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Influence of Frozen State Reactions on Freeze-Dried Foods

Marcus Karel* and James M. Flink

The quality of freeze-dried foods depends strongly on chemical reactions and on physical phenomena which occur during freezing and during the maintenance of frozen state before and during vacuum dehydration. The following specific aspects are discussed: effects of freezing conditions on water binding by freeze-dried foods; effects of freezing on drying behavior; rates of chemical reactions in frozen foods; and effects of freezing and freeze-drying on retention of flavor in foods.

The quality of freeze-dried foods is potentially superior because the process is characterized by a low drying temperature, the preservation of food's original shape and appearance, and substantial flavor retention. These desirable characteristics depend on the maintenance of macroscopic, microscopic, and molecular morphology since low drying temperature is only practical if the rate of sublimation is substantial, even at low temperatures and vapor pressures of ice. The porosity of the dry layer must therefore be high, and there must be no excessive impedance to vapor flow due to collapsing pores and capillaries.

Preservation of shape, texture, and appearance requires that internal structure be maintained.

Flavor retention depends on the formation of microregions where flavor compounds are locked within a matrix of solids (Flink and Karel, 1970a). When this matrix is disrupted, flavor loss increases.

During freezing events occur which develop the structure and therefore predetermine the properties of subsequently dried material. Ideally, the thermal history of freeze-dried materials (Figure 1) is such that while moisture content is high, the temperature is low enough to prevent mobility and structural changes, and conversely that when the temperature finally rises, the moisture content has become low enough to achieve the same result. Freezing variables, including rate, minimum temperature during freezing, and the temperature of the frozen layer during drying, affect reactions in subsequent stages of product life.

STATE OF WATER AND OF SOLUTES IN FROZEN AND FREEZE-DRIED SYSTEMS

In foods some of the total water is bound to hydrophilic compounds and does not crystallize. Most of the water, however, does separate out during freezing in crystals of pure ice. During freeze-drying in aqueous systems containing hydrophilic polymers and smaller solutes the water partitions into three states; water bound to the polymer, ice crystals, and concentrated solution.

Among systems with this characteristic behavior is muscle, which contains proteins, water, and electrolytes. Other protein- or polysaccharide-containing foods behave similarly. In these systems, the electrolytes and other solutes may also appear in the following forms: bound to the polymer; in concentrated solutions; (or upon removal of water) in amorphous precipitates or in crystalline form. Gal (1969) studied the casein–water–NaCl system and found NaCl to separate into different forms (Table I).

The state of the water bound to polymers has been frequently investigated using various physical and physicochemical methods of analysis. In frozen systems that portion of water which remains unfrozen, while less "free" than water in solutions, is more mobile and therefore more "free" than water in ice crystals. This conclusion is supported by most recent nuclear magnetic resonance (Dehl, 1970; Kuntz *et al.*, 1969) and infrared work (Falk *et al.*, 1970).

Energy of binding of unfreezable water has also been studied. Up to 0.5 g of water per g of proteins and of other hydrophilic polymers does not freeze, but calorimetric studies on water sorbed on proteins and polymers show that only less than 0.1 g per g has a significant heat of adsorption, and that this heat of adsorption is usually less than the latent heat of fusion. There are, however, a few reported heats of adsorption, usually involving polyelectrolytes which exceeded heats of fusion (Amberg, 1957).

The thermodynamic confirmation of the greater mobility of the nonfreezing water in muscle-derived foods compared to water in ice is also shown by Riedel (1966), who studied the apparent specific heat of frozen beef. The specific heat for "bound" water (unfreezable water) calculated by Riedel was intermediate between that for ice and

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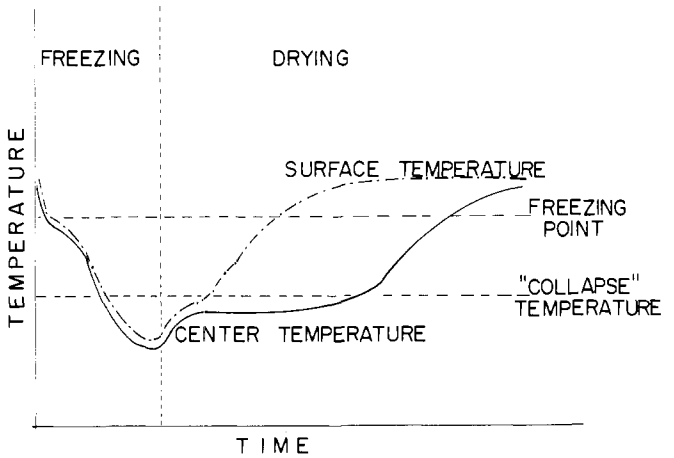


Figure 1. Thermal history of freeze-dried materials.

that for pure unfrozen water. Since specific heat reflects molecular mobility, "bound" water is evidently less bound than water in ice.

