionic charge.

In order to account for the effect of ionic strength on stability constants, activities rather than actual concentrations should be used in making equilibrium calculations. Activity in this regard is defined as:

\[ a_A = f_A [A] \]

where \( a_A \) is the activity of species \( A \), \( f_A \) is the activity coefficient, and \([A] \) is the concentration of species \( A \). In general, \( f_A \) decreases as ionic strength increases. At very low ionic strengths, \( f_A \) approaches 1 and activity equals concentration. Data in Figure 4 indicate that ionic strength can have a marked effect on activity coefficients, especially when the charge on the ion is large.
Unfortunately, it is difficult to determine activity coefficients, especially in complex systems like foods, so activity coefficients generally are assumed, improperly, to be 1. This being the case, one may wonder about the utility of stability constants for predicting the forms of metal ionic in foods. The problem can be partly overcome by expressing stability constants at a given ionic strength and assuming that effects of ionic strength will be similar for all species. Thus, it is reasonable to conclude from the large stability constant for the Fe(EDTA) complex (Table 1) that EDTA (ethylenediamine tetraacetic acid) added to a food will complex much of the iron in that food. On the other hand, it may not be reasonable to conclude that copper will displace iron from a histidine complex since the stability constants, while different, are similar in magnitude.

9.2.2.4 The Chelate Effect

The stabilities of complexes resulting from reactions between Lewis acids and bases are proportional to the driving force of the reaction. This can be described quantitatively by the Gibbs equation for free energy:

$$G = H - T S$$

where $G$ is the free energy change in a system undergoing transformation, $H$ is the enthalpy change, $T$ is absolute temperature, and $S$ is the entropy change.

Reactions will occur spontaneously (but at unspecified rates) when $G$ is negative, and as $G$ becomes more negative, the reaction becomes more favorable. Note that for a spontaneous reaction to occur, enthalpy of the system must decrease or entropy must increase. A decrease in enthalpy means that the total electronic energy of the system decreases. An increase in entropy means the randomness of the system increases.

A chelate is a complex resulting from the combination of a metal ion and a multidentate ligand such that the ligand forms two or more bonds with the metal, resulting in a ring structure that includes the metal ion. The term chelate is derived from *chele*, the Greek word for claw. Thus a chelating ligand (also called a chelating agent) must contain at least two functional groups capable of donating electrons. In addition, these functional groups must be spatially arranged so that a ring containing the metal ion can form. Chelates have greater thermodynamic stabilities than similar complexes that are not chelates, a phenomenon referred to as the “chelate effect.” Several factors interact to affect the stability of a chelate. Kratzer and Vohra [23] summarized these factors as follows:
1. Ring size. Five-membered unsaturated rings and six-membered saturated rings tend to be more stable than larger or smaller rings.

2. Number of rings. The greater the number of rings in the chelate, the greater the stability.

3. Lewis base strength. Stronger Lewis bases tend to form stronger chelates.

4. Charge of Ligand. Charged ligands form more stable chelates than uncharged ligands. For example, citrate forms more stable chelates than citric acid.

5. Chemical environment of the donating atom. Relative strengths of metal-ligand bonds are shown below in decreasing order.

   - Oxygen as donor: H₂O > ROH > R₂O
   - Nitrogen as donor: H₃N > RNH₂ > R₃N
   - Sulfur as donor: R₂S > RSH > H₂S

6. Resonance in chelate ring. Enhanced resonance tends to increase stability.

7. Stearic hindrance. Large bulky ligands tend to form less stable chelates.

Thus, chelate stabilities are affected by many factors and are difficult to predict. However, the concept of Gibbs free energy is useful for explaining the chelate effect. Consider the example of Cu²⁺ complexing with either ammonia or ethylenediamine [38]:

\[
\text{Cu(H}_2\text{O)}^2+ + 2\text{NH}_3 \rightarrow [\text{Cu(H}_2\text{O)}_4(\text{NH}_3)_2]^2+ + 2\text{H}_2\text{O}
\]

( \( H = -46 \text{ kJ mol}^{-1}; \ S = -8.4 \text{ J K}^{-1} \text{ mol}^{-1}; \text{ and log } \theta = 7.7.)

\[
\text{Cu(H}_2\text{O)}^2+ + \text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \rightarrow [\text{Cu(H}_2\text{O)}_4(\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2)]^{2+} + 2\text{H}_2\text{O}
\]

( \( H = -54 \text{ kJ mol}^{-1}; \ S = +23 \text{ J K}^{-1} \text{ mol}^{-1}; \text{ and log } \theta = 10.1)

Both complexes have two nitrogens bound to a single copper ion (Fig. 5), and yet the stability of the ethylenediamine complex is much greater than that of the ammonia complex (log of formation constant is 10.1 and 7.7, respectively). Both enthalpy and entropy contribute to the difference in stabilities, but the entropy change is a major factor in the chelate effect. Ammonia, a monodentate ligand, forms one bond to copper while ethylenediamine, a bidentate ligand, forms two. The difference in entropy change is due to the change in the number of independent molecules in solution. In the first reaction, the number of molecules is equal on both sides of the equation so the entropy change is small. The chelation reaction, on the other hand, results in a net increase in the number of independent molecules in solution, and, thus, an increase in entropy.

**FIGURE 5**

Cupric ion (Cu²⁺) complexed with ammonia (left) and chelated with ethylene (right) in an aqueous system.
Ethylenediamine tetraacetate ion (EDTA) provides an even more dramatic illustration of the chelate effect [34]. EDTA is a hexadentate ligand. When it forms a chelate with a metal ion in solution, it displaces six water molecules from the metal, and this has a large effect on the entropy of the system (Fig. 6):

Moreover, EDTA chelates contain five-membered rings, which also enhance stability. EDTA forms stable chelates with many metal ions and is widely used as a food additive.

Chelates are very important in foods and in all biological systems. Chelating agents may be added to foods to sequester mineral ions, such as iron or copper, to prevent them from acting as prooxidants. Furthermore, most complexes resulting from metal ions and food molecules are chelates.

9.3 Nutritional Aspects of Minerals

9.3.1 Essential Mineral Elements

What do we mean when we say an element is “essential for life?” Several definitions have been proposed. A widely accepted definition is: An element is essential for life if its removal from the diet or other route of exposure to an organism “results in a consistent and reproducible impairment of a physiological function” [42]. Thus, essentiality can be demonstrated by feeding diets low in a particular element to humans or experimental animals and watching for signs of impaired function.

Human requirements for essential minerals vary from a few micrograms per day up to about 1 g/day. If intakes are low for some period of time, deficiency signs will develop. Conversely, excessively high intakes can result in toxicity. Fortunately, for most minerals the range of safe and adequate intake is fairly wide, so deficiency or toxicity is relatively rare provided a varied diet is consumed. This concept is illustrated in Figure 7.

This broad range of safe and adequate intakes is possible because higher organisms have homeostatic mechanisms for dealing with both low and high exposures to essential nutrients.

![Diagram of EDTA and Ca^{2+} chelated with EDTA](FIGURE%206)

**FIGURE 6**
Ethylenediamine tetraacetic acid (EDTA) and calcium (Ca^{2+}) chelated with EDTA.
Homeostasis may be defined as the processes whereby an organism maintains tissue levels of nutrients within a narrow and constant range. In higher organisms, homeostasis is a highly complex set of processes involving regulation of absorption, excretion, metabolism, and storage of nutrients. Without homeostatic mechanisms, intakes of nutrients would have to be very tightly controlled to prevent deficiency or toxicity. Homeostasis can be overridden when dietary levels are excessively low or high for extended periods of time. Persistently low intakes of mineral nutrients is not uncommon, especially in poor populations where access to a variety of foods is often limited. Toxicities caused by high dietary intakes of minerals are less common, although much has been made recently of possible links between chronic diseases and high dietary levels of iron.

9.3.2 Recommended Dietary Allowances for Mineral Nutrients (U.S.)

Recommended dietary allowances (RDAs) are defined as “the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons” [14]. RDAs are established by estimating the requirement for the absorbed nutrient, adjusting for incomplete utilization of the ingested nutrient, and incorporating a safety factor to account for variability among individuals. Thus RDA values are greater than the requirement, usually by two standard deviations above the mean. This means that individuals with nutrient intakes below the RDA do not necessarily have an inadequate intake. However, when a significant fraction of a population has an intake that is significantly below the RDA for an extended period of time, the probability that deficiency will occur in some individuals increases.

