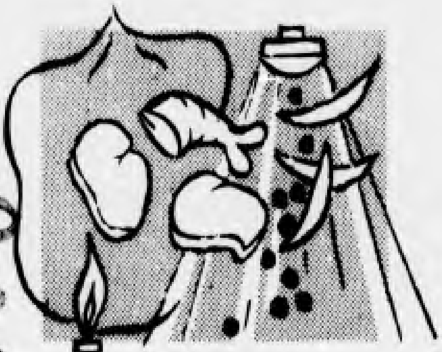
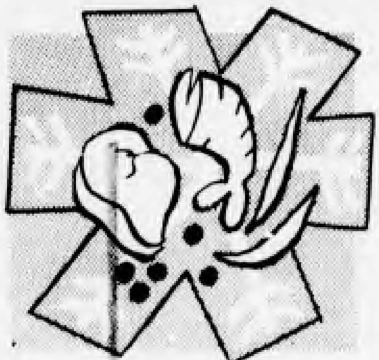
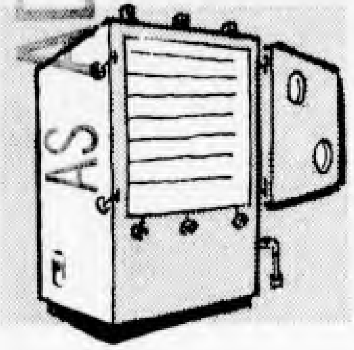


FREEZE-DRYING OF FOODS





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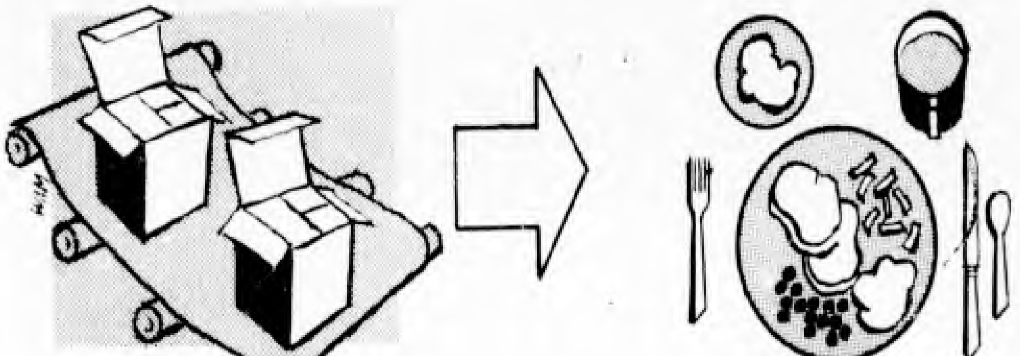

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FREEZE-DRYING OF FOODS

Proceedings of a Conference

SHORELAND HOTEL
CHICAGO, ILLINOIS
APRIL 12-14, 1961

Sponsored by

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES
QUARTERMASTER RESEARCH AND ENGINEERING
COMMAND
U. S. ARMY QUARTERMASTER CORPS
AND
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Division of Engineering and Industrial Research

Edited by
FRANK R. FISHER

NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL
Washington, D. C.

1962

This publication is available from the

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES

1819 W. Pershing Road
Chicago 9, Illinois

Library of Congress
Catalog Card No. 62-60065

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FOREWORD

The increased and novel demands imposed by modern warfare, together with recognition of deficiencies in existing military rations, have combined to provide the basis for the development of more effective feeding systems for the Armed Forces. Two feeding systems, both based on unitized meals prepared from dehydrated components, have been developed to meet several different military situations. In preparing dehydrated components for such meals, extensive use has been made of freeze-drying as the only means presently known for attaining a high degree of acceptance and allowing rapid reconstitution prior to use. However, regardless of the success attained by these new feeding systems during extensive field trials, acceptance by the Armed Forces must also be contingent upon an evaluation of our resources for producing specific freeze-dried components in amounts consistent with projected requirements.