In frozen systems, therefore, water appears to exist in a number of states of increasing mobility, the partition between which depends strongly on temperature.

The state of water in freeze-dried systems may be affected by the past history, particularly by the rate of freezing. MacKenzie and Luyet (1967) determined the sorption isotherms of ox muscle, prefrozen at various rates and then freeze-dried. Table II shows sorption parameters calculated from their data which show that the rate of freezing does not affect the B.E.T. monolayer value, but that when muscle is frozen quickly the monolayer water has a higher binding energy. They also show that at a given constant relative humidity, a quickly frozen and

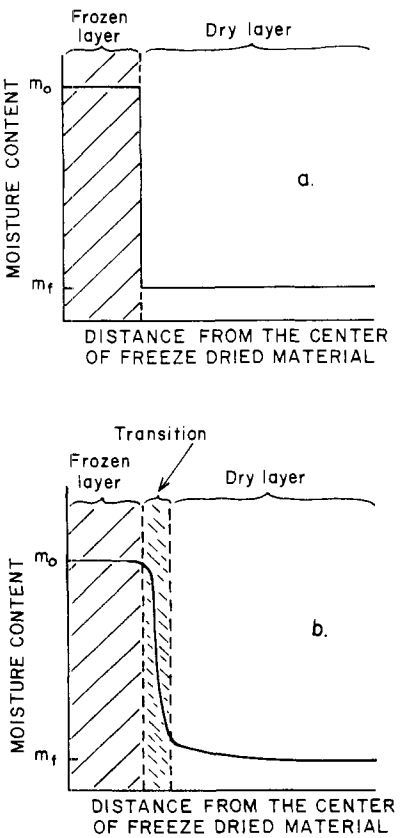


Figure 2. Moisture profiles in freeze-drying materials; a, ideal; b, actual.

Table I. Properties of NaCl in Freeze-Dried Casein + NaCl Systems[a]

Bound to casein	Free, noncrystalline	Crystalline
Unable to crystallize	Crystallizes given sufficient water to impart mobility	Crystalline
Increases water sorption of casein	Sorbs water until crystallization starts	Nonhygroscopic
No X-ray reflection	No X-ray reflection	X-ray reflection

[a] Gal (1969).

then dried sample holds more water than slowly frozen dried samples.

Of several possible explanations for these effects we favor the following. Slow freezing causes a high salt concentration to act on muscle protein long enough to cause dislocations and aggregations. The total number of accessible hydrophilic sites does not change, hence there is no effect of rate on B.E.T. monolayer. The access to these sites, however, is more difficult, and this is thermodynamically equivalent to reducing the energy of water binding by the amount of energy required to open the path to a binding site, and it similarly explains the requirement for a higher relative humidity for a given amount of water bound by the protein.

The question of mobility of "bound" and of unfrozen water is important to reactions in frozen and in freeze-dried foods. Water has complex functions in these reactions, since it may act as solvent, reactant (in hydrolyses), product (in condensations), or modifier of catalytic or inhibitory activity of other substances.

The presence or absence of water-binding compounds strongly affects these functions of water (Heidelbaugh *et al.*, 1971).

EFFECTS OF FREEZING ON DRYING BEHAVIOR

During freeze drying heat is transported from the surface to the frozen layer interface, and water is transported from this interface to the surface. Ideal and actual moisture gradients in freeze drying are shown in Figure 2.

The temperature and moisture gradients in the freeze-drying product depend strongly on the drying layer's properties, which are partially "set" during freezing. For instance, if solute migration is possible during freezing, an impermeable film which impedes drying by substantially lowering its rate can form at the surface (Quast and Karel, 1968). Slush freezing can prevent the formation of such a film (Quast and Karel, 1968), or it may be removed mechanically. Slow freezing produces bigger crystals, hence usually bigger pores and better mass flow during drying and reconstitution.

