13 Non-Fickian Mass Transfer in Fruit Tissue

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Abstract

Many fruit processing unit operations involve the application of heat or immersion of the fruit in different types of solutions, causing significant mass transfer of various food components as well as of exogen solutes. The mass transfer process may have important consequences on food texture, nutritional value, and quality in general. Application of minimal processing to obtain convenience fresh-like foods with extended shelf life is gaining interest worldwide, and this type of process often involves mass transfer in living tissues. A better understanding of mass transfer phenomena is crucial for controlling and optimizing food processing. The increasing awareness of this importance is revealed by the large amount of research in this field in recent years. The current state of the art is still dominated by the Fickian approach (implying use of lumped effective diffusion coefficients).
However, transport in living tissues requires basic knowledge of biology, as the cell structure plays a major role in the transport mechanisms. On the other hand, evidence supplied from the food-processing area shows changes in the pattern of mass transfer in some species with temperature and environmental conditions that may be intimately related to phenomena at the cell level. Therefore, the function, the mechanisms of transport of the biological membrane, and its fate during various processing conditions are most important for the food process engineer. This review covers cases of non-Fickian behavior, including convection phenomena due to turgor and buoyancy, and agitation effects, passive membrane transport, and active membrane transport. The possibility of application of the thermodynamic approach as a basis for mass transfer modeling when mechanisms other than Fickian diffusion are present is covered as well.

13.1 INTRODUCTION

Food processing implies a wide spectrum of operating temperatures, from freezing up to cooking and frying temperatures, and sometimes extreme conditions of other environmental factors such as ambient humidity, osmotic pressure, or water chemical potential, which are quite different from physiological conditions. These conditions induce mass transfer of water and other components that is quite important in food-processing unit operations such as drying, blanching, osmotic treatment (dehydration or impregnation), cooking, and frying, because of its influence on texture and overall quality. The material is divided into three parts concerning the effects of low-, intermediate-, and high-temperature conditions, respectively.

Mass transfer elucidation, in general, requires the knowledge of structure aspects and driving force aspects. Before addressing the processing aspects, we anticipate two parts on structure and driving force, starting with cell walls, which are the first internal constraints of the system, hindering the free diffusion of certain components—namely, macromolecules. Living cell membranes present special mechanisms of mediated and active transport that enable the metabolic activities of the cells and of the whole plant organism. A number of textbooks cover these subjects thoroughly; therefore, in the first part of this review, we intend to provide only an engineering-oriented short description. We also propose a classification scheme for transport processes to clarify terminology that sometimes leads to confusion.

We discuss the driving force aspects following the thermodynamic approach as being the most plausibly fundamental. In the thermodynamic ensembles of Gibbs and Callen, the very important concept of chemical potential is derived from either $G$, the free enthalpy function, or from $S$, the entropy function. The Gibbs ensemble, using measurable parameters such as temperature $T$, pressure $P$, and number of moles $n_i$ of any component $i$ to be the thermodynamic coordinates, leads to the definition of measurable thermodynamic quantities, e.g., of the chemical potential. The entropy ensemble, taking extensive properties as thermodynamic coordinates, leads to the deduction of all important driving forces and provides the basis for the theory of irreversible thermodynamics. The coupling of fluxes and driving forces could poten-

13.2 STRUCTURE ASPECTS

13.2.1 THE CELL WALL AS AN ULTRAFILTRATION MEMBRANE

From the mass transfer point of view, the cell wall behaves as an ultrafiltration (UF) membrane that hinders the entrance of foreign particulate matter, viruses, etc., into the cell. It also controls the transport of macromolecules, such as proteins, from and to the cell. Like a UF membrane, the cell wall allows water, sugars, and salts to pass freely, while macromolecules are hindered, except in the case of the symplastic mode of direct cell-to-cell transfer of macromolecules through the plasmodesmata. In the latter case, common in mass transfer from a mother cell to the daughter cells, the hindering effect of the cellular membranes is also absent. The cell wall, therefore, acts as a UF membrane in the apoplastic mode, i.e., cell wall to cell wall, eventually involving the plasmalemma membrane, with the singularity of symplastic transport of macromolecules through the plasmodesmata. Plasmodesmata are tortuous channels with a diameter of 30–60 nm, lined by plasma membrane at the periphery, and containing the desmotubule (tubular plasma membrane). Cell walls typically have 1–15 of these channels per square micrometer.

As with any UF membrane, data and properties of interest are thickness, constituting material (charge and hydrophilic/hydrophobic properties), pore size, and cutoff. Thickness is basically determined by the primary wall that ranges from 100–1000 nm, because the other two parts of the intermediate lamella (less than 30 nm) and the second wall (which is found in specialized cells only, with the main function of cell type recognition) are much thinner. Because of the cellulose nature of the constituting material, cell walls are normally hydrophilic, but they may become hydrophobic in some cases (lignification, encrustation on the epidermis/endodermis). Lignification may also affect (reduce) water and dissolved solute permeability. The (net) charge of the cell wall is negative, due to uronic acid residues of pectin and xylans.

The pore size (3.5–5.5 nm) is of the same order of magnitude of typical pore sizes of a “good,” i.e., sharp, cutoff UF membrane with cutoff values between 10 and 50 kDa, depending on the size and shape of the macromolecule. Similar values are shown by synthetic polymeric or inorganic UF membranes. Using the concept of hydrodynamic volume value for polymers, however, it is possible to obtain a unique cutoff value for macromolecules such as dextrans, PEGs, and proteins. For the sake of simplicity, some cells specialized in secreting larger macromolecules are not considered in this review. Our main interest is “normal” cells of the parenchymatic tissue, like the ones in potato tubers or in fruits such as apples.