Fortunately, considerable activity is under way in many segments of industry on the development and production of freeze-dried products for the civilian food market. In addition, a considerable reservoir of information applicable to the freeze-drying of food and other biological material has been developed in industrial establishments, in the laboratories of many educational institutions, and in other research organizations both in the United States and abroad.

It was the intent of the Committee charged with the design of this conference to bring together scientists with specialized knowledge relevant to the various aspects of freeze-drying technology and to encourage exchange of ideas and information on the broadest basis possible. It was hoped that additional perspectives would be supplied by a number of outstanding scientists from abroad. Although precise evaluation will never be possible, the ultimate objective of this conference is sought in the generation and implementation of new ideas and in the identification and stimulation of productive research which eventually will lead to more efficient processing, to superior products, and to a more attractive economic outlook for freeze-dried foods. Realization of any of the above objectives should be reflected in an expansion of our capacity for the freeze-drying of food, thus providing needed support for a significant advance in the feeding of our Armed Forces.

Since this conference was oriented to scientists already familiar with much of the contemporary activity in the freeze-drying of food, no effort was made to review current knowledge, to survey the most recent developments, or to emphasize the technology of specific food products. Rather, the papers presented were intended to identify problems, to explore solutions, to introduce new ideas, and to stimulate creative thinking. Whatever permanent values are forthcoming from this conference must be credited to the members of the Conference Planning Committee for the organization and execution of the program, to the Staff of the Advisory Board on Quartermaster Research and Development for the coordination of arrangements, to the conference participants, and especially to the authors who put a great deal of time and effort in the preparation of manuscripts.

M. C. BROCKMANN
Chief, Meat Products Branch
Quartermaster Food and Container
Institute for the Armed Forces

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Proceedings of a Conference

on

FREEZE-DRYING OF FOODS

SESSION NO. 1

MINIMIZING PRODUCT DAMAGE—BASIC BIOCHEMICAL AND BIOPHYSICAL APPROACHES

HAROLD T. MERYMAN, *Moderator*

INTRODUCTORY SURVEY OF BIOPHYSICAL AND BIOCHEMICAL ASPECTS OF FREEZE-DRYING

HAROLD T. MERYMAN

The subjects for discussion by this panel are the basic biophysical and biochemical events which may lead to product alteration. Knowledge of the exact nature of changes taking place during freeze-drying is very cloudy, and it is impossible arbitrarily to catalog the events occurring. Rather than to attempt the impossible, therefore, this paper will introduce the subject by first examining the general mechanism of the freeze-dying process in the hope that this analysis will lead to a clearer understanding of freeze-drying and that we will then be in a more advantageous position to discuss the kinds of product change that may occur during the different phases of the process.

Biophysics of Freeze-Drying

The process of dehydrating a frozen specimen can be divided into three steps: 1) the introduction of heat to supply the energy necessary for sublimation, 2) the transfer of water vapor from the subliming ice crystal through the already dried shell of the specimen, and 3) the removal of water vapor that reaches the specimen surface. These three steps in the drying process are analyzed in detail below.

Heat Transfer and Sublimation. An ice crystal is composed of pure water rather rigidly confined in a crystal lattice. Within the restrictions of its position, the water molecule continues to indulge in random thermal motion. Statistically there is a certain probability that a molecule may, as the result of this thermal motion, pop out of its hole and become free of the restraints of the crystal. The possibility that a molecule will leave its position in the ice crystal depends on the degree of violence of its random thermal motions and the expectation that a particularly violent motion will project it past the energy barrier that restrains it. The violence of the random motion is a function of temperature—in fact, this is a kind of definition of temperature. With a reduction in temperature the thermal agitation of the water molecule is less and the prospects that it may leave its position diminish.