The beneficial effect of slow freezing on mass transfer rates is often offset by the structure's collapse if the temperature of the frozen layer is too high. Ideally, the vapor

Table II. Effect of Freezing Rate on Water Sorption by Freeze-Dried Ox Muscle[a]

	Relative sorption parameters			
	B.E.T. monolayer		Amount sorbed	
Relative freezing rate	Amount	Energy	20% relative humidity	60% relative humidity
1	0.99	0.39	0.55	0.88
100	0.94	0.77	0.73	0.94
20,000	1.00	1.00	1.00	1.00

[a] MacKenzie and Luyet (1967).

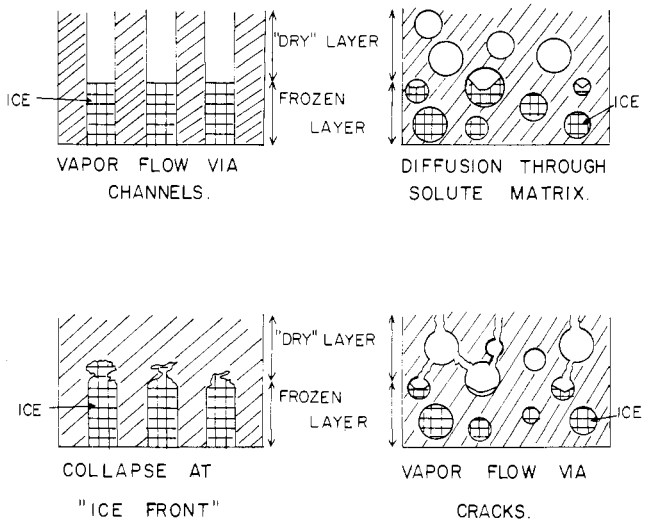


Figure 3. Representation of types of vapor flow in freeze-drying.

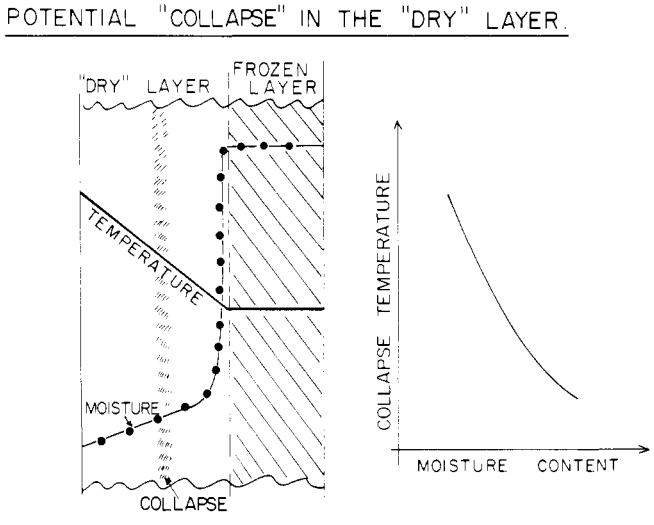


Figure 4. Potential collapse of structure in the partially dry layer of freeze-drying materials.

Table III. Collapse Temperatures for Different Solutes

	Collapse temperature, °C		
Sucrose	−25[a]	−32[b]	−22[c]
Glucose	−40	−40	−40.5
Lactose	−19	−31	−21
Dextran	−2	−10	
Orange juice			−20
NaCl	−22		

[a] Ito (1971). [b] MacKenzie (1967). [c] King (1972).

Table IV. Effect of Freezing Rate on Freeze-Dried Bananas[a]

	Rehydration ratio	Brown pigment, Klett units	Ascorbic acid, mg/kg
Immediately after drying			
Frozen slowly	1.14	18	215
Frozen quickly	1.0	10	350
After 9 months at 30°			
Frozen slowly	0.74	24	77
Frozen quickly	0.66	18	125

[a] Maia and Luh (1970).

flows through the pores and channels left by ice crystals. However, if freezing produces isolated ice crystals surrounded by a solid matrix, then the vapor must diffuse through the solids (Figure 3). A similar situation results if the matrix collapses at the ice front and seals the channels. However, the matrix can be cracked, particularly in rapidly frozen systems.