Mass transfer modeling takes into account both the structure of the medium across which the mass transfer occurs and the applied driving force. In commercial ultrafiltration or microfiltration membranes, the applied driving force is a pressure difference. However, in almost all cases, secondary concentration differences also
play an important role, and the chemical potential of the component studied becomes the driving force for the transfer of that component. It is known that, in the extended form, the chemical potential includes both pressure and concentration gradients, and even electrical potential gradients, in the case of ions or charged molecules (known in the last case also as electrochemical potential). This is the universal driving force for mass transfer and is also valid in the living cell, except in the case of active transport.

Because the cell wall acts as a UF membrane separating macromolecules that are hindered from passing and microsolute (ions, sugars, organic acids) that move freely both to and from the cell, the resistance of the cell wall to the transport of small solutes is negligible when compared with cell membrane transport. An exception occurs when specific interactions are established between a small solute and the cell wall (as, for example, calcium, participating in reactions with pectin molecules in the middle lamella). On the other hand, macromolecules represent the other extreme case of infinite cell wall resistance (or zero permeability). Although the cutoff values reported for the cell wall indicate the possibility of movement of macromolecules with low molecular mass, we will not consider these cases, as they touch applications outside the interest of this review. Water should also be mentioned, as it plays an interesting role due to the matric potential created by bound water in the cell wall.

13.2.2 Cell Membranes

Description

Every living cell is surrounded by a cell membrane, the plasma membrane, which defines the periphery of the cell, separating its content from its surroundings. Even organelles inside of the cell are surrounded by membranes. The membranes are composed of a bilayer of polar lipids, mostly phospholipids, but also include other components such as cholesterol and triacyl glycerol. Both the internal and the external surfaces of a membrane are hydrophilic (because of the polar phospholipid heads). The fatty acyl chains in the interior of the membrane form a fluid, hydrophobic region. Integral membrane proteins float in this sea of lipid, held by hydrophobic interactions in their nonpolar amino acid side chains. Both proteins and lipids are free to move laterally in the plane of the bilayer. In a plant cell, there is also a vacuole, which is surrounded by a membrane, the tonoplast. The vacuole represents, especially in mature cells, a big part of the cell volume, sometimes as much as 90%. It contains ions, metabolites, and digestive enzymes that degrade and recycle macromolecular components no longer useful to the cell. Because of the high solute concentration in the vacuole, water passes into it by osmosis. This mechanism creates a pressure on the cytosol and on the cell wall. This turgor pressure within the cells stiffens the plant tissue, so the vacuole provides a physical support to the plant cell.

Data reported on turgor pressure (estimated or modeled values) for roots, in general, falls in the range of 0.432–0.534 MPa; barley roots (measured values) show a narrower range of 0.31–0.50 MPa. In general, 0.5 MPa or 5 bar seems to be a typical order of magnitude for full turgor values. Several procedures may be used to measure tissue osmotic pressure, such as pressure volume (PV) method (theoretically more sound, but time consuming), methods based on sap expression (rapid but with errors due to cell disruption), and other methods based on measurements of water chemical potential.

Classification of Transport Mechanisms

Some proteins are constructed as channels for ion transport. Their amino acid sequences span the membrane several times, forming a transmembrane channel. The basal permeability, i.e., the permeability of the phospholipidic phase, is usually expressed in the same units as synthetic polymeric or inorganic membranes, i.e., as fluxes divided by the driving force. On the other hand, the permeability of channels is given as conductance or as numbers of ions transported through a channel per second. The same format is used for mediating protein transporters, both passive and active. Active transport uses energy to transport species against the electrochemical potential gradient. Most primary active transporters use ATP, but some use light or substrate oxidation. Secondary transport occurs when uphill transport of one solute is coupled to the downhill flow of a different solute (the lactose symporter from Escherichia coli is a typical example). This transporter utilizes the proton electrochemical gradient generated by the respiratory electron transport chain to drive the uptake of lactose into the cell. In this case, two different solutes (protons and lactose) are simultaneously transferred across the membrane; therefore, the term symporter is applied. Antiporters, on the other hand, couple the transport of solutes in opposite directions, for example, the Band 3 from erythrocyte, responsible for the transport of Cl– and HCO3– in opposite directions across the red blood cell membrane. Permeases, translocases, and carriers are other terms found in the literature applied to protein transporters other than primary active transporters. Engineers should also have in mind the terms high-affinity and low-affinity transporters. This level of affinity is quantified by the Km values and may be described in analogy to enzymes, as the affinity of the protein transporters to the substrates they transport.

Water passes through channels and pores in the membrane and also diffuses, to some extent, through the phospholipidic phase (simple diffusion). Some dissolved gases (e.g., O2, N2, and CH4) are transported by simple diffusion through the phospholipidic phase. This transport could be explained by the hydrophobic nature of the phospholipidic phase, once gas solubilization is favored in this phase. However, for the transport of oxygen, mediated transport has been reported as well. Some small organic molecules, e.g., urea, and some organic acids (e.g., acetic acid, lactic acid, salicylic acid) also pass through the phospholipidic phase. Amino acids and sugars are transported by active secondary symport transport in the intestine by coupling to the flow of sodium. In bacteria, lactose is transported in the same way, using protons instead of sodium. Glucose enters the erythrocyte by facilitated diffusion via a specific glucose permease, which allows glucose to enter into the cell at a rate about 50,000 times greater than its unaided diffusion through a lipid layer.
Glucose also has been found to be transported through facilitated transport in plant cells. Chloride (Cl⁻) and bicarbonate (HCO₃⁻) are cotransported across the erythrocyte membrane in an antipor processes. The chloride-bicarbonate exchanger increases the permeability of the membrane to bicarbonate by a factor of more than a million. Calcium (Ca²⁺) is transported into the cells in vertebrate animals to keep the calcium concentration low inside of the cell. This is an antipor process with three sodium ions for each calcium ion, and it uses the transmembrane Na⁺ gradient. The free Ca²⁺ of the cytoplasm in animal cells has been reported to be very low, between 10⁻⁶ and 10⁻⁸ M depending on the cell type. This low level of free Ca²⁺ has to be maintained against a Ca²⁺ concentration in the extracellular fluid, which is a few orders of magnitude higher.