The transition from the solid to the vapor is a transition from a restricted to a relatively unrestricted state with an according increase in the amount of energy possessed by the molecule. This additional energy, the latent heat of sublimation, is acquired by each molecule that makes such a transfer of state. The energy itself is derived primarily from the environment in the form of heat. Thus, as each molecule transfers from ice to vapor, a discrete amount of heat is removed from the environment and, unless replaced from without, the temperature of the environment will fall. A temperature reduction in turn serves to reduce the probability of further sublimation. The end result of such a closed cycle is the attainment of a temperature so low that sublimation cannot take place.

If heat is introduced into the system at a fixed rate it will support continuing sublimation at a fixed rate. The temperature of the drying boundary will be self-determining. If sublimation takes place at a rate more rapid than the supply of heat will support, the temperature will fall until a level is reached at which the rate of sublimation is exactly equivalent to that which the heat input can maintain. Conversely, if the temperature is too low, the sublimation rate will be less than that which the incoming heat could support, there will be an excess of incoming heat and the temperature will rise until again sublimation exactly equals that supportable by heat input. Drying boundary temperature, then, is a variable depending upon the rate at which heat is provided and the rate of vapor loss.

In the foregoing discussion we have assumed that all subliming water molecules have been promptly removed from the system. In real systems this is rarely the case and a second factor influencing specimen temperature must be considered.

When sublimation from ice takes place within a closed container, an increasing concentration of water vapor develops in the space around the ice. This creates an opportunity for water vapor molecules to return to the ice crystal should they happen to strike a suitable site with the proper energy and orientation. Obviously, the higher the concentration of vapor molecules surrounding the ice, the greater the possibility that a return will take place. Each time a vapor molecule returns to the solid state, latent heat is returned to the environment exactly as it was acquired during sublimation. Eventually, an equilibrium will be established between the subliming and recondensing vapor providing there is no introduction or removal of heat from the system. If heat is introduced the temperature of the specimen will rise, the sublimation rate will increase, and the concentration of vapor molecules in the space will increase. This in turn increases the rate at which molecules return to the crystal, and a new equilibrium will be established at the higher specimen temperature with an increase in surrounding vapor concentration.

In practice, then, the temperature of the specimen at the drying boundary is determined by the rate of heat input and the rate of vapor transfer away from the boundary. It is very important to understand that the drying boundary temperature is not a fixed value but is the resultant of these two factors. At temperatures down to at least −40°C the rate at which water molecules are leaving the ice crystal is still very great, and if no resistance to the departure of vapor is present very large drying rates are possible even at these low temperatures. This activity is experimentally demonstrated in experiments by Greaves (1) both on the drying of distilled-water ice and of specimens from which the dried shell is scraped continuously. It is only when there is a resistance to the evolution of water vapor that the water vapor pressure at the drying boundary rises with an accompanying rise in specimen temperature.

Water Vapor Transfer Away from the Drying Boundary. Under conventional freeze-drying circumstances, an increasing shell of dried material develops as the drying boundary progresses into the specimen. All water vapor produced by sublimation at the drying boundary must pass by diffusion through this barrier of increasing thickness. As a concentration of water vapor molecules develops at the drying boundary, diffusion toward an area of lesser concentration will take place. The coefficient of diffusion, a measure of the degree of obstruction introduced by the dried shell, is not an easy figure to predict. The shell may have cracks, vascular pathways, or holes left by sublimed ice crystals which provide intermittent low resistance pathways. But regardless of the exact nature of this resistance to diffusion, it is very high. Stephenson (2), in a very thorough analysis of the subject, estimates that a dried shell 1 mm thick introduces "a reduction in drying rate by a factor smaller than .001 relative to the maximum rate of sublimation of ice at the same temperature."