The RDA committee has published RDAs for only 7 of the 20 known essential minerals (Table 2). An RDA is established only when data are sufficient to determine a reliable value. The RDA Committee has, however, published “estimated minimum requirements” for several minerals where data to support an RDA are not adequate. These are sodium, chloride, and potassium (Table 3). “Estimated safe and adequate intakes” have been published for copper, manganese, fluoride, chromium, and molybdenum (Table 4).

Deficiencies of the minerals listed in Table 3 and 4 are rare in the United States. However, for many people, intakes of sodium tend to be higher than desirable for optimal health. Epidemiological data show that blood pressure is positively associated with level of salt intake. Populations with salt intakes greater than 6 g/day have progressively increasing blood pressure with age, while blood pressure rise with age is not seen in populations with salt intakes below 4.5 g/day [8]. Thus, dietary guidelines for the prevention on chronic disease usually include the recommendation that intakes of salt be less than 6 g/day. Sodium chloride is 40% sodium by weight, so 6 g salt provides 2400 mg sodium.
TABLE 2 Recommended Dietary Allowances (RDAs) for the Mineral Nutrients

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Age (years) or condition</th>
<th>Calcium (mg)</th>
<th>Phosphorus (mg)</th>
<th>Magnesium (mg)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Iodine (μg)</th>
<th>Selenium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0.0–0.5</td>
<td>400</td>
<td>300</td>
<td>40</td>
<td>6</td>
<td>5</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.5–1.0</td>
<td>600</td>
<td>500</td>
<td>60</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Children</td>
<td>1–3</td>
<td>800</td>
<td>800</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>800</td>
<td>800</td>
<td>120</td>
<td>10</td>
<td>10</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>7–10</td>
<td>800</td>
<td>800</td>
<td>170</td>
<td>10</td>
<td>10</td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>Males</td>
<td>11–14</td>
<td>1200</td>
<td>1200</td>
<td>270</td>
<td>12</td>
<td>15</td>
<td>150</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>15–18</td>
<td>1200</td>
<td>1200</td>
<td>400</td>
<td>12</td>
<td>15</td>
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<td>50</td>
</tr>
<tr>
<td></td>
<td>19–24</td>
<td>1200</td>
<td>1200</td>
<td>350</td>
<td>10</td>
<td>15</td>
<td>150</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>25–50</td>
<td>800</td>
<td>800</td>
<td>350</td>
<td>10</td>
<td>15</td>
<td>150</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>51+</td>
<td>800</td>
<td>800</td>
<td>350</td>
<td>10</td>
<td>15</td>
<td>150</td>
<td>70</td>
</tr>
<tr>
<td>Females</td>
<td>11–14</td>
<td>1200</td>
<td>1200</td>
<td>280</td>
<td>15</td>
<td>12</td>
<td>150</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>15–18</td>
<td>1200</td>
<td>1200</td>
<td>300</td>
<td>15</td>
<td>12</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>19–24</td>
<td>1200</td>
<td>1200</td>
<td>280</td>
<td>15</td>
<td>12</td>
<td>150</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>25–50</td>
<td>800</td>
<td>800</td>
<td>280</td>
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<td>55</td>
</tr>
<tr>
<td></td>
<td>51+</td>
<td>800</td>
<td>800</td>
<td>280</td>
<td>10</td>
<td>12</td>
<td>150</td>
<td>55</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td>1200</td>
<td>1200</td>
<td>320</td>
<td>30</td>
<td>15</td>
<td>175</td>
<td>65</td>
</tr>
<tr>
<td>Lactating</td>
<td>1st 6 months</td>
<td>1200</td>
<td>1200</td>
<td>355</td>
<td>15</td>
<td>19</td>
<td>200</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>2nd 6 months</td>
<td>1200</td>
<td>1200</td>
<td>340</td>
<td>15</td>
<td>16</td>
<td>200</td>
<td>75</td>
</tr>
</tbody>
</table>

Source: Adapted from Ref. 14.

9.3.3 Bioavailability

It has been recognized for at least a century that the concentration of a nutrient in a food is not necessarily a reliable indicator of the value of that food as a source of the nutrient in question. This led nutritionists to develop the concept of nutrient bioavailability. Bioavailability may be defined as the proportion of a nutrient in ingested food that is available for utilization in metabolic processes. In the case of mineral nutrients, bioavailability is determined primarily by the efficiency of absorption from the intestinal lumen into the blood. In some
<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (kg)</th>
<th>Sodium (mg)</th>
<th>Chloride (mg)</th>
<th>Potassium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.–5</td>
<td>4.5</td>
<td>120</td>
<td>180</td>
<td>500</td>
</tr>
<tr>
<td>6–11</td>
<td>8.9</td>
<td>200</td>
<td>300</td>
<td>700</td>
</tr>
<tr>
<td>Years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11.0</td>
<td>225</td>
<td>350</td>
<td>1000</td>
</tr>
<tr>
<td>2–5</td>
<td>16.0</td>
<td>300</td>
<td>500</td>
<td>1400</td>
</tr>
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<td>6–9</td>
<td>25.0</td>
<td>400</td>
<td>600</td>
<td>1600</td>
</tr>
<tr>
<td>10–18</td>
<td>50.0</td>
<td>500</td>
<td>750</td>
<td>2000</td>
</tr>
<tr>
<td>&gt;18</td>
<td>70.0</td>
<td>500</td>
<td>50</td>
<td>2000</td>
</tr>
</tbody>
</table>

Source: Adapted from Ref. 14.
TABLE 4 Estimated Safe and Adequate Daily Dietary Intakes of Selected Minerals

<table>
<thead>
<tr>
<th>Category</th>
<th>Age (years)</th>
<th>Cu (mg)</th>
<th>Mn (mg)</th>
<th>F (mg)</th>
<th>Cr (g)</th>
<th>Mo (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0–0.5</td>
<td>0.4–0.6</td>
<td>0.3–0.6</td>
<td>0.1–0.5</td>
<td>10–40</td>
<td>15–30</td>
</tr>
<tr>
<td></td>
<td>0.5–1</td>
<td>0.6–0.7</td>
<td>0.6–1.0</td>
<td>0.2–1.0</td>
<td>20–60</td>
<td>20–40</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>1–3</td>
<td>0.7–1.0</td>
<td>1.0–1.5</td>
<td>0.5–1.5</td>
<td>20–80</td>
<td>25–50</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>1.0–1.5</td>
<td>1.5–2.0</td>
<td>1.0–2.5</td>
<td>30–120</td>
<td>30–75</td>
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<td></td>
<td>7–10</td>
<td>1.0–2.0</td>
<td>2.0–3.0</td>
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<td>50–150</td>
</tr>
<tr>
<td></td>
<td>11+</td>
<td>1.5–2.5</td>
<td>2.0–5.0</td>
<td>1.5–2.5</td>
<td>50–200</td>
<td>5–250</td>
</tr>
<tr>
<td>Adults</td>
<td>1.5–3.0</td>
<td>2.0–5.0</td>
<td>1.5–4.0</td>
<td>50–200</td>
<td>75–250</td>
<td></td>
</tr>
</tbody>
</table>

Source: Adapted from Ref. 14.

cases, however, absorbed nutrients may be in a form that cannot be utilized. For example, iron is bound so tightly in some chelates that even if the iron chelate is absorbed, the iron will not be released to cells for incorporation into iron proteins; rather, the intact chelate will be excreted in the urine.

Bioavailabilities of mineral nutrients vary from less than 1% for some forms of iron to greater than 90% for sodium and potassium. The reasons for this wide range are varied and complex, since many factors interact to determine the ultimate bioavailability of a nutrient.

9.3.3.1 Nutritional Utilization of Minerals

The process of mineral nutrient utilization may be described as follows [29]. In the mouth, the food is masticated while salivary amylase begins the process of starch digestion. At this stage, only limited changes in mineral species occur. Next, the food is swallowed and enters the stomach where the pH is gradually lowered to about 2 by gastric acid. At this stage, dramatic changes occur in mineral species. Stabilities of complexes are changed by the altered pH and by protein denaturation and hydrolysis. Minerals may be released into solution and may reform complexes with different ligands. In addition, transition metals such as iron may undergo a valence change when the pH is reduced. The redox behavior of iron is strongly pH dependent. At neutral pH, even in the presence of excess reducing agents like ascorbic acid, ferric iron will not be reduced. However, when the pH is lowered, ascorbic acid rapidly reduces Fe$^{3+}$ to Fe$^{2+}$. Since Fe$^{2+}$ has lower affinity than Fe$^{3+}$ for most ligands, this reduction will promote the release of iron from complexes in food.