Studies with intact red blood cells have shown that Ca²⁺ is expelled from the cytoplasm by an active energy consuming transport mechanism through the plasma membrane. Similar experiments with plant cells (tobacco protoplasts, carrot cells, pea epicotyls, onion roots) have provided evidence that, similar to animal cells, plant cells maintain a low cytoplasmic concentration of free Ca²⁺, mainly by an active efflux mechanism in the plasma membrane. Intracellular organelles and compartments also take part in the regulation of the cytoplasmic concentration of free Ca²⁺. Potassium is the most abundant cation, in higher plants, and is crucial for Nutrition growth tropism and osmoregulation. K⁺ accumulation can be rate limiting for agricultural production. K⁺ is also a key factor for nutrition value of foods. Whereas Ca²⁺ is maintained at low concentrations in the cytoplasm, the uptake of K⁺ by the cell guarantees high intracellular concentrations. This implies active transport, as first studied in animal cells. Potassium (K⁺) is transported into cells of animals by an active antipor cotransport with sodium (Na⁺). The enzyme Na⁺K⁺ATPase couples breakdown of one ATP to the simultaneous movement of both three Na⁺ and two K⁺ ions, against their concentration gradients. This mechanism creates a higher potassium concentration inside the cell.

The cell's membrane potential, the difference between the electrical potential inside and outside the cell, is partly determined by the Na⁺K⁺ATPase and potassium channels. These channels regulate a wide range of functions, including salt and water flow from kidney cells and guard cells, insulin release from pancreatic β-cells, electrical excitability and synaptic plasticity in neurons, and even the rate at which the heart beats. Potassium channels of known sequence fall into two distinctly related families, the voltage-gated and the so-called inwardly rectifying channels. These two classes of potassium channels share the same basic pore design. The activity of voltage-gated channels, which have six putative membrane-spanning domains in each subunit, is insensitive to changes in the membrane potential. These channels are typically activated at membrane potentials above the resting potential (the potential that causes no net flow of ions across the membrane, typically around ~60 mV). In animals, this is slightly above the potassium equilibrium potential, while in plants, it is slightly below it. Inwardly rectifying potassium channels, by contrast, have only two putative transmembrane spanning domains in each subunit and are sensitive to changes in the potassium concentration. They are effectively unidirectional, as they allow a much larger potassium influx than efflux, allowing them to control the resting potential without causing massive potassium loss. All potassium channels are at least 100 times more permeable to potassium ions than to sodium ions and allow a flux greater than a million potassium ions per second. Because sodium has a smaller radius than potassium, it is unlikely that selectivity is achieved by physical occlusion (sieving effect). Other features, such as multiple binding sites arranged in a single file along each pore channel, may explain this selectivity. K⁺ channels were first studied in animal cellular tissues, but evidence of the existence of such channels in plants has also been reported. Analogies between the mechanisms of animal and plant K⁺ channels have also been found. For example, it was shown that the blockade of the K⁺ channel of Chara contraria by Cs⁺ and tetraethylammonium resembles that of K⁺ channels in animal cells. In a recent publication concerning potassium uptake by roots of higher plants, it was found that the transport mechanism was based on a K⁺−H⁺ cotransporter. This way, when a plant is deprived of potassium, a high-affinity K⁺ uptake takes place in the roots. Sodium (Na⁺) transport is important for halophytic plants. Na⁺ transport was studied in the marine euryhaline alga, Enteromorpha intestinalis in seawater (465 mM Na⁺) and in low salinity medium [Artificial Cape Bank Spring Water (ACBSW), 25.5 mM Cl⁻, 20.4 mM Na⁺, 0.5 mM K⁺]. Most of the Na⁺ of the Enteromorpha tissue was bound to the fixed negative charges of the cell wall, and this binding has, in previous studies, led to great overestimates of the intracellular Na⁺ of this plant. The Na⁺ flux in Enteromorpha plants in seawater was about 3 nmol m⁻²s⁻¹ and in low-salinity plants was about 0.2 nmol m⁻²s⁻¹. Sodium in Enteromorpha is far from electrochemical equilibrium (more than ~100 mV) in plants in both seawater and ACBSW medium so that Na⁺ is actively excluded from the cells. The plasmalemma has a very low Na⁺ permeability (seawater, 3 pm s⁻¹; ACBSW plants, either 3 or 100 pm s⁻¹, depending on the compartmentation model used).

Typical Transport Rates

Typical transport rates are found in textbooks, such as Reference 7, both for basal and specialized permeability. While basal permeability is expressed in units of fluxes divided by molar concentrations in ms⁻¹, specialized permeabilities are expressed, in literature, in number of ions or molecules transported per unit time and per transporter. To enable comparison, the units of basal permeabilities should be expressed in numbers of transported molecules or ions by dividing the former units by membrane surface for a typical apple plasmalemma cell membrane. In the case of ion transport, the permeabilities are sometimes expressed in conductance. Significant differences are apparent in the permeabilities of different species between the two main categories (basal and special) but also within each category. For the components allowed to pass the phospholipidic phase, the permeabilities are comparable to values of hindered diffusion in solids. The basal permeability of the ions and the sugars is two to three orders of magnitude lower than water or urea; that is, the phospholipidic phase can be considered almost impermeable to ions and sugars. Ion permeabilities in channels demonstrate comparable transport rates as water and small organic compounds in the phospholipidic phase (ca 10⁶ ions per second per
transporter). It should not be forgotten, however, that channel transport, as happens with all forms of protein-mediated transport, occurs only at fractions of time when the channels are open, a parameter that is difficult to know in practice. It is especially difficult to know this parameter for a plant tissue after harvesting and under processing conditions. Therefore, a strict comparison between the two types of permeability is not possible. A comparison within the special permeability between the transport rates of channels and transporters that change their conformation shows differences of many orders of magnitude (100–1000 molecules per second per transporter), the active transport rates being by far the slower ones (ca 30 per second per transporter).