The question whether vapor transfer within the dried shell is by molecular or viscous flow is generally more of academic than of practical interest. The fact remains that the dried shell will offer a resistance to flow regardless of the nature of the flow and that the rate of vapor flow will depend upon the resistance of the shell and the water vapor concentration gradient existing between the drying boundary and the specimen surface. The only pertinent observation to be made regarding the type of flow in the dried shell is that at higher pressures, when the mean free path is substantially shorter than the average path distance in the solid shell, the diffusion rate will be a function of absolute pressure. At pressures where the mean free path is in excess of the average pathway in the shell, diffusion rates become independent of absolute pressure so that theoretically further improvements in the vacuum system are without beneficial effect on mass transfer within the shell.

It is imperative to recognize that the only force driving water vapor from the drying boundary to the specimen surface is a concentration gradient. One often encounters the intuitive notion that when the specimen is placed in a vacuum chamber the vacuum will tend to "suck" water out of the specimen. Nothing could be further from the truth. The only effect of an external vacuum on circumstances within the dried shell will be to reduce the concentration of air molecules present in interstices of the tissue and thus somewhat to reduce the resistance to water vapor flow. The water vapor itself is diffusing along a concentration gradient which is the sole motivation for its net transfer.

Reduction of Vapor Pressure at the Specimen Surface. Since the only motive force to effect a transfer of vapor across the dried shell is a water vapor concentration gradient, the steepness of this gradient will determine the rate of vapor transfer. The gradient can be increased by raising the concentration of vapor at the drying boundary, but since vapor pressure at this location is directly related to temperature, there is a limit to which the vapor pressure here can be increased without developing too high a temperature at the drying boundary. The gradient can also be steepened by reducing the vapor pressure at the specimen surface to a minimum. Much of the design of freeze-drying equipment is directed toward this latter goal. The ideal is, of course, to maintain a zero vapor pressure around the specimen. This would require that each molecule that reached the surface could continue on without impediment so that no congregation of vapor molecules would remain to constitute a finite external vapor pressure.

Summary. Above all, one must recognize that an understanding of the whole transcends the importance of any one of the parts. All the elements of the freeze-drying process: heat flow into the specimen, drying boundary temperature, vapor flow through the dried shell, chamber design, absolute pressure, and condenser temperature are all interdependent and no one element can be modified without affecting the others.

A most useful device for clarifying this interdependence is the electrical analogue as illustrated in Fig. 1.

In this analogue the battery, B_1, represents a heat source supplying energy to the specimen. This energy flows through R_1, representing the resistance to heat flow of the frozen specimen. The condenser, C_1, represents the drying boundary. Its capacity is actually very small, representing the accumulation of water vapor in the vicinity of the drying boundary. The voltage at point B will represent the temperature of the drying boundary. Condenser C_2 discharges through resistors R_2 and R_3 which represent respectively the resistance to vapor flow of the drying boundary and of the space between specimen and condenser plates. The battery, B_2, represents the condenser plates. Reducing the condenser temperature is comparable to a reduction in voltage at point D due to the reverse polarity of battery B_2. In the actual freeze-drying system the current flow from B to D will represent vapor rather than heat flow, and the voltage difference across resistors, R_2 and R_3, will represent water vapor pressure differential rather than temperature differential. However, since water vapor flow can be equated directly with the energy required for the latent heat of fusion, the analogy remains quite valid.