In the next stage of digestion, the partially digested food in the stomach is emptied into the proximal small intestine, where pancreatic secretions containing sodium bicarbonate and digestive enzymes raise the pH and continue the process of protein and starch digestion. In addition, lipases begin digesting triacylglycerols. As digestion proceeds, more new ligands are formed and existing ligands are altered in ways that undoubtedly affect their affinities for metal ions. Thus a further reshuffling of mineral species occurs in the lumen of the small intestine, resulting in a complex mixture of soluble and insoluble and high and low molecular weight species. Soluble species may diffuse to the brush border surface of the intestinal mucosa where they may be taken up by the mucosal cell or pass between cells (the paracellular route). Uptake can be facilitated by a membrane carrier or ion channel; may be an active, energy requiring process; may be saturable; and may be regulated by physiological processes.

Clearly, the process of mineral absorption and the factors that affect it are extremely complex. Moreover, speciation of minerals in the gastrointestinal tract, although known to occur,
poor bioavailability of calcium from spinach and pinto beans is probably due to high concentrations of oxalate and phytate, respectively.

### 9.3.3.3 Iron Bioavailability

Iron bioavailability is determined almost totally by the efficiency of iron absorption in the intestine. Total iron intake, composition of the diet, and iron status of the individual consuming the diet all play a role in determining the amount of iron absorbed.

Diets in industrialized countries like the United States consistently provide about 6 mg iron per 1000 kcal (4186 kJ) [6]. Iron species in foods may be broadly grouped as either heme or nonheme. Heme iron is firmly bound in the center of a porphyrin ring and does not dissociate from this ligand until after it is taken up by intestinal mucosal cells. It occurs primarily as hemoglobin or myoglobin and thus is found exclusively in meat, poultry, and fish. Virtually all of the iron in plant foods and approximately 40–60% of the iron in animal tissues is nonheme iron. It is bound primarily to proteins but may also be complexed with citrate, phytate, oxalate, polyphenolics, or other ligands.

The bioavailability of heme iron is relatively unaffected by composition of the diet and is generally significantly greater than that of nonheme iron. The bioavailability of nonheme iron varies markedly depending on composition of the diet. It is widely assumed that nonheme iron from all sources in a meal (foods as well as fortification iron) enters a common pool during digestion and that absorption of iron from this pool is determined by the totality of ligands present in the small intestine at the site of absorption.

Several enhancers and inhibitors of nonheme iron absorption have been identified. Enhancers include meat, poultry, fish, ascorbic acid, and EDTA (in diets where bioavailabilities are low). Inhibitors include polyphenolics (tannins in tea, legumes, and sorghum), phytates (present...
in legumes and whole-grain cereals), some plant proteins (especially legume proteins), calcium, and phosphates.

The overall bioavailability of iron in a diet is determined by complex interactions of the enhancers and inhibitors present. Iron absorption from diets composed primarily of roots, tubers, legumes, and cereals, with limited meat and ascorbic acid, may be only about 5% even in people with poor iron status. Such a diet would provide only about 0.7 mg absorbable iron per day, a quantity too small to meet the needs of many individuals. Iron absorption from diets based on roots, cereals, and legumes that contain some meat, poultry, or fish and some foods high in ascorbic acid may be about 10%. These diets provide about 1.4 mg of absorbable iron per day, an amount that is adequate for most men and postmenopausal women but inadequate for up to 50% of women of child-bearing age. Diets composed of generous quantities of meat, poultry, fish, and foods high in ascorbic acid provide over 2 mg absorbable iron per day, an amount sufficient to meet the needs of nearly all healthy persons [6].

9.3.4 Minerals of Particular Nutritional Concern

For various reasons, deficiencies are common for some mineral elements and rare or nonexistent for others. Moreover, there are large variations in prevalences of specific deficiencies across geographical and socioeconomic divisions. Human dietary deficiencies have been reported for calcium, cobalt (as vitamin B\textsubscript{12}), chromium, iodine, iron, selenium, and zinc [17]. Calcium, chromium, iron, and zinc occur in bound form in foods, and bioavailabilities may be low depending on the composition of the food or meal. Thus, deficiencies of these minerals result from a combination of poor bioavailability and low intakes.

Iodine is present in foods and water predominantly as the ionic, unbound form and has high bioavailability. Iodine deficiency is caused primarily by low intakes. Selenium is present in foods as selenomethionine, a chelate, but it is efficiently utilized so deficiency is caused by low intakes. Vitamin B\textsubscript{12} deficiency is a problem only with persons on strict vegetarian diets, which are low in this vitamin. These observations further illustrate the complexities of the mineral bioavailability story. Some bound forms have low bioavailability while other bound forms have high bioavailability. Unbound forms generally have high bioavailability. Current thinking on bioavailability and mineral deficiencies is summarized in Figure 8.

In the United States, deficiencies of calcium and iron have received the most attention. In

![Figure 8](image-url)

**Figure 8**

Essential minerals grouped by chemical form (free ions in solution or bound to food ligands), bioavailability, and occurrence of nutritional deficiency. *As vitamin B\textsubscript{12}. (From Ref. 17.)
developing countries, iron and iodine have been targeted because of high prevalences of deficiencies among these populations.

9.4 Mineral Composition of Foods

9.4.1 Ash

“Ash” is included in nutrient databases as one of the proximate components of foods. It provides an estimate of the total mineral content of foods [16]. Methods for determination of ash in specific foods and food groups are described in official publications [3]. Minerals in the ash are in the form of metal oxides, sulfates, phosphates, nitrates, chlorides, and other halides. Thus, ash content overestimates total mineral content by a considerable extent since oxygen is present in many of the anions. It does, however, provide a crude idea of mineral content and it is required for calculation of total carbohydrate in the proximate analysis scheme.

9.4.2 Individual Minerals

Individual minerals in foods are determined by ashing the food, dissolving the ash (usually in acid), and measuring mineral concentrations in the resulting solution [16, 19]. Both chemical and instrumental methods are used to measure mineral concentrations, but instrumental methods are generally more rapid, precise, and accurate. Atomic absorption spectroscopy has been available since the 1960s and is still widely used. It is a reliable technique but can measure only one mineral at a time. Inductively coupled plasma spectrometers have gained popularity in recent years primarily because they are capable of quantifying several mineral elements simultaneously from a single sample [28].

Listed in Table 7 are concentrations of some minerals in selected foods. Sources include the U.S. Department of Agriculture database, journal articles, and manufacturers’ data. Values are means; data from individual foods may vary substantially from the data reported.

9.4.3 Factors Affecting the Mineral Composition of Foods

Many factors interact to affect the mineral composition of foods, so compositions can vary greatly.

9.4.3.1 Factors Affecting the Mineral Composition of Plant Foods

In order for plants to grow, they must take up water and essential mineral nutrients from the soil. Once taken up by plant roots, nutrients are transported to other parts of the plant. The ultimate composition of the edible parts of plants is influenced and controlled by fertility of the soil, genetics of the plant, and the environment in which it grows (Fig. 9). The degree to which mineral content can vary even within a plant species is illustrated by wheat grain. For grain grown in Australia, North America, and the United Kingdom, zinc concentrations range from 4.5 to 37.2 mg/kg and iron from 23.6 to 74.7 mg/kg [5].

9.4.3.2 Adequacy of Plant Foods for Supplying the Nutrient Needs of Animals and Humans

Several questions are pertinent. Do plants and humans require the same mineral nutrients? Are the concentrations of mineral nutrients in plants sufficient to meet human requirements? Can mineral concentrations in plants be altered by agricultural or genetic means to enhance the nutritional quality of plants? Are plants grown on depleted soils nutritionally inferior to plants grown on more fertile soils?

The list of essential minerals for plants is similar but not identical to the list for humans.
F, Se, and I are essential for humans but not for most plants. Thus, we might expect to see human deficiencies of these elements in populations that depend on plants grown locally where soil concentrations of these elements are low. In fact, serious human deficiencies of selenium and iodine do exist in several areas of the world [41].

For nutrients required by both plants and animals, we might expect human deficiencies to be less of a problem because the elements will necessarily be present in plant foods. Unfortunately, concentrations of minerals in plants are sometimes too low to meet human needs, or the minerals may be present in forms that cannot be efficiently utilized by humans (see earlier section on bioavailability). These situations apply, respectively, to calcium and iron. The calcium content of some plants is extremely low. Rice, for example, contains only about 10 mg calcium per 100 kcal. Thus, persons consuming rice-based diets must depend on other foods to meet calcium requirements. Iron is more uniformly distributed in plant foods than calcium but its bioavailability can be extremely poor, so diets based on cereals and legumes are often inadequate in iron.