13.3 DRIVING FORCE ASPECTS

13.3.1 CONCENTRATION APPROACHES: MICHAELIS–MENTEN VS. FICKIAN

Physiologists have modeled mediated (channeled, facilitated, and active) transport with regard to the influence of the concentration on the rate of transport. In these kinetic studies, it was shown that the protein transporter or carrier follows a typical Michaelis–Menten behavior similar to enzymatically catalyzed reactions. The concentration of the transferred component (substrate) is noted by \( S \), and the flux, known as transport velocity, by \( V \). \( V_{\text{max}} \) and \( K_m \) have analogous meanings. In this context, the maximum transport rate and \( K_m \) the concentration of the transferred component when \( V \) equals \( V_{\text{max}}/2 \). The kinetics of mediated transport may be described by

\[
V = \frac{V_{\text{max}} S}{S + K_m}
\]  

(13.1)

and, if competitive inhibition occurs, by

\[
V = \frac{V_{\text{max}} S}{S + K_m(1 + I K_i)}
\]  

(13.2)

where \( I \) and \( K_i \) stand for the concentration and the inhibition constant of the inhibitor.

Using similar terminology, simple Fickian diffusion may be described by

\[
V = K_o S
\]  

(13.3)

where \( K_o \) is a mass transfer coefficient corresponding to the ratio between the diffusion coefficient \( D \) and the membrane thickness \( L \). Equations (13.1) through (13.3) imply steady state conditions. For steady state simultaneous diffusion and mediated transport (without inhibition), the kinetics equation becomes

\[
V = \frac{V_{\text{max}} S}{S + K_m} + K_o S
\]  

(13.4)

if uptake is considered through a cell membrane, as, for example, uptake of water through both the phospholipidic phase and through a protein carrier. Transport away from the cell could be similarly considered, and generalization of Equation (13.4) to this situation is straightforward. Low \( K_m \) values mean a high affinity of the protein transporter for the component being transported (the substrate), while high \( K_m \) values imply the opposite (low-affinity transport). When considering unsteady coupled diffusional and mediated transport, modeling may be based on the generalized transport equation using a source term, \( K \), to account for the flux due to the mediated transport, in a way analogous to the approach followed for enzymatic reactions.3

13.3.2 IRREVERSIBLE THERMODYNAMICS

Wherever a membrane is present in a system, trials to model water and solute transport using a single diffusivity or permeability coefficient for each species have proved to be inadequate, because the coupling between fluxes and driving forces previewed by irreversible thermodynamics was not considered. There is an interactive effect of the driving forces for water and solutes on their movement. Thermodynamic driving forces are based on chemical potentials rather than on concentrations, thus including both composition forces (activities or concentrations) and pressure or even voltage potentials. Based on thermodynamic driving forces, the transport equations for biological membranes were developed for a typical system containing a solvent (water) and a solute. The driving force was expressed in terms of chemical potentials of water and the solute, and using the entropy production theorem of irreversible thermodynamics (IT) and the Onsagers law of reciprocity, the following equations and inequalities for the volumetric flux, \( J_v \) (conjugate to a pressure-driving force) and the diffusive flux, \( J_D \), conjugate to the osmotic pressure or composition driving force, were proposed.

\[
J_v = L_p \Delta P + L_{pD} \Delta \Pi
\]

\[
J_D = L_{DP} \Delta P + L_D \Delta \Pi
\]  

(13.5)

For the phenomenological coefficients, entropy considerations require that

\[
L_p > 0, \quad L_D > 0 \quad \text{and} \quad L_p L_D > L_{pD}^2
\]

(The cross-phenomenological coefficients \( L_{pD} \) and \( L_{DP} \) are equal, by Onsagers law.) After a number of simplifications for dilute solutions, i.e., use of concentration instead of activity in the composition term, neglecting the solute contribution in the volumetric flux and using an average concentration \( C_m \) within the membrane, the two transport equations yield the following equations:
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include the mediated transport in the IT approach? In general, as stated above, one possibility might be to use the generalized transport equation (from the second law of Fick) and include the mediated transport rate in the source term as if it were a reaction term.

Using the IT approach, the action of the protein carrier or transporter could be coupled to the transport of the solute. Acting driving forces should be the driving force of diffusion, which is the chemical potential of the solute and the affinity, $A$, of the protein carrier’s reaction.

Let us define affinity. In the entropy ensemble, Prigogine gives the following expression for the entropy production term, per unit time, $\dot{s}$ for a system into which heat transfer, mass transfer, and chemical reaction occur:

$$ s = 1/T \cdot d\Phi/dt + \mu_i/T \cdot dn_i/dt + A/T \cdot d\xi/dt $$

In this equation, the generalized driving forces are $1/T$ for heat transfer, $\mu_i/T$ for mass transfer, and $A/T$ for chemical reaction, where $T$, $\mu_i$, and $A$ stand for temperature, chemical potential of a given component $i$, and chemical affinity, respectively. The fluxes are $d\Phi/dt$ for heat transfer, $dn_i/dt$ for mass transfer (in number of moles of the component $i$), and $V = d\xi/dt$ for the chemical reaction, $\xi$ being the extent of the reaction. The affinity $A$ of a reaction in general is related to the stoichiometric coefficients and the chemical potentials of the reaction components as follows:

$$ A = \sum v_i \mu_i $$

Ideas also exist for coping with active transport, where coupling with the reaction of ATP hydrolysis could be considered. The solute’s flux against its own chemical potential gradient can be understood as an effect of major affinity (the driving force of ATP reaction) on that flux rather than the effect of the conjugative driving force (chemical potential of the solute). Mathematically, this implies a negative cross-coefficient combined with high affinity values, which can easily be fulfilled in those cases.