Collapse often occurs at a fixed temperature similar to recrystallization temperature (Ito, 1971; King, 1972; MacKenzie, 1966) when the matrix is sufficiently mobile to allow flow under the influence of various forces. King (1972) suggests that surface tension is the force responsible for flow, but thermal or gravitational forces or other gradients may also cause flow. Resistance to flow depends on the viscosity of concentrated solution, and for a given solute the viscosity depends solely on concentration, which in an equilibrium situation depends only on temperature. Typical collapse temperatures are shown in Table III. Note that if moisture content falls below saturated solution level, mobility of an amorphous matrix depends on moisture content as well as on temperature; hence collapse can occur at areas other than *ice surface* (Figure 4).

EFFECTS OF CHEMICAL REACTIONS IN THE FROZEN STATE ON FREEZE-DRIED FOOD QUALITY

In the life cycle of freeze-dried foods, the duration of the frozen state is small; most of the potentially deteriorative reactions occur either during storage in the dry state or in the hydrated state above freezing after rehydration or before freezing (Karel, 1969).

However, in some situations reactivity in frozen state may cause a problem. In unblanched fruit and vegetables, enzymes may be active in the frozen state, resulting in quality deterioration or in buildup of intermediates which then participate in reactions in the dry state. This type of behavior has been reported by Goldblith *et al.* (1963) and more recently by Maia and Luh (1970), who studied the storage stability and quality of freeze-dried bananas (Table IV). Slowly frozen, freeze-dried bananas apparently undergo some enzymatic browning in the frozen state and also lose significant amounts of ascorbic acid compared with bananas frozen quickly in a freon coolant. Incidentally, rehydration after drying is somewhat better in the slowly frozen samples, presumably because of the large pores created by slow freezing.

However, slow freezing may be more detrimental to water-holding capacity of freeze-dried meat, fish, and crustaceans than quick freezing. The texture of frozen muscle-derived foods is poor and generally water-holding capacity is low. Water of rehydration is held more loosely than in refrigerated or frozen and thawed foods. Changes in the morphology of the myofibrils and decreases in ATPase activity of the actomyosin of muscle and particularly a decrease in salt solubility of the muscle proteins accompany the development of textural defects.

These changes are due to aggregation of muscle protein, but the details of these aggregative reactions are still unclear. Textural changes may be due to one or all of the following in the actomyosin complex: aggregation or crosslinking of undenatured protein; denaturation of proteins followed by aggregation; or interaction of the native or denatured proteins with lipids or carbohydrates.

The occurrence of the above events in actomyosin filaments is now well substantiated, and myosin is also probably the site of the crosslinks. While various secondary and valence bonds have been proposed, the evidence seems good that -S-S- bond formation is involved. The crosslinks apparently form in frozen state and are stabi-

Table V. Effect of Freeze-Drying on Myosin[a]

	ATPase activity	
	pH 6.0	pH 7.0
Before freezing	100	100
After thawing	76	92
After freezing drying	25	48

[a] Yasui and Hashimoto (1966).

lized or made irreversible by drying. Andrews and Levitt (1967) have shown in a model system (Thiogel) that this reaction proceeds rapidly in the frozen state, but can be retarded by sucrose, glycerol, and DMSO.

Further support is provided by Buttkus (1970), who presented evidence for disulfide formation and other aggregative reactions in myosin solutions at −10°. ATPase activity changes have also supported the concept that reactivity in the frozen state before freeze drying affects the state of freeze-dried muscle. Goldblith *et al.* (1963) have shown that freezing conditions affected the rate of ATPase inactivation in subsequent storage of freeze-dried fish and shrimp. Yasui and Hashimoto (1966), working with proteins extracted from muscle, observed that solubility and ATPase activity decreased due to freezing as well as drying (Table V).

Reactions in the frozen state may initiate protein change, and these changes might become extensive and irreversible during drying and storage. The following features are likely. Myosin is the protein responsible for the aggregation. Myosin is not extensively denatured before crosslinking and is not dissolved from filaments by the salt solutions concentrated by freezing. Links form between adjacent myosin filaments. Secondary bonds as well as disulfide bonds are involved. Temperature of drying is of key importance to the rate of formation of irreversible aggregates.

Peroxidation of lipids can also result in very extensive and very complex protein-lipid reactions (Karel, 1969). Because the frozen state facilitates energy transfer and because freezing stress causes polymer breaks and free radical formation, these reactions may be affected by freezing factors (Patat and Högner, 1964). However, these reactions occur in storage, which we will not discuss here further.

FLAVOR RETENTION IN FREEZE-DRIED FOODS

Food materials are generally freeze-dehydrated by placing the frozen food in a high vacuum environment. Contrary to what one might expect, compounds of different vapor pressures do not have different volatile retentions (Table VI). The retention of the organic volatiles is based largely on the properties of the solute which forms the amorphous matrix of the freeze-dried solid.