While it is possible in some cases to enhance the nutritional quality of crops through agronomic practices and plant breeding, the movement of mineral nutrients from the soil to the plant and from the plant to the animal or human is an extremely complicated process. Soils differ considerably in their mineral composition. Moreover, the concentration of an element in the soil may not be a good indicator of the amount that can be taken up by plant roots, since the chemical form of the element and soil pH have marked effects on mineral bioavailability to plants. For example, increasing soil pH by adding lime will lower availability of iron, zinc, manganese, and nickel to plants and will increase availability of molybdenum and selenium [47]. Also, plants generally possess physiological mechanisms for regulating amounts of nutrients taken up from the soil. Therefore, we might expect that attempts to alter the mineral composition of food crops would meet with mixed results. For example, application of fertilizer does not significantly increase iron, manganese, or calcium content of food crops [47]. On the other hand, fertilization with zinc at levels in excess of the zinc requirement of the plant has been shown to increase the level of zinc in pea seeds [48].

9.4.3.3 Factors Affecting the Mineral Composition of Animal Foods

Mineral concentrations in animal foods vary less than mineral concentrations in plant foods. In general, changes in dietary intake of the animal have only a small effect on mineral concentrations in meat, milk, and eggs. This is because homeostatic mechanisms operating in the animal regulate tissue concentrations of essential nutrients.

9.4.3.4 Adequacy of Animal Foods for Supplying the Nutrient Needs of Humans

The composition of animal tissues is similar to that of humans; thus we might expect animal foods to be good sources of nutrients. Meat, poultry, and fish are good sources of iron, zinc, phosphate, and cobalt (as vitamin B₁₂). These products are not good sources of calcium unless bones are consumed, which is usually not the case. Also, the iodine content of animal foods may be low. Dairy products are excellent sources of calcium. Thus, consumption of a variety of animal foods along with a variety of plant foods is the best way to ensure adequate intakes of all essential minerals.

9.4.4 Fortification

Fortification of the U.S. food supply began in 1924 with the addition of iodine to salt to prevent goiter, a prevalent public health problem in the United States at the time. In 1933, the American
<table>
<thead>
<tr>
<th>Quantity</th>
<th>Food</th>
<th>Weight (g)</th>
<th>kcal</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
<th>Na</th>
<th>K</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 egg</td>
<td>Scrambled</td>
<td>100</td>
<td>157</td>
<td>57</td>
<td>13</td>
<td>269</td>
<td>290</td>
<td>138</td>
<td>2.1</td>
<td>2.0</td>
<td>0.06</td>
<td>8</td>
</tr>
<tr>
<td>1 slice</td>
<td>White bread</td>
<td>28</td>
<td>75</td>
<td>35</td>
<td>6</td>
<td>30</td>
<td>144</td>
<td>31</td>
<td>0.8</td>
<td>0.2</td>
<td>0.04</td>
<td>8</td>
</tr>
<tr>
<td>1 slice</td>
<td>Whole wheat bread</td>
<td>28</td>
<td>70</td>
<td>20</td>
<td>26</td>
<td>74</td>
<td>180</td>
<td>50</td>
<td>1.5</td>
<td>1.0</td>
<td>0.10</td>
<td>16</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Spaghetti, cooked without salt</td>
<td>70</td>
<td>99</td>
<td>5</td>
<td>13</td>
<td>38</td>
<td>1</td>
<td>22</td>
<td>1.0</td>
<td>0.4</td>
<td>0.07</td>
<td>19.0</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Brown rice, cooked</td>
<td>98</td>
<td>108</td>
<td>10</td>
<td>42</td>
<td>81</td>
<td>5</td>
<td>42</td>
<td>0.4</td>
<td>0.6</td>
<td>0.01</td>
<td>13.0</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>White rice, parboiled, cooked</td>
<td>88</td>
<td>100</td>
<td>17</td>
<td>11</td>
<td>37</td>
<td>3</td>
<td>32</td>
<td>1.0</td>
<td>0.3</td>
<td>0.08</td>
<td>8.30</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Black beans, cooked</td>
<td>86</td>
<td>113</td>
<td>24</td>
<td>61</td>
<td>120</td>
<td>1</td>
<td>305</td>
<td>2.0</td>
<td>1.0</td>
<td>0.18</td>
<td>6.9</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Red kidney beans</td>
<td>89</td>
<td>112</td>
<td>25</td>
<td>40</td>
<td>126</td>
<td>2</td>
<td>356</td>
<td>3.0</td>
<td>0.9</td>
<td>0.21</td>
<td>1.9</td>
</tr>
<tr>
<td>1 cup</td>
<td>Whole milk</td>
<td>244</td>
<td>150</td>
<td>291</td>
<td>33</td>
<td>228</td>
<td>120</td>
<td>370</td>
<td>0.1</td>
<td>0.9</td>
<td>0.05</td>
<td>3.0</td>
</tr>
<tr>
<td>1 cup</td>
<td>Skim milk/nonfat milk</td>
<td>245</td>
<td>86</td>
<td>302</td>
<td>28</td>
<td>247</td>
<td>126</td>
<td>406</td>
<td>0.1</td>
<td>0.9</td>
<td>0.05</td>
<td>6.6</td>
</tr>
<tr>
<td>1.5 oz.</td>
<td>American cheese, processed</td>
<td>43</td>
<td>159</td>
<td>261</td>
<td>10</td>
<td>316</td>
<td>608</td>
<td>69</td>
<td>0.2</td>
<td>1.3</td>
<td>0.01</td>
<td>3.8</td>
</tr>
<tr>
<td>1.5 cup</td>
<td>Cheddar cheese</td>
<td>43</td>
<td>171</td>
<td>305</td>
<td>12</td>
<td>219</td>
<td>264</td>
<td>42</td>
<td>0.3</td>
<td>1.3</td>
<td>0.01</td>
<td>6.0</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Cottage cheese, creamed, small curd</td>
<td>105</td>
<td>108</td>
<td>63</td>
<td>6</td>
<td>139</td>
<td>425</td>
<td>89</td>
<td>0.1</td>
<td>0.4</td>
<td>0.03</td>
<td>6.3</td>
</tr>
<tr>
<td>1 cup</td>
<td>Yogurt, low-fat, plain</td>
<td>227</td>
<td>144</td>
<td>415</td>
<td>10</td>
<td>326</td>
<td>159</td>
<td>531</td>
<td>0.2</td>
<td>2.0</td>
<td>0.10</td>
<td>5.5</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Ice cream, regular vanilla</td>
<td>67</td>
<td>134</td>
<td>88</td>
<td>9</td>
<td>67</td>
<td>58</td>
<td>128</td>
<td>0.1</td>
<td>0.7</td>
<td>0.01</td>
<td>4.7</td>
</tr>
<tr>
<td>1 each</td>
<td>Baked potato with skin</td>
<td>202</td>
<td>220</td>
<td>20</td>
<td>55</td>
<td>115</td>
<td>16</td>
<td>844</td>
<td>2.8</td>
<td>0.7</td>
<td>0.62</td>
<td>1.8</td>
</tr>
<tr>
<td>Quantity</td>
<td>Food</td>
<td>Weight (g)</td>
<td>kcal&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Ca</td>
<td>Mg</td>
<td>P</td>
<td>Na</td>
<td>K</td>
<td>Fe</td>
<td>Zn</td>
<td>Cu</td>
<td>Se</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------</td>
<td>------------</td>
<td>------------------</td>
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<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>1 each</td>
<td>Peeled potato, boiled</td>
<td>135</td>
<td>116</td>
<td>10</td>
<td>26</td>
<td>54</td>
<td>7</td>
<td>443</td>
<td>0.4</td>
<td>0.4</td>
<td>0.23</td>
<td>1.2</td>
</tr>
<tr>
<td>3 each</td>
<td>Broccoli, raw spears</td>
<td>453</td>
<td>126</td>
<td>216</td>
<td>114</td>
<td>297</td>
<td>123</td>
<td>1470</td>
<td>4.0</td>
<td>2.0</td>
<td>0.40</td>
<td>0.9</td>
</tr>
<tr>
<td>3 each</td>
<td>Broccoli spear, cooked from fresh</td>
<td>540</td>
<td>151</td>
<td>249</td>
<td>130</td>
<td>318</td>
<td>141</td>
<td>1575</td>
<td>4.5</td>
<td>2.1</td>
<td>0.23</td>
<td>1.1</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Raw carrot, grated</td>
<td>55</td>
<td>24</td>
<td>15</td>
<td>8</td>
<td>24</td>
<td>19</td>
<td>178</td>
<td>0.3</td>
<td>0.1</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>0.5 cup</td>
<td>Cooked carrots, from frozen</td>
<td>73</td>
<td>26</td>
<td>21</td>
<td>7</td>
<td>19</td>
<td>43</td>
<td>115</td>
<td>0.4</td>
<td>0.2</td>
<td>0.05</td>
<td>0.9</td>
</tr>
<tr>
<td>1 each</td>
<td>Tomato, fresh, whole, average</td>
<td>123</td>
<td>26</td>
<td>6</td>
<td>14</td>
<td>30</td>
<td>11</td>
<td>273</td>
<td>0.6</td>
<td>0.1</td>
<td>0.09</td>
<td>0.6</td>
</tr>
<tr>
<td>0.75 cup</td>
<td>Tomato juice, canned</td>
<td>183</td>
<td>31</td>
<td>17</td>
<td>20</td>
<td>35</td>
<td>661</td>
<td>403</td>
<td>1.0</td>
<td>0.3</td>
<td>0.18</td>
<td>0.4</td>
</tr>
<tr>
<td>0.75 cup</td>
<td>Orange juice prepared from frozen</td>
<td>187</td>
<td>83</td>
<td>17</td>
<td>18</td>
<td>30</td>
<td>2</td>
<td>356</td>
<td>0.2</td>
<td>0.1</td>
<td>0.08</td>
<td>0.4</td>
</tr>
<tr>
<td>1 each</td>
<td>Orange, average, 2 5/8 in diameter</td>
<td>131</td>
<td>60</td>
<td>52</td>
<td>13</td>
<td>18</td>
<td>0</td>
<td>237</td>
<td>0.1</td>
<td>0.1</td>
<td>0.06</td>
<td>1.2</td>
</tr>
<tr>
<td>1 each</td>
<td>Apple with peel, 2.75 in diameter</td>
<td>138</td>
<td>80</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>159</td>
<td>0.3</td>
<td>0.1</td>
<td>0.06</td>
<td>0.6</td>
</tr>
<tr>
<td>1 each</td>
<td>Banana (peeled weight)</td>
<td>114</td>
<td>85</td>
<td>7</td>
<td>32</td>
<td>22</td>
<td>1</td>
<td>451</td>
<td>0.4</td>
<td>0.2</td>
<td>0.12</td>
<td>1.1</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Beef round, roasted</td>
<td>85</td>
<td>205</td>
<td>5</td>
<td>21</td>
<td>176</td>
<td>50</td>
<td>305</td>
<td>1.6</td>
<td>3.7</td>
<td>0.08</td>
<td>–</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Veal, round, roasted</td>
<td>85</td>
<td>160</td>
<td>6</td>
<td>28</td>
<td>234</td>
<td>68</td>
<td>389</td>
<td>0.9</td>
<td>3.0</td>
<td>0.13</td>
<td>–</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Chicken, white meat, roasted</td>
<td>85</td>
<td>140</td>
<td>13</td>
<td>25</td>
<td>194</td>
<td>63</td>
<td>218</td>
<td>0.9</td>
<td>0.8</td>
<td>0.04</td>
<td>–</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Chicken, leg meat, roasted</td>
<td>85</td>
<td>162</td>
<td>10</td>
<td>20</td>
<td>156</td>
<td>77</td>
<td>206</td>
<td>1.1</td>
<td>2.4</td>
<td>0.07</td>
<td>–</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Salmon, cooked</td>
<td>85</td>
<td>183</td>
<td>6</td>
<td>26</td>
<td>234</td>
<td>56</td>
<td>319</td>
<td>0.5</td>
<td>0.4</td>
<td>0.06</td>
<td>–</td>
</tr>
<tr>
<td>3 oz.</td>
<td>Salmon, canned, with bones</td>
<td>85</td>
<td>130</td>
<td>25</td>
<td>277</td>
<td>458</td>
<td>231</td>
<td>0.9</td>
<td>0.9</td>
<td>0.07</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