### 13.4 EFFECTS OF PROCESSING CONDITIONS

#### 13.4.1 Low-Temperature Effects

The food engineer interested in low-temperature processes (cooling, freezing, thawing) can take advantage of literature data on responses of plant cells (cell walls and cell membranes) to low temperatures. Plant physiologists have studied those effects during cold acclimation studies of plants cultivated in cold climates and frozen environments. Data on effects of low-temperature conditions on the integrity of cell membranes function is of great interest. Physical disruption of tissue during freezing...
is often attributed to ice crystal formation, but this mechanism is insufficient to explain the wide, observed variations in the response of different species to chilling or freezing. Other mechanisms suggested to explain these differences are disulphide bond formation between membrane-bound protein molecules (upon thawing, those bonds remain intact, affecting protein mobility and activity);\(^{19,20}\) phase transition;\(^{21,22}\) this suggests a primary (reversible) event associated with a threshold temperature, and secondary effects (such as increase of membrane permeability as a result of membrane protein dysfunction or overall membrane dysfunction) that may follow until general cellular degradation; and, although there is no full agreement between physiologists, the importance of membrane phospholipid composition (which can explain acclimation phenomena: certain plants show the ability to fight chilling injury by inducing alterations in fatty acids—the more the latter become unsaturated, the more successful the cold hardening). Membrane composition alteration is also known as membrane turnover.

Susceptibility to chilling injury implies an increased risk to freezing injury. Both in freezing and in chilling, membrane integrity is lost, and the metabolic activity is altered, and it has been suggested that the injury mechanisms are the same for the two cases. Nevertheless, important differences are noticeable and should be stressed. Both the electrolytic leakage and the electrical impedance methods have been reported to be applicable for detection of average membrane damage in complex tissues, whereas DTA can be applied to evaluate major and minor freezing points of water. Work with juvenile and mature apple trees (cv. Spur Mac) has shown that the normalized electrical impedance test appears to measure changes in the cell wall fluids within the tissues, while electrolytic determination is based on average changes of the diffusion-limited effect of electrolytes. These differences between the tests' basic principles appear to account for the lack of a complete linear fit between Znf and electrolytic leakage. Znf measurements were found to predict hardness in juvenile and mature apple trees throughout the winter period.\(^{23}\)

Chilling has different effects, depending on light conditions. Under dark conditions, ion leakage indicates that the plasmalemma membrane plays a major role, as well as the tonoplast and mitochondrial membranes. This crucial importance of the plasmalemma is explained by its function as ion transporter and its role in keeping cell wall integrity. Light induces restricting photosynthetic ability, and, in this case, plastids are the sites of major importance. The plasmalemma has been considered as the primary site of freezing injury, the decisive step being freeze-induced water transport from the interior of the cell to the intercellular spaces. The cell membrane regulates the level of supercooling of the cytoplasm by controlling the water flux through an alteration of membrane permeability, although details of this mechanism are not yet fully understood. This prevents ice formation from extending from intercellular to intracellular spaces. Maintenance of membrane integrity upon thawing is critical for the survival of the cell. The altered water status at the membrane interface, due to dehydration, is said to be of particular interest in freezing injury. Compositional changes, phase transitions, lipid peroxidation within plant membranes, and their effects on membrane fluidity have also been considered important. Needs for further studies are based on the following:

1. Similarities and differences between chilling and freezing injury must be further explored.
2. A single mechanism is insufficient to account for all low-temperature and acclimation responses, where subtle rather than dramatic changes may play an important role. At the cell membrane level, lipid-protein interactions and interfacial phenomena need more investigation.
3. Biophysical studies must go beyond phase transitions and fluidity. Diffusion and mass transport related aspects, for example, how cell membrane permeability is affected by temperature, constitute challenges for future studies. Ion transport is of particular importance—the role of Ca\(^{2+}\) on cold acclimation mechanisms involving the cell membrane is likely to be quite relevant.
4. Although focusing on the cell membrane is important, we believe that the role of the cell wall has been almost totally neglected. The cell wall constitutes the first barrier of the cell to the extracellular environment, both mechanically and functionally. It is possible, for example, that freezing injury starts with ice nucleation in the intercellular capillaries, and this may cause mechanical damage to the plant cell walls. Those could serve as additional nucleation sites for both extracellular and intracellular ice formation. Furthermore, damage of the cell walls would leave the cell membrane unprotected, with accumulation of various species on its surface as a consequence. This could accelerate cell membrane responses and deteriorative mechanisms.