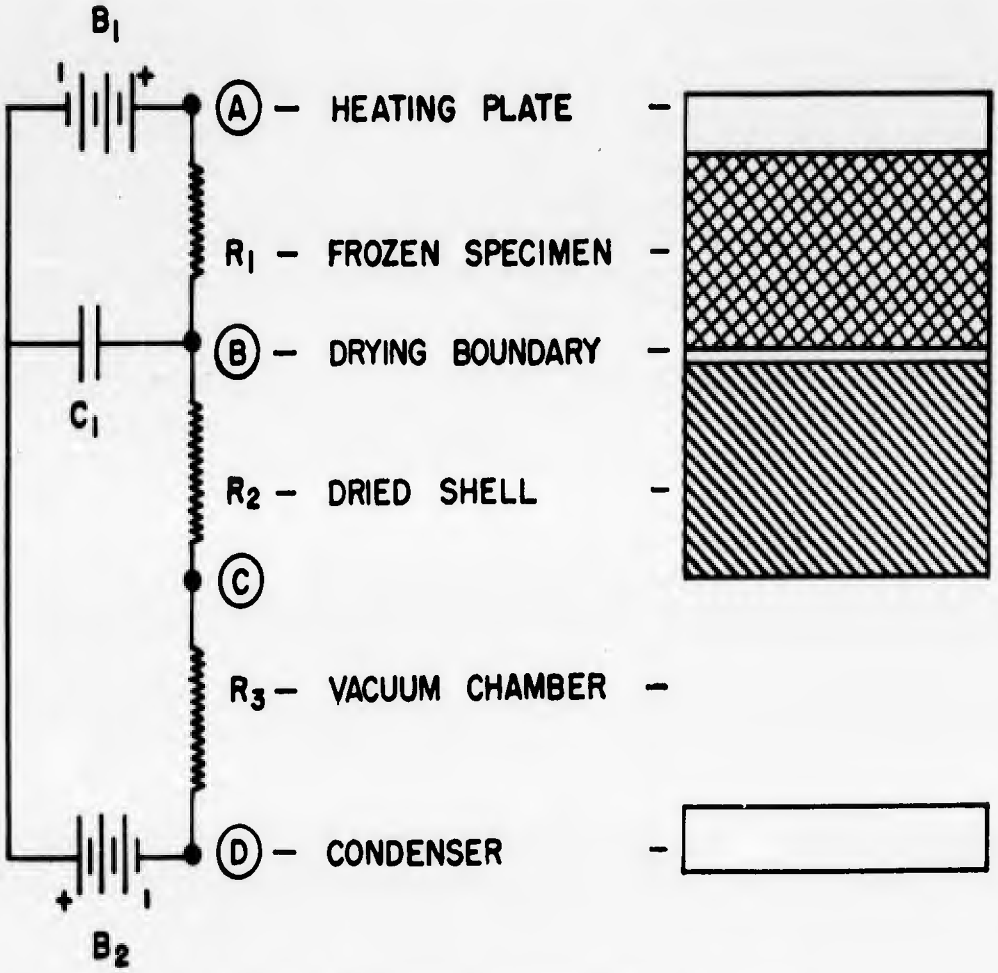

FIGURE 1. Electrical analogue of freeze-drying.

Putting a freeze-drying operation through its paces in terms of the analogue now becomes quite simple. The voltage at B (drying boundary temperature) is completely floating, depending on the relative resistance of R_1 (heat transfer) and R_2 and R_3 (vapor transfer), and on the total voltage difference between A and D (heater temperature and condensing plate vapor pressure). In the extreme case of the drying of distilled-water ice or the Greaves scraping method, resistance R_2, the dried shell resistance, is eliminated. Since R_3 can be assumed to be small, the voltage at B will be much closer to that at D than A regardless of the current flow which can be quite large. In the actual freeze-drying operation this means that despite a high rate of heat flow through the specimen and an equivalently large vapor flow leaving it, the drying boundary temperature can remain low. If R_2 were gradually re-introduced, the total resistance of the system would increase, current flow would correspondingly decrease and the voltage at B would rise relative to A and D. In the freeze-drying system this corresponds to the introduction of dried shell resistance with a reduction in the total amount of vapor evolved and an increase in the drying boundary temperature.

In the terms of the electrical analogue, it becomes quite simple to visualize the influence of various elements of the system or to compare, for example, the difference between a constant voltage supply at B_1 (fixed-temperature heater plate) or a constant current supply (constant-wattage heater plate). If a radiant heat input is to be used, one must introduce an additional resistor, R_o, between points A and B to represent a temperature gradient between the heat source and the specimen surface. The value of this resistance can be altered by the introduction of conductive or convective heat transfer at higher chamber pressures.