<sup>2</sup>kcal × 4.186 = kJ.

*Note:* Values are mg per serving, except Se is g per serving.

*Source:* Refs. 12 and 43.
Plants obtain mineral nutrients from the soil solution surrounding the roots. Sources of these minerals include fertilizer, decaying organic materials, and weathering rocks. The minerals are taken up in the roots by a selective process and transported upward to all parts of the plant. The whole process is regulated according to instructions encoded in the plant's genetic material. (From Ref. 1.)

Medical Association recommended that vitamin D be added to milk to prevent rickets. In the early 1940s, food fortification was expanded further when it became apparent that many young adults were failing Army physical exams due to poor nutritional status. In 1943, the government issued an order making enrichment of flour with iron, riboflavin, thiamin, and niacin mandatory. Since the introduction of fortification back in the 1920s there has been a dramatic reduction in the prevalences of many nutrient deficiency diseases in the United States, including iron deficiency and goiter. While general improvements in diets were major factors in this improvement in nutritional status, fortification undoubtedly deserves much of the credit for the low prevalences of nutrient deficiency diseases in the United States today.
In the United States, fortification of foods with iron and iodine remains widespread. In addition, calcium, zinc, and other trace minerals are sometimes added to breakfast cereals and other products. Infant formulas contain the largest number of added minerals since they must be nutritionally complete.

Iron has received far more attention than the other minerals because of the high prevalences of iron deficiency anemia and because of technological and stability problems associated with the addition of iron to foods. Thus, iron fortification deserves detailed attention here.

The first recorded recommendation for iron fortification was made in 4,000 BC by a Persian physician named Melampus [35]. He recommended that sailors consume sweet wine laced with iron filings to strengthen their resistance to spears and arrows and to enhance sexual potency. Widespread iron fortification began in the United States in 1943 when War Food Order No. 1 made enrichment of white flour sold in interstate commerce mandatory. Federal regulations no longer require flour enrichment, but many state regulations do. If flour and other cereal products are enriched, permissible levels of addition are specified in FDA regulations (Table 8).

Addition of iron to foods is a difficult balancing act because some forms of iron catalyze oxidation of unsaturated fatty acids and vitamins A, C, and E. In many cases, forms that are highly bioavailable are also the most active catalytically, and forms that are relatively chemically inert tend to have poor bioavailability. Numerous iron sources have been studied for their effect on oxidative stability and nutritional efficacy. These sources are listed in Table 9.

Ferrous sulfate is the cheapest, most bioavailable, and most widely used iron source for food fortification. It is routinely used as the reference standard in iron bioavailability studies because of its high bioavailability (Table 9). Results of several studies have indicated that off odors and off flavors occur in bakery products made from flour that was heavily fortified with ferrous sulfate and stored for extended periods of time [4]. Barrett and Ranum [4] made the following recommendations for minimizing oxidation problems in bakery products that have been fortified with ferrous sulfate:

1. Ferrous sulfate is the preferred iron source for addition at the bakery.

2. Ferrous sulfate may be used to fortify wheat flour provided iron levels are kept below 40 ppm and the flour is stored at moderate temperatures and humidities for periods not to exceed 3 months.

3. Ferrous sulfate should not be used to fortify flour that may be stored for extended periods.

<table>
<thead>
<tr>
<th>Food</th>
<th>Iron (mg/lb) (shall contain)</th>
<th>Calcium (mg/lb) (may contain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enriched flour</td>
<td>20</td>
<td>960</td>
</tr>
<tr>
<td>Enriched rice</td>
<td>Not less than 16</td>
<td>Not less than 500</td>
</tr>
<tr>
<td></td>
<td>Not more than 32</td>
<td>Not more than 750</td>
</tr>
<tr>
<td>Enriched corn grits</td>
<td>Not less than 13</td>
<td>Not less than 500</td>
</tr>
<tr>
<td></td>
<td>Not more than 26</td>
<td>Not more than 1000</td>
</tr>
<tr>
<td>Enriched macaroni products</td>
<td>Not less than 13</td>
<td>Not less than 500</td>
</tr>
<tr>
<td></td>
<td>Not more than 16.5</td>
<td>Not more than 625</td>
</tr>
<tr>
<td>Enriched bread, rolls, and buns</td>
<td>12.5</td>
<td>600</td>
</tr>
</tbody>
</table>

*Note:* Forms of iron and calcium used must be harmless and assimilable.