The retention of organic volatiles has been considered to result from surface adsorption of the volatile on the dry layer of the freeze-drying sample (Rey and Bastien, 1962) or from an entrapment mechanism which immobilizes the volatile compounds within the amorphous solute matrix (Chandrasekaran and King, 1971; Flink and Karel, 1972; Thijssen and Rulkens, 1968). A simple experiment shows that the retention phenomena depend on local entrapment rather than adsorption (Flink and Karel, 1970a,b). Maltose solutions were frozen in layers, some of which were volatile-containing and others were volatile-free. After freeze-drying, the layers were separated and analyzed separately for the volatile. The results (Table VII) show that volatile retention is localized in those areas at which the volatile is initially present. This experiment demonstrates that adsorption is not a retention mechanism, since volatile escaping from the lowest layer of Sample A was not retained on the upper dry layers. Further, volatile retention is not affected by the passage of water vapor through the volatile-containing dry layer from the volatile-free maltose layers as shown for Sample B. In a companion experiment sections of the freeze-dried cake were cut from a sample perpendicular to the mass transfer axis. Essentially uniform retention was observed for the whole sample, which supports the observations that volatile retention is determined locally in the food material and not by surface adsorption. The gross structure of the freeze-dried material is freely permeable to the flow of volatile from the lower freeze-drying levels, and the retained volatile is not located on the surface of the dry layer but within the amorphous solute matrix.

Table VI. Retention of Organic Volatiles in Freeze-Dried Carbohydrate Solutions[a]

	Retention g of volatile/100 g of carbohydrate		Volatile vapor pressure at −20°F, μ
Volatile	Maltose	Dextran-10	
Acetone	2.01	0.03	11,500
2-PrOH	2.71	0.30	760
1-PrOH	2.41	0.17	280
1-AmOH	1.37	1.41	20

[a] Initial volatile content: 4 g/100 g of carbohydrate.

Table VII. Retention of 2-Propanol in Specified Layers of Freeze-Dried Maltose Solutions[a]

	Sample A		Sample B	
	Before freeze-drying	After freeze-drying	Before freeze-drying	After freeze-drying
Top layer	0	0	4	2.52
Middle layer	0	0.05	0	0.05
Bottom layer	4	2.73	0	0.02

[a] 2-Propanol content is g/100 g of solids.

The physical aspects by which the volatile is entrapped within the amorphous solute matrix are only partially understood. Two mechanisms, selective diffusion (Thijssen, 1971) and microregions (Flink and Karel, 1970a), represent perhaps macro- and microviews of the same basic phenomenon. The size of the entrapments is small, since grinding and evacuation of the dry material does not release any volatile. Recently the size of the microregions has been shown to vary with, among other things, the solubility of the organic volatile in the aqueous solution. Retained hexanal (about 1 g of hexanal per 100 g of maltodextrin) freeze-dried from an aqueous 20% maltodextrin solution appeared in the optical microscope within the amorphous solute matrix as 2- to 6-mμ droplets (Figure 5) (Flink and Gejl-Hansen, 1972). Concurring evaluations have been made with a scanning electron microscope. The numbers and sizes of droplets observed in the optical microscope for a series of *n*-alcohols qualitatively indicated that droplet size and number increased with alcohol molecular weight.

The addition of sufficient water to the dry material will cause volatile loss, the extent of which depends on the amount of water added and the particular solute matrix. This is seen in Figure 6 for the humidification of freeze-dried lactose containing 2-propanol. Note that a sample may be humidified at 20% relative humidity without volatile loss (Figure 6).

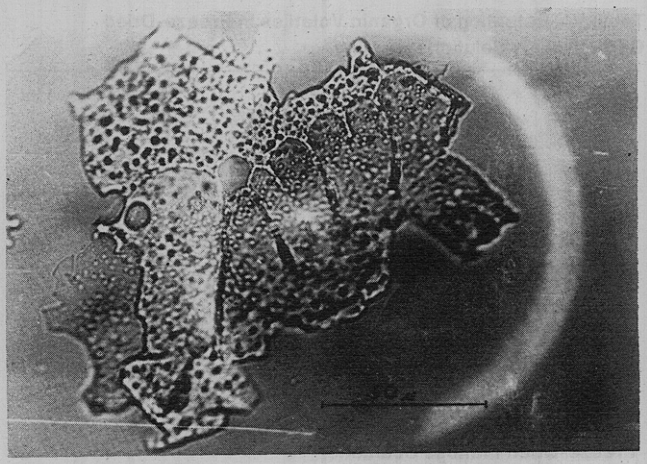


Figure 5. Micrograph of hexanal droplets in a freeze-dried solution originally containing 1% hexanal and 20% maltodextrin (400×).