13.4.2 Intermediate-Temperature Effects

Between low-temperature and high-temperature conditions, there is a wide spectrum of the quasi-ambient temperature conditions, common in postharvest, minimal processing, storage, and distribution. Minimal processing involves a large number of different operations such as grading, cutting, mild heat treatments, etc. Minimally processed products are living, damaged tissues that require refrigeration and adequate packaging. Therefore, understanding the involved phenomena at the cell level is most important in this case. The boundary between minimal processing and conventional processing is not very clear, and some processes, such as osmotic dehydration, lie exactly in this boundary. Osmotically treated products do not show a fresh-like appearance, but the final product has a water activity well within the range of minimally processed products, and processing temperatures are below the critical temperature at which membrane protein denaturation occurs (this temperature will be further referred to as \(T_p\)). Nonthermal processing methods such as high pressure, electrical or magnetic fields, ohmic heating, microwaves, etc., are also milder treatments, although already out of the range of minimal processing. These applications are at an early development stage, and their effect on cell structure has only been incipiently studied, although there is evidence that they increase cell membrane permeability.\(^{24-25}\)
Effects on Cell Walls

Cell walls are susceptible to minimal processing unit operations such as cutting, sizing, slicing, or grinding. The consequences are that cut cells will show less resistance to oxidative browning and to the entrance of bacteria as compared with intact cells. The effects of divalent ions on the cell wall strengthening (through the interaction with the pectin methyl esterase -PME- enzyme action and pectin molecule bridging) is well documented, not only for low and ambient temperature conditions but also in blanching and osmotic dehydration.23,26-28

The effects of divalent cations on the response of apple fruit tissue to accelerated aging, temperature, or osmotic stress have been evaluated29 by measuring ethylene evolution, electrolyte leakage, and membrane microviscosity. Apple tissue slices, incubated in an isotonic sorbitol solution at 25°C for 24 h, underwent rapid aging, as expressed by a sharp drop in ethylene production (70–90%) and leakage of potassium from the tissue. At a higher temperature and in hypotonic medium, these symptoms were accelerated and enhanced. Addition of Ca²⁺ to the isotonic incubation medium inhibited ethylene production during the first 6 h, but thereafter partially prevented the drop in ethylene production that took place in the aged tissue slices and completely prevented K⁺ leakage beyond 4 h of incubation. The influence of Ca²⁺ on the physical state of the membranes was observed by an increase in membrane microviscosity relative to control, as determined by the fluorescence depolarization technique. Using fluorescent probes that bind to different sites in the membrane, it was found that the effect of Ca²⁺ was more pronounced at the membrane surface and diminished toward the hydrophobic region of the membrane bilayer. The effect of Mg²⁺ on ethylene production and membrane microviscosity was similar though weaker. However, membrane permeability, as expressed by K⁺ leakage, was unaffected by Mg²⁺ at similar concentrations. Ca²⁺ is suggested to inhibit stress-induced senescence by maintaining membrane integrity.

Glenn et al. investigated the effect of Ca²⁺ on various parameters of apple fruit senescence. Distinct and specific changes in polypeptide and phosphoprotein patterns were observed in Ca²⁺-treated fruit, when compared to control fruits. Transmission electron micrographs of the cell showed Ca²⁺ to be effective in maintaining the cell wall structure, particularly the middle lamella. Furthermore, increase in fruit Ca²⁺ reduced CO₂ and C₃H₄ evolution and altered chlorophyll content, ascorbic acid level and hydraulic permeability.27

Postharvest calcium treatment is, however, sometimes insufficient to accomplish cold storage preservation. Peaches harvested in mature-green stage were dipped in a CaCl₂ solution at 40°C for 2.5 min and wrapped in PVC film of 15 μm thickness and placed in constant cold storage (0°C and RH 90–95%). Half of the samples were then submitted to intermittent warming for 48 h at ambient temperature (21°C). In spite of the high level of calcium, the cold-stored apples showed ripening and cold injury symptoms. Intermittent warming to ambient temperature gave more satisfactory results.28

Other effects related to cell walls are linked to the enzymatic hydrolysis of cell wall components caused by slicing. Pectinolytic and proteolytic enzymes liberated from cells damaged by slicing could diffuse into inner tissues. High migration rates of macromolecules through, for example, kiwi fruit tissue, determined with labeled enzymes, were observed (penetration rate, 1 mm/h). Compared with normal maturation conditions, a difference in cell wall hydrolysis was noticed, proteopectin solubilization predominating in the former case.

Effects on Cell Membranes

When heating or treating a plant cellular tissue with osmotic solutions, loss of membrane functions, including changes in transport and permeability characteristics of membranes, may occur. In both cases (heating, immersion in osmotic solutions) the role of divalent ions such as Ca²⁺ on the strengthening of the structure has been identified.20,27,28

Millimolar concentrations of extracellular Ca²⁺ have been reported to be necessary for proper membrane function and to protect the cell against adverse conditions of low pH, toxic ions, and nutrient imbalance. Ca²⁺ may influence membrane structure due to its ability to induce asymmetrical distribution of negative phospholipids in membrane bilayers and alter membrane fluidity. Ca²⁺ has been found to be effective in preventing senescence-related increases in membrane microviscosity.25 Under modified atmosphere packaging (MAP) conditions, the transport rates of the respiratory (or respiration-related) gases O₂ and CO₂ are very important. Slicing of tuber or root tissue (for example, potato) could invoke an immediate two- to fourfold increase in respiration.

Taking into account that gas permeability in the cell is controlled by the cell membranes, slicing obviously causes damage at the cell membrane level. Study of the cell membrane structure changes with temperature could explain the anomalous Arrhenius patterns sometimes observed.23 In a number of tissues, low temperatures may induce a rise in respiration, probably due to phase transitions of the phospholipidic phase.26 A typical example is potato tubers, where storage at 1°C evokes a respiration rise above that observed at 10°C.21 Special care should, therefore, be taken when applying the Arrhenius equation to predict the respiration of a fresh product at a given temperature.