*Source:* Ref. 13.
TABLE 9 Iron Sources Used in Food Fortification and Their Bioavailabilities

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Formula</th>
<th>Fe content (g/kg fortificant)</th>
<th>Human</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulfate</td>
<td>FeSO₄ · 7H₂O</td>
<td>200</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ferrous lactate</td>
<td>Fe(C₃H₅O₃)₂ · 3H₂O</td>
<td>190</td>
<td>106</td>
<td>—</td>
</tr>
<tr>
<td>Ferric phosphate</td>
<td>FePO₄ · xH₂O</td>
<td>280</td>
<td>31</td>
<td>3-46</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>Fe₄(P₂O₇)₃ · 9H₂O</td>
<td>250</td>
<td>—</td>
<td>45</td>
</tr>
<tr>
<td>Ferric sodium pyrophosphate</td>
<td>FeNaP₂O₅ · 2H₂O</td>
<td>150</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>Fe₇NH₆(C₆H₅O₇)ₓ</td>
<td>165-185</td>
<td>—</td>
<td>107</td>
</tr>
<tr>
<td>Elemental Fe</td>
<td>Fe</td>
<td>960-980</td>
<td>13-90</td>
<td>8-76</td>
</tr>
</tbody>
</table>

*Relative biological value is the bioavailability relative to ferrous sulfate which is set at 100.

**Source:** Adapted from Refs. 35 and 21.

4. Concentrated premixes containing ferrous sulfate and wheat flour for later addition to flour should not be used because rancidity may develop in the premix.

When fortification with ferrous sulfate is likely to cause problems in a food, other sources are commonly used. In recent years, elemental iron powders have been the sources of choice for fortification of flour for home use, breakfast cereals, and infant cereals. These are all products with long shelf lives.

As the name implies, elemental iron powders consist of elemental iron in a finely divided form. These forms are nearly pure iron with some contamination with other trace minerals and iron oxides. Elemental iron is insoluble in water and thus it is likely that it must be oxidized to a higher oxidation state before it can be absorbed from the intestine. Presumably, this oxidation occurs in the stomach when the iron is exposed to stomach acid:

$$\text{Fe}^+ + 2\text{H}^+ \rightarrow \text{Fe}^{2+} + \text{H}_2 \uparrow$$

Three different types of elemental iron powders are available [32].

Reduced iron: This form is produced by reducing iron oxide with hydrogen or carbon monoxide gas and then milling to a fine powder. It is the least pure of the three types, and purity depends largely on the purity of the iron oxide used [32].

Electrolytic iron: This form is produced by the electrolytic deposition of iron onto a cathode made of flexible sheets of stainless steel. The deposited iron is removed by flexing the sheets and it is then milled to a fine powder. The purity of electrolytic iron is greater than of reduced iron. The main impurity is the iron oxide that forms on the surface during grinding and storage [32].

Carbonyl iron: This form is produced by heating scrap or reduced iron in the presence of CO under high pressure to form iron pentacarbonyl, Fe(CO)₅. The pentacarbonyl is then decomposed by heating to yield a very fine powder of high purity [32].
Elemental iron powders are relatively stable and do not appear to cause serious problems with oxidation in foods. However, the bioavailability of the powders is variable, probably due to differences in particle size. Iron powders are dark gray in color and do cause a slight darkening of white flour, but this is not considered to be a problem [4].

Sodium iron EDTA has not yet been approved by the U.S. Food and Drug Administration for use as an iron fortificant in the United States. However, disodium and calcium disodium EDTA have been approved and are widely used. Sodium iron EDTA is attractive as an iron fortificant because it is relatively stable in foods, it does not catalyze the formation of undesirable flavors and odors in most foods, it is not markedly affected by dietary inhibitors of iron absorption, and it may actually enhance the bioavailability of intrinsic food iron in some low-bioavailability foods [49].

There has been reluctance to used iron EDTA in foods because of concern over possible adverse effects of excessive levels of EDTA in the diet. However, recent surveys have shown that dietary levels of EDTA in the United States are substantially lower than previously thought. This finding may cause the FDA to approve iron EDTA for use in the United States [49].

9.4.5 Effects of Processing

Mineral elements, unlike vitamins and amino acids, cannot be destroyed by exposure to heat, light, oxidizing agents, extremes in pH, or other factors that affect organic nutrients. In essence, minerals are indestructible. Minerals can, however, be removed from foods by leaching or physical separation. Also, the bioavailabilities of minerals may be altered by the factors mentioned earlier.

The most important factor causing mineral loss in foods is milling of cereals. Mineral elements in grain kernels tend to be concentrated in the bran layers and the germ. Thus, removal of bran and germ leaves pure endosperm, which is mineral poor. Mineral concentrations in whole wheat, white flour, wheat bran, and wheat germ are shown in Table 10. Similar losses occur during milling of rice and other cereals. These are substantial losses. During fortification of milled products in the United States, iron is the only mineral commonly added.

Retention of calcium in cheese can be dramatically affected by manufacturing conditions. In cheeses where the pH is low, substantial losses of calcium occur when the whey is drained. Calcium contents of various cheeses are shown in Table 11. Compositions are expressed both as mg/100 g cheese and as a Ca:protein ratio. The latter expression gives a better comparison of Ca losses because the water content of cheeses varies from one variety to another. Cottage cheese has the smallest calcium concentration because the pH at time of whey removal is typically less than 5 [15].

### Table 10: Concentrations of Selected Trace Minerals in Wheat and Milled Wheat Products

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Whole heat</th>
<th>White flour</th>
<th>Wheat germ</th>
<th>Millfeeds (bran)</th>
<th>Loss from wheat to flour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>43</td>
<td>10.5</td>
<td>67</td>
<td>47–78</td>
<td>76</td>
</tr>
<tr>
<td>Zinc</td>
<td>35</td>
<td>8</td>
<td>101</td>
<td>54–130</td>
<td>78</td>
</tr>
<tr>
<td>Manganese</td>
<td>46</td>
<td>6.5</td>
<td>137</td>
<td>64–119</td>
<td>86</td>
</tr>
<tr>
<td>Copper</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>7–17</td>
<td>68</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.6</td>
<td>0.5</td>
<td>1.1</td>
<td>0.5–0.8</td>
<td>16</td>
</tr>
</tbody>
</table>

*Note:* Values are mg mineral/kg product.

*Source:* Adapted from Ref. 36.
### Table 11: Protein, Calcium, and Phosphate Contents of Selected Cheeses

<table>
<thead>
<tr>
<th>Cheese variety</th>
<th>Protein (%)</th>
<th>Ca (mg/100 g)</th>
<th>Ca:protein (mg/g)</th>
<th>PO₄ (mg/100 g)</th>
<th>PO₄:protein (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottage</td>
<td>15.2</td>
<td>80</td>
<td>5.4</td>
<td>90</td>
<td>16.7</td>
</tr>
<tr>
<td>Cheddar</td>
<td>25.4</td>
<td>800</td>
<td>31.5</td>
<td>860</td>
<td>27.3</td>
</tr>
<tr>
<td>Emmenthal</td>
<td>27.9</td>
<td>920</td>
<td>33.1</td>
<td>980</td>
<td>29.6</td>
</tr>
</tbody>
</table>

*Source: Adapted from Refs. 15 and 25.*

Cheddar and Emmenthal cheeses, the whey is normally drained at pH 6.1 and 6.5, respectively, and this causes the calcium content of Emmenthal cheese to be greater than that of cheddar [25].

Since many minerals do have significant solubility in water, it is reasonable to expect that cooking in water would result in some losses of minerals. Unfortunately, few controlled studies have been done. In general, boiling in water causes greater loss of minerals from vegetables than steaming [24]. Mineral losses during cooking of pasta are minimal for iron but more than 50% for potassium [24]. This is predictable because potassium is present in foods as the free ion while iron is bound to proteins and other high-molecular-weight ligands in the food.

### 9.5 Chemical and Functional Properties of Minerals in Foods

Even though minerals are present in foods at relatively low concentrations, they often have profound effects on physical and chemical properties of foods because of interactions with other food components. Details of mineral-food interactions for the broad array of minerals found in foods are given mainly in other chapters, and these interactions as well as their roles are summarized in Table 12. A more detailed treatment of selected minerals follows.