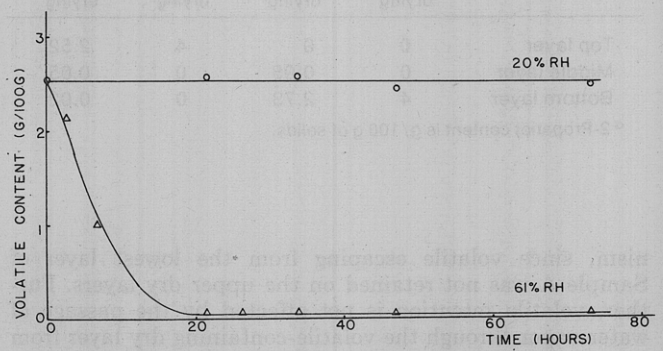
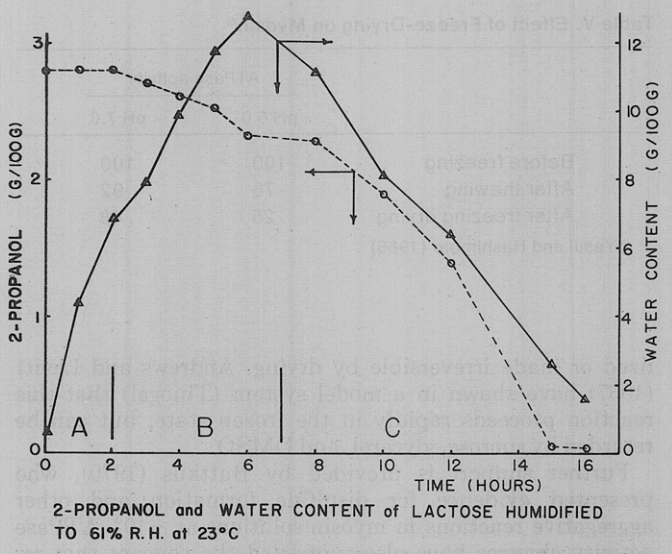


Figure 6. Loss of 2-propanol from freeze-dried lactose solutions during exposure to two different relative humidities.



2-PROPANOL and WATER CONTENT of LACTOSE HUMIDIFIED TO 61% R.H. at 23°C

Figure 7. Loss of 2-propanol from freeze-dried lactose solutions showing correlation between water loss due to lactose crystallization and the volatile loss.

Table VIII. Retention of 2-Propanol in Freeze-Dried Solutions

Carbohydrate concentration (20%) in solution	2-Propanol concentration in solution, ppm	2-Propanol retention, %	
		Frozen rapidly	Frozen slowly
Maltose	10,000	71	85
Maltose	100	10	84
Dextran	100	43	88
Maltose	200	8	87
Dextran	200	63	82

Humidification at 61% relative humidity is presented in more detail in Figure 7. No volatile loss occurs until after water content has reached a critical level corresponding to the B.E.T. monolayer sorption level of 6.2 g of H_2O per 100 g of lactose (Region A). Volatile loss continues slowly through Region B until the water reaches the next critical level, where lactose crystallizes. At this point major structural changes occur as the solute changes from amorphous to crystalline, eventually rejecting completely both water and volatile from the solute matrix.

Water's influence on volatile loss is due to changes in the matrix structure caused by the breakdown of carbohydrate–carbohydrate hydrogen bonds which had stabilized the solute matrix in the amorphous state.

Further evidence of the importance of maintaining matrix structure was obtained by humidifying at reduced temperature; even after equilibrium to high moisture contents, only an observable collapse of the solute matrix will cause volatile loss.

As noted above, maintenance of a solute matrix structure is essential to retain organic volatiles after freeze drying. Equally important is the maintenance of the solute structure during freeze drying.