Product Change from Freeze-Drying

There is always a danger in any conference on a specialized subject that one may lose perspective regarding the position of that specialty relative to the rest of the world. In the case of freeze-drying, it is all too easy to forget the fact that freeze-drying is a means to an end rather than an end in itself. The purpose of freeze-drying in the context of this meeting can be defined as a means of producing a product which will be stable on storage at normal ambient temperatures and reconstitute to be as close to the original product as possible. It is the end that is important, and we must be careful not to attribute to freeze-drying virtues that it does not possess—nor allow it to become something sacred. If drying at temperatures higher than those considered proper for true freeze-drying still yields an acceptable product, then it is unfair to criticize the procedure because it does not follow the strict criteria for true freeze-drying. In the freeze-drying of foodstuffs more than any other material, one can justifiably say that "the proof of the pudding is in the eating."

When biological material is dried from the liquid state, two important phenomena occur with the progressive removal of water. First, the forces of surface tension cause progressive contraction of the specimen which eventually leads to a grossly shrunken and insoluble result. Second, the removal of water creates a progressively concentrated solution which can become chemically injurious. Since chemical reactions are always dependent upon temperature and time, these latter alterations could be avoided either by accelerating the drying process or reducing the temperature at which it takes place. If the temperature is reduced sufficiently the water freezes, but it should be recognized that freezing is merely incidental to reduction in temperature and is not necessarily desirable in ameliorating the chemical effects of drying.

In fact, the only specific inherent virtue in drying from the frozen state lies in the prevention of shrinkage and the maintenance of solubility. Shrinkage is prevented by eliminating surface tension during drying, and solubility is maintained by creating a dried product that is highly porous and effectively presents an extremely large surface area (presuming no chemical changes affecting solubility).

It may, at first blush, appear that a discussion of the freeze-drying of living cells or of highly labile biological materials is not particularly germane to a discussion of the preservation of food. However, it is well known to all that the taste, texture, and storage qualities of many freeze-dried foods leave much to be desired. In fact, this is so consistent an observation that one often gets the impression from the food-drying engineer that such changes are inevitable. One might be led to accept this inevitability were it not for the observation that other specialized techniques of drying can result in products of extreme stability, even to the preservation of living cells. To dismiss these results as involving materials unrelated to food or requiring procedures uneconomical or unadaptable to the food industry is to assume voluntary blinders. If it is possible to preserve plasma proteins or microorganisms with great stability, then it follows that it should also be possible to attain the same successful results with other biological materials. The challenge is to determine which aspects of the specialized or idealized process can improve the quality of dried foods since the possibility always exists that these factors may indeed turn out to be applicable to large production techniques. It would be unwise to dismiss the results of more idealized drying techniques simply because the techniques as a whole may not be suitable or economical for foods.

Justified by the preceding remarks, the second portion of this paper will be a survey of the areas in which mechanical or chemical injury may take place, using as an experimental system the most sensitive of all indicators, living cells.

Research on the drying of living cells is still at a very elementary stage, although there has been a substantial amount of experience on an empirical basis with the drying of viruses, bacteria, and a few protozoa (3). Only two mammalian cell types, both highly specialized, have been successfully dried and reconstituted (4). Concerning none of these examples do we have any real understanding of the mechanisms by which cell damage is done or avoided. Experimental observations have not as yet been related to a theory of mechanism so that much of this discussion can be only speculative in nature.

The drying of purified proteins has been very successful, and it has been well established that for good recovery and maximum storage life the material should be as dry as possible. With bacteria, however, it has been found that if desiccation approaches complete dryness the recovery on reconstitution is generally substantially reduced. The best survival from drying appears to be attained with about 1% residual water. On the other hand, it also appears to be generally true with bacteria that the presence of residual water results in poor storage characteristics. Thus, for practical purposes the residual moisture requirements for high recovery from drying and for survival for long periods of storage are incompatible