#### 9.5.1 Calcium

Besides its structural role in plants and animals, calcium plays a major regulatory role in numerous biochemical and physiological processes. For example, calcium is involved in photosynthesis, oxidative phosphorylation, blood clotting, muscle contraction, cell division, transmission of nerve impulses, enzyme activity, cell membrane function, and hormone secretion.

Calcium is a divalent cation with a radius of 0.95 Å. Its multiple roles in living cells are related to its ability to form complexes with proteins, carbohydrates, and lipids. Calcium binding is selective. Its ability to bind to neutral oxygens, including those of alcohols and carbonyl groups, and to bind to two centers simultaneously allow it to function as a cross-linker of proteins and polysaccharides [10]. This property has numerous consequences in foods.

The functional role of calcium in milk and milk products has been studied extensively and serves as an example of mineral interactions in a food system (see Chap. 14). Milk contains a complex mixture of minerals including calcium, magnesium, sodium, potassium, chloride, sulfate, and phosphate. Calcium in milk is distributed between the milk serum and the casein micelles. The calcium in serum is in solution and comprises about 30% of the total milk calcium. The remainder of the calcium is associated with casein micelles and is present primarily as colloidal calcium phosphate. It is likely that association of submicelles involves calcium bridges between phosphate groups esterified to serine residues in casein and inorganic phosphate ions.

Calcium and phosphate play an important functional role in the manufacture of cheese.
Addition of calcium prior to renneting shortens coagulation time [25]. Curds with lower Ca contents tend to be crumbly, while cheeses higher in Ca are more elastic.

9.5.2 Phosphates

Phosphates occur in foods in many different forms, both as naturally occurring components of biological molecules and as food additives with specific functions.

In living systems, phosphates serve a variety of functions. For example, adenosine triphosphate (ATP) is the principle source of energy in cells. Phosphoproteins (ferritins) are involved in iron storage. Phospholipids are major components of membranes. Hydroxyapatite, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, comprises the mineral phase of bone. Sugar phosphates, such as glucose 6-phosphate, are key intermediates in carbohydrate metabolism.

A voluminous literature exists on the use of phosphates in foods. See Ellinger [11] and Molins [30] for in-depth treatments of this topic. Several phosphates are approved food additives. These include phosphoric acid, the orthophosphates, pyrophosphates, tripolyphosphates, and higher polyphosphates. Structures are shown in Figure 10.

Phosphate food additives serve many functions including acidification (soft drinks), buffering (various beverages), anticaking, leavening, stabilizing, emulsifying, water binding, and protection against oxidation. The chemistry responsible for the wide array of functional properties of phosphates is not fully understood but undoubtedly is related to the acidity of protons associated with phosphates on the charge on phosphate ions. At pH levels common in foods, phosphates carry negative charges and polyphosphates behave as polyelectrolytes. These negative charges give phosphates strong Lewis-base character and thus strong tendencies to bind metal cations. An ability to bind metal ions may underlie several of the functional properties noted earlier. It should be mentioned, however, that there is considerable controversy about mechanisms of phosphate functionality, particularly as it relates to enhanced water-holding capacity in meats and fish.

9.5.3 Iron

Iron is the fourth most abundant element in the earth's crust and is an essential nutrient for nearly all living species. In biological systems, iron is present almost exclusively as chelates with proteins. Iron plays many key roles in biological systems, including oxygen transport and storage in higher animals (hemoglobin and myoglobin), ATP generation (iron-sulfur proteins and cytochromes), DNA synthesis (ribonucleotide reductase), and chlorophyll synthesis. Unfortunately, free iron can be toxic to living cells. Presumably, this toxicity results from the generation of activated species of oxygen, which in turn can promote lipid oxidation or attack DNA molecules (see later discussion).

In order to avoid the toxic consequences of free iron, virtually all living cells have a mechanism for storing extra iron intracellularly in a nontoxic form. The iron is sequestered in the interior of a hollow protein shell called apoferritin. This protein shell is composed of 24 polypeptide subunits arranged as a sphere. Iron is deposited in the cavity of the shell as polymeric ferric oxyhydroxide. Up to 4500 atoms of iron can be stored in one ferritin shell [46]. Ferritin iron is essentially a cellular reserve that can be mobilized when iron is needed for the synthesis of hemoglobin, myoglobin, or other iron proteins.

In spite of its abundance in the environment, iron deficiency in human, some farm animals, and crops grown on some soils is a problem of major proportions. For example, Schrimshaw [37] estimated that two-thirds of children and women of childbearing age in most developing countries suffer from iron deficiency. The presence of iron deficiency in the United
## TABLE 12 Nutritional and Functional Roles of Minerals and Mineral Salts/Complexes in Foods

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Food sources</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>Low and variable in foods, component of some antacids and leavening agents</td>
<td>Essential nutrient: Possibly essential, evidence not conclusive. Deficiency unknown. Leavening agent: As sodium aluminum sulfate (Na₂SO₄·Al₂(SO₄)₃) Texture modifier</td>
</tr>
<tr>
<td>Bromine</td>
<td>Brominated flour</td>
<td>Essential nutrient: Not known to be essential to humans. Dough improver: KBrO₃ improves baking quality of wheat flour. It is the most used dough improver.</td>
</tr>
<tr>
<td>Calcium</td>
<td>Dairy products, green leafy vegetables, tofu, fish bones</td>
<td>Essential nutrient: Deficiency leads to osteoporosis in later life. Texture modifier: Forms gels with negatively charged macromolecules such as alginites, low-methoxy pectins, soy proteins, caseins, etc. Firms canned vegetables when added to canning brine.</td>
</tr>
<tr>
<td>Iodine</td>
<td>Iodized salt, seafood, plants and animals grown in areas where soil iodine is not depleted</td>
<td>Essential nutrient: Deficiency produces goiter and cretinism. Dough improver: KIO₃ improves baking quality of wheat flour.</td>
</tr>
<tr>
<td>Iron</td>
<td>Cereals, legumes, meat, contamination from iron utensils and soil, enriched products</td>
<td>Essential nutrient: Deficiency leads to anemia, impaired immune response, reduced worker productivity, impaired cognitive development in children. Excessive iron stores may increase risk of cancer and heart disease. Catalyst: Fe²⁺ and Fe³⁺ catalyze lipid peroxidation in foods. Color modifier: Color of fresh meat depends on valence of Fe in myoglobin and hemoglobin: Fe²⁺ is red, Fe³⁺ is brown. Forms green, blue, or black complexes with polyphenolic compounds. Reacts with S²⁻ to form black FeS in canned foods. Enzyme cofactor: Lipoygenase, cytochromes, ribonucleotide reductase, etc.</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Whole grains, nuts, legumes, green leafy, vegetables</td>
<td>Essential nutrient: Deficiency rare. Color modifier: Removal of Mg from chlorophyll changes color from green to olive-brown</td>
</tr>
</tbody>
</table>

(Table continued on next page)
<table>
<thead>
<tr>
<th>Mineral</th>
<th>Foods sources</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>Whole grains, fruits, vegetables</td>
<td>Essential nutrient: Deficiency extremely rare. Enzyme cofactor: pyruvate carboxylase, superoxide dismutase.</td>
</tr>
<tr>
<td>Nickel</td>
<td>Plant foods</td>
<td>Essential nutrient: Deficiency in humans unknown. Catalyst: Hydrogenation of vegetable oils-finely divided, elemental Ni is the most widely used catalyst for this process.</td>
</tr>
<tr>
<td>Phosphates</td>
<td>Ubiquitous, animal products tend to be good sources</td>
<td>Essential nutrient: Deficiency rare due to presence in virtually all foods. Acidulant: $H_3PO_4$ in soft drinks. Leavening acid: $Ca(HPO_4)_2$ is a fast-acting leavening acid. Moisture retention in meats: Sodium tripolyphosphate improves moisture retention in cured meats. Emulsification aid: Phosphates are used to aid emulsification in comminuted meats and in process cheeses.</td>
</tr>
<tr>
<td>Potassium</td>
<td>Fruits, vegetables, meats</td>
<td>Essential nutrient: Deficiency rare. Salt substitute: KCl may be used as a salt substitute. May cause bitter flavor. Leavening agent: Potassium acid tartrate.</td>
</tr>
<tr>
<td>Selenium</td>
<td>Seafood, organ meats, cereals (levels vary depending on soil levels)</td>
<td>Essential nutrient: Keshan disease (endemic cardiomyopathy in China) was associated with selenium deficiency. Low selenium status may be associated with increased risk for cancer and heart disease. Enzyme cofactor: Glutathione peroxidase.</td>
</tr>
<tr>
<td>Sodium</td>
<td>NaCl, MSG, other food additives, milk; low in most raw foods</td>
<td>Essential nutrient: Deficiency is rare; excessive intakes may lead to hypertension. Flavor modifier: NaCl elicits the classic salty taste in foods. Preservative: NaCl may be used to lower water activity in foods. Leavening agents: Many leaving agents are sodium salts, e.g., sodium bicarbonate, sodium aluminum sulfate, sodium acid pyrophosphate.</td>
</tr>
<tr>
<td>Zinc</td>
<td>Meats, cereals</td>
<td>Essential nutrient: Deficiency produces loss of appetite, growth retardation, skin changes. Marginal deficiency exists in United States but extent is unknown. Pronounced deficiency was documented in populations in the Middle East. ZnO is used in the lining of cans for proteinaceous foods to lessen formation of black FeS during heating. Zn can be added to green beans to help stabilize the color during canning.</td>
</tr>
</tbody>
</table>
States and other industrialized countries is lower than that in many developing countries but remains a persistent problem.