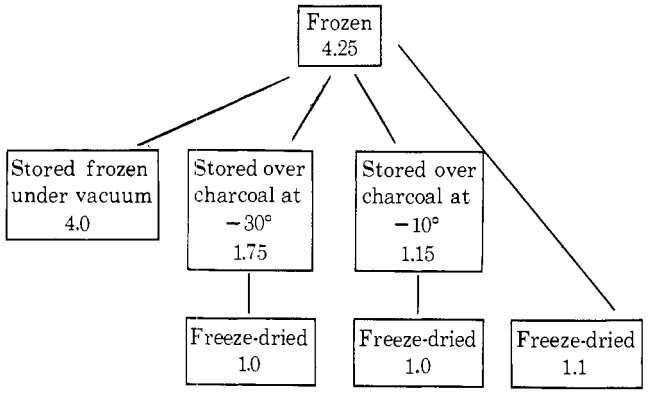
The influence of frozen-layer temperature on volatile retention during freeze drying has been demonstrated recently by Thijssen (1971), Bellows (1972), and Ettrup-Petersen *et al.* (1972). They showed that if the ice-front temperature is allowed to be higher than the collapse temperature for the solute matrix, a sizable volatile loss will occur. This is due to the viscous flow of the solute matrix ("Collapse") which results in the degradation of the matrix structure.

The influence of the freezing conditions on subsequent volatile retention has been noted by virtually every researcher studying volatile retention in freeze drying. Differences in solute phase separation and concentration, matrix pore size, and concentrated solute phase thickness which are thought to be important in controlling volatile retention result from variations in the freezing rate and final sample temperature. Table VIII gives typical data which show that slow freezing will result in up to tenfold improvement of volatile retention compared to very rapid freezing (Flink and Labuza, 1972).

Recently, some investigations of the volatile-retaining properties of frozen materials have given results for different treatments of frozen aqueous maltodextrin solutions (Gejl-Hansen, 1971; Steijvers, 1971) (Table IX). Of particular interest was the exposure of the frozen solutions to activated charcoal under vacuum and the resultant partial hexanal loss (Gejl-Hansen, 1971). This observation seems to indicate that the volatile can be trapped in the frozen matrix as well as in the dry state. Table IX further shows that freeze-drying the samples equilibrated with charcoal gives a further volatile loss only when the frozen layer or ice-front temperature is higher than the equilibration temperature, and thus that volatile retention in the frozen system is temperature dependent.

Most recently Lambert, working with Flink and Karel, has studied *n*-butanol loss from the frozen aqueous solution (Lambert, 1972). When equilibrated at −10° over activated charcoal in the presence of ice (to prevent water loss from the samples), only 25% of the butanol was lost (Figure 8). The remaining 75% was strongly retained and was not removed by transferring the samples to a desiccator which contained fresh activated charcoal. While the mechanism for this retention in the absence of a solute phase is not fully understood, apparently ice crystals are fully capable of entrapping organic volatile, though this

Table IX. Retention of Hexanal in Solutions of 20% Maltodextrin in Water. (g of Hexanal/100 g of Maltodextrin)



entrapment cannot persist through any freeze-drying process.

The retention of organic volatile compounds in the freeze-drying process is dependent on the control of processing steps. The first step is the formation of the concentrated solute phase during freezing and control of this step to give a solute matrix structure capable of retaining volatile. To maintain this structural arrangement in the frozen sample at high moisture contents, the temperature must be kept below the collapse temperature during freeze drying. After drying, the volatile-containing sample will be stable to wide ranges in temperature, but the structure must still be maintained by control of the sample moisture content.

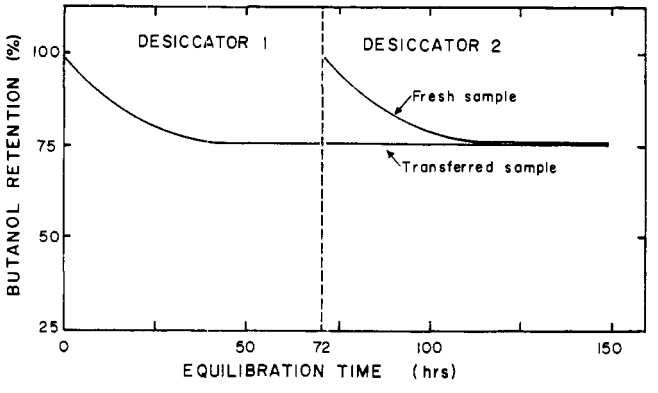


Figure 8. Retention of butanol in a frozen solution of 1% butanol in water during frozen storage over activated charcoal.

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