13.4.3 High-Temperature Effects

Physical Changes

Studies on biological membrane behavior are mainly done by physiologists under physiological conditions, and the high temperatures in physiologist’s language are restricted to the 35–45°C interval. Although this temperature range is important to the food engineer, knowledge of the behavior of cell membranes at temperatures higher than the denaturation temperature (T > Td) is of utmost importance, because many food-processing operations are carried out at these temperatures (canning, aseptic processing, drying, etc.). A few studies have been reported on the behavior of membranes at these temperatures, both by physiologists and engineers.
Paszewski and Spiewka, for instance, studied the changes in the electrical resistance of internodal giant cells of Characeae in a range of temperatures from 4 to 48°C. Cells of Characeae, because they are large, have often been used as models for plant cellular tissues. In the whole temperature range examined, the electrical resistance of the cell membranes underwent from five to nine distinct changes; the overall changes were approximately hyperbolic while, in certain narrow temperature ranges, they were nomenclature and, in others, linear. Arrhenius plots of these dependencies provided data that suggest a specific, stepwise change in permeability to ions, produced by temperature changes and probably connected with corresponding modifications of cell membrane fluidity. This seems to support the view that the cell membranes of the examined plant species may exist in different, discrete, physiological states, characteristic of certain temperature ranges. Values of the unitary resistance \( r_u \) (kΩ cm\(^2\)) were found in the region of 10–50 and 20–90 for two different species of Characeae; i.e., for Nitelopsis obtusa and Lychnothamnus barbatus, respectively.

The higher resistance values refer to 4°C and the lower ones to 48°C. In an earlier study, the cytoplasmic membranes of sugar beet and sugar parenchyma tissues fresh and also during heat treatment (78°C, 30 min) and mechanical stressing (pressures of 350.105 Pa, for cane only) were observed by electron microscopy. Whereas, in the intact cell membrane, the pores were 0.3 µm and round, after treatment, they became oval, creating channels doubled in size, of a diameter much greater than needed by sucrose and other sugar molecules to pass through. The interpretation given by the authors is disputable, because evidence suggests that the transport of sugar molecules in fresh, untreated tissues is protein mediated and thus not dependent on pore size. Nevertheless, their results show evident alterations suffered by cell membranes at processing temperatures and pressures. Concerning the effects of high temperatures on cell walls and the relation to texture properties of plant tissues, we have recently published a critical review focusing on potato. Cell walls are known to be thermal resistant. On heating, they may become thinner and mechanically weaker, but they can keep the cell structure even at cooking conditions.

**Determination of \( T_d \)**

There is considerable evidence of a discontinuity in the temperature dependence of mass transfer rates of various solutes from the cells of plant tissues (leaching) immersed in treatment solutions (brine, blanching, osmotic). Discontinuities are also sometimes found when solutes are transferred in the opposite direction, from the solution to the tissue (uptake). Thus, above a critical temperature \( T_d \), the apparent diffusivities seem to increase one and two orders of magnitude for solutes such as sugars (glucose, fructose) and ions (K\(^+\), Ca\(^{2+}\)), respectively, compared with the respective values at \( T < T_d \). Because the above solutes present a very low basal permeability and are transferred by mediated transport, this discontinuity clearly reflects an abrupt change of the integrity of the protein phase of the cell membranes that is responsible for the mediated transport.

For nonmediated solute transport but high basal permeability solutes (for example, acetic acid), experiments done at ESB, Porto, have shown that the temperature dependence of this solute in plant tissue was as smooth as in a gel; i.e., a medium lacking cell structure. In another work on batch pickling of carrots at temperatures below \( T_d \), increase of the apparent diffusivity of electrolytes with concentration may suggest clear evidence of the mediated transport by membrane proteins being intact. In general, discontinuities in the Arrhenius dependence of apparent diffusivities offer a diagnosis possibility for the detection of the transport mechanism of a given component, either leaching from or uptake from a plant tissue material in a given temperature interval.

In the case of potatoes, however, another phenomenon occurs at an interval of temperatures close to 55°C, namely the gelatinization of starch granules, making the determination of \( T_d \) more difficult. Until a collaborative study was undertaken by Bahia Blanca and Lund Universities, there was no way to differentiate the effects due to membrane denaturation or starch gelatinization. The aim of this study was twofold, to discriminate between the two mechanisms and to more accurately determine \( T_d \). Starch (potato) and nonstarch (apple) commodities were used, and the experiments were carried out in isotonic media to limit the transport to the solute only (in this case, potassium). In those experiments, the tissue was first preheated to a given temperature in the range of 25–75°C, and then the loss rate of the solute was measured at ambient temperature so as to avoid the interference of the Arrhenius temperature dependence of the mass transfer rates. The findings of this study were, in our opinion, very interesting. Under similar experimental conditions, apples and potatoes have both shown a sharp increase of the potassium loss from the tissue to the isotonic solution at temperatures between 45 and 50°C. The pattern of the sharp increase was, however, different for the two commodities tested.

A third interesting finding was that, for temperatures beyond 60°C, a slight decrease in the potassium loss rate, rather than an increase, was observed. A possible explanation of this behavior is related to interactions with the cell wall and the thermostability of the PME enzyme; phase transition of the cell membrane may also be associated, but more investigation is needed to clarify this point.

A fourth finding provides strong evidence that ATPase activity shows likewise patterns; i.e., with sharp changes, as above, in the temperature intervals, where the sharp changes in observed mass transfer rates occur.

**Modeling of \( T_d \)**

By far, the Fickian approach is the dominating basis for modeling mass transfer in food processing (unit operations such as blanching, drying, frying, etc.). This approach is based on the use of an overall, apparent or effective, diffusion coefficient.
that accounts for a number of important phenomena and anomalies, such as shrinkage, simultaneous heat transfer, concentration effects (reflecting the difference between activity of a component and its concentration), the anisotropic structure of the media, and sometimes even multicomponent effects. Application of irreversible thermodynamics (IT) in food processing is uncommon, and the Maxwell-Stefan's approach (well suited for multicomponent transport) is even more rare. To our knowledge, the only study based on Maxwell-Stefan's approach is a Ph.D. thesis concerning mass transfer in a model food ternary system composed of water, protein, and sugar. \[5, 44\]

The need for a more fundamental approach considering both true driving forces (activities or chemical potentials) and the cellular structure is being increasingly considered to be of great importance. This approach would allow better insight on the mechanisms of mass transfer at cell level and their relationship to food product quality.\[5-8, 35-36, 41, 53-56\]

To accomplish this objective, the food engineer needs a mathematical model that may be chosen from among the Fickian approach modified to include other effects such as source terms, multicomponent effects, and shrinkage; irreversible thermodynamics; Maxwell-Stefan's approach; and a physical model to simulate the plant or vegetable tissue cellular structure, both under physiological conditions, and its changes upon processing (cell size, intercellular spaces, shrinkage, cress formation in some cases acting as a dynamic membrane, etc.). Convection phenomena in relation to osmotic treatment of fruits and vegetables has been studied by the team of Prof. Fito, in Valencia, who has considered the so-called hydrodynamic mechanism in the mass transfer inside intercellular spaces. This mechanism is enhanced when vacuum conditions are applied to the tissue with a twofold aim: increasing the driving force per se between the intracellular phase and the outer phase through the increase of the pressure term of the chemical potential and increasing the driving force by replacing the air contained initially in the intercellular spaces by the osmotic solution, where the chemical potential of water is lower than it is in the air.\[55, 56\] The chemical potential as the driving force has also been considered for convective air drying\[53-54\] and also by Le Maguer et al. for osmotic dehydration.\[53-54\] Characterization of the osmotic solution in terms of its chemical potential for both water and solutes has also been studied.\[45\] The equation for the water chemical potential of the cell is given by

\[
\mu = \nu_s P + RT \ln a_s + V_{wm} \psi_w
\]

where \(P\) is the hydrostatic pressure (due to the turgor pressure) of the water solution in the vacuole, \(V_{wm}\) is the molar volume of water, \(a_s\) is the water activity corresponding to the effect of soluble solids in the cell, and \(\psi_w\) is the so-called matric potential term, corresponding to the effect of the insoluble solids on the water chemical potential.\[45\] If \(\mu_w\) the reference potential of the water in its pure state, is set equal to zero, and all the terms are divided by the molar volume, the units of each term of the chemical potential are those of pressure; the components of the water chemical potential are, therefore, the pressure potential \((\psi_P)\), the matric potential \((\psi_m)\), and the solute potential \((\psi_s)\). Attention should be paid to avoid confusion with the more rigorous term of thermodynamics solute chemical potential, which refers to the chemical potential of the solute, whereas the term solute potential as used in biology is the effect of the solutes on the water chemical potential. Then, the solute term is negative, because the presence of soluble solids in a solution lowers the water activity, and the pressure term is positive, because it increases the tendency of water to leave the vacuole, and it therefore increases the chemical potential. The matric term is also negative, but it can be neglected in many cases (for example, in the case of relatively high-porosity commodities such as apples), since its contribution to the total water potential becomes very small.\[5\]

Whereas, for the mathematical modeling, the food engineer has access to a reasonably large body of literature, information on how to proceed with the second problem, i.e., the cellular structure simulation, is scarce. Therefore, we shall focus the discussion in this section on cellular structure modeling. Starting from the simple Fickian approximation, a biphasic model of a porous medium, expressing the effective diffusivity as the diffusivity in an aqueous solution corrected for porosity and tortuosity, would be a clear improvement. This has already been attempted by some authors. More sophisticated methods imply the use of the Voronoi tessellation or the averaging volume method introduced by Whitaker and applied to food tissues by References 6, 53-54, and 58.

The Voronoi tessellation is based on a random division of a plane (or a volume) in a number of polygons (or polyhedra). It is based on the observation that the plant cells seem to have a polyhedral shape (polyhedral plane cross section) with a variable number of faces, from 9-20, averaging 13.8.\[57\] After the division of a plane (or volume) into a number of polygons (or polyhedra), according to appropriate constraints, some of them are randomly chosen to represent the intercellular spaces or pores, respecting the real porosity experimentally observed.

Matteo et al.\[57\] used the Voronoi tessellation to simulate the shrinkage and deformation of cellular tissue during dehydration. The same method was also applied to model penetrometric texture studies in apples. A further step in this structure simulation would be to couple it with the study of mass transfer properties of the processed tissues.

The averaging volume method applied by Rotstein and Crapiste in foods aimed at a more detailed and realistic picture of the cellular structure in a way that the cell compartments, such as the vacuole, the cytoplasm, the cell wall, and the intercellular spaces, constitute the phases over which the volume averaging of the mass transfer properties was made. In this model, the cellular membranes were taken into account as interfaces between the phases. Modeling of the drying of apples and potatoes was performed.

### 13.5 Conclusion: Need for Future Research

There is an obvious need to focus research on mass transfer at the cell level on biological tissues undergoing processing and to develop more fundamental mathe-
tactical models, taking into account all the driving forces acting in the systems studied. Knowledge of the thermodynamics of the system cell and its environment is a prerequisite.

More research is needed for the determination of the membrane deterioration temperature ($T_d$) in various systems, covering not only thermal processing conditions but also different types of environments and application of pressure, electrical, and magnetic fields.

Thermodynamic modeling of solutions containing sugars and ions, typically found in the vacuole of the plant cells, as well as the processing solutions (blanching, osmotic) also have to be studied. There is an obvious gap compared with similar data reported in classical chemical engineering (hydrocarbon compounds, petrochemical organic chemistry). Modeling of the cellular structure is also in its infancy, only a little having been done until now, but emerging studies are providing clear paths for future research. At the cell level, studies have to be clearly accompanied by adaptation of powerful analytical tools [e.g., particle-induced X-ray emission (PIXE) or fluorescence microscopy] to the special features of plant cells.

REFERENCES