The paradox of high prevalences of nutritional deficiency of a nutrient present in such abundance in the environment may be explained by the behavior of iron in aqueous solutions. Iron is a transition element, which means that it has unfilled d orbitals. Its oxidation state in most natural forms is either +2 (ferrous) or +3 (ferric). Ferrous iron has six \( d \) electrons while ferric iron has five. In aqueous solutions under reducing conditions, the ferrous form preeminent. Ferrous iron is quite soluble in water at physiological pH levels. In the presence of molecular oxygen, however, aqueous Fe\(^{2+}\) may be oxidized to Fe\(^{3+}\).

\[
\text{Fe}^{2+}_{\text{aq}} + \text{O}_2 \rightarrow \text{Fe}^{3+}_{\text{aq}} + \text{O}_2^-
\]

The hydrated Fe\(^{3+}\) will then undergo progressive hydrolysis to yield increasingly insoluble ferric hydroxide species [7]:

\[
\text{Fe}(\text{H}_2\text{O})^2+ + \text{H}_2\text{O} \rightarrow \text{Fe}(\text{H}_2\text{O})_2(\text{OH})^2+ + \text{H}_3\text{O}^+ \rightarrow \rightarrow \text{Fe(OH)}_3
\]

Because this hydrolysis reaction occurs readily except at very low pH, the concentration of free ferric ions in aqueous systems is vanishingly small. The predominance of low-solubility forms of iron explains why it is so poorly available.
It is well established that iron can promote lipid peroxidation in foods. Iron appears to catalyze both the initiation and propagation stages of lipid peroxidation. The chemistry is exceedingly complex, but several probable mechanisms have been suggested. In the presence of thiol groups, ferric iron promotes the formation of the superoxide anion [50]:

\[
\text{Fe}^{3+} \text{RSH} \rightarrow \text{Fe}^{2+} + \text{R}^{+} + \text{H}^{+} \\
\text{RSH} + \text{R}^{+} + \text{O}_2 \rightarrow \text{RSSR} + \text{H}^{+} + \text{O}_2
\]

The superoxide anion may then react with protons to form hydrogen peroxide or reduce ferric iron to the ferrous form:

\[2\text{H}^{+} + 2\text{O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2\]
\[\text{Fe}^{3+} + \text{O}_2 \rightarrow \text{Fe}^{2+} + \text{O}_2\]

Ferrous ion promotes decomposition of hydrogen peroxide to hydroxyl radicals by the Fenton reaction:

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^{-} + \cdot\text{OH}
\]

The hydroxyl radical is highly reactive and may rapidly generate lipid free radicals by abstracting hydrogen atoms from unsaturated fatty acids. This initiates the lipid peroxidation chain reaction.

Iron can also catalyze lipid peroxidation by accelerating decomposition of lipid hydroperoxides present in foods:

\[
\text{Fe}^{2+} + \text{LOOH} \rightarrow \text{Fe}^{3+} + \text{LO} + \cdot\text{OH}
\]

or

\[
\text{Fe}^{3+} + \text{LOOH} \rightarrow \text{Fe}^{2+} + \text{LOO} + \cdot\text{H}
\]

The rate of the first reaction is greater than the second by an order of magnitude. This explains why ascorbic acid may function as a prooxidant in some food systems since it can reduce ferric iron to the ferrous form.

**9.5.4 Nickel**

While nickel deficiency has never been documented in humans, there is substantial evidence of its essentiality in several animal species [14]. The primary significance of nickel from a food processing perspective is its use as a catalyst for the hydrogenation of edible oils [31] (see Chap. 5). The hydrogenation reaction takes place on the surface of the catalyst. Thus catalytic activity is a function of the surface area of the catalyst. For this reason, catalysts are prepared as a finely divided power of elemental Ni dispersed in a solid support. When the catalyst is mixed with the oil, and hydrogen gas is introduced, double bonds in the fatty acid residues in the triacylglycerol molecules attach to the surface of the Ni through a \(\pi\)-bonding interaction and the bond opens. H\(_2\) also binds to the Ni and the H-H bond is cleaved. Thereafter, several possibilities exist (Fig. 11): (a) transfer of two hydrogens form the Ni to carbons on the fatty acid and subsequent dissociation of the fatty acid from the Ni leaving a saturated C-C bond; (b) dissociation of the fatty acid from the Ni before transfer of hydrogens, leaving the original CIS-unsaturated species; (c) rotation around the C-C bond on the carbon attached to the catalyst followed by dissociation from the catalyst before transfer of hydrogens, leaving a \textit{trans} double bond; or (d) transfer of hydrogens to the double bond followed by removal of different hydrogens.
before the fatty acid disassociates, leaving a double bond (either cis or trans) shifted one position. Thus hydrogenation results in a mixture of products.

The situation is even more complicated when the fatty acid contains two or more double bonds. This is because methylene hydrogens are labile and may be easily removed by the catalyst. Rearrangement of electrons then results in conjugation of double bonds, and subsequent hydrogenation of one of the double bonds yields a mixture of cis and trans isomers in different positions in the molecule.

The physical and nutritional properties of the hydrogenated oil are determined by the degree of saturation, the location of the double bonds, and the fraction of trans double bonds. Thus, it is important to adjust operating conditions (mainly hydrogen pressure and temperature) to achieve a desirable final product [2].

9.5.5 Copper

Copper, like iron, is a transition element and exists in foods in two oxidation states Cu$^{1+}$ and Cu$^{2+}$. It is a cofactor in many enzymes including phenolase and is at the active center of hemocyanin, an oxygen carrying protein in some arthropods. Both Cu$^{1+}$ and Cu$^{2+}$ bind tightly to organic molecules and thus exist primarily as complexes and chelates in foods. On the negative side, copper is a potent catalyst of lipid oxidation in foods.

An intriguing functional role of copper has been exploited in Western cuisine for at least 300 years [26]. Many recipes for meringues specify copper bowls as the preferred vessel for whipping egg whites. A common problem with egg white foams is collapse resulting from overwhipping. Presumably, foam stability is reduced when the proteins at the air-liquid interface
are excessively denatured by whipping. Egg white contains conalbumin, a protein analogous to the plasma iron-binding protein transferrin. Conalbumin binds Cu\(^{2+}\) as well as Fe\(^{3+}\), and the presence of bound copper or iron stabilizes conalbumin against excessive denaturation [33].

9.6 Summary

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. These species undergo complex changes during processing, storage, and digestion of foods. With the exception of group IA and VIIA elements, minerals exist in foods as complexes, chelates, or oxyanions. While understanding of the chemical forms and properties of many of these mineral species remains limited, their behavior in foods often can be predicted by applying principles of inorganic, organic, physical, and biological chemistry.

The primary role of minerals in foods is to provide a reliable source of essential nutrients in a balanced and bioavailable form. In cases where concentrations and/or bioavailabilities in the food supply are low, fortification has been used to help assure adequate intakes by all members of the population. Fortification with iron and iodine has dramatically reduced deficiency diseases associated with these nutrients in the United States and other industrialized countries. Unfortunately, it has not been possible to fortify appropriate staple foods in many developing countries, leaving hundreds of millions of people in these countries to suffer the tragic consequences of iron and/or iodine deficiency.

Minerals also play key functional roles in foods. For example, minerals may dramatically alter the color, texture, flavor, and stability of foods. Thus minerals may be added to or removed from foods to achieve a particular functional effect. When manipulation of concentrations of minerals in foods is not practical, chelating agents such as EDTA (when allowed) can be used to alter their behavior.

Bibliography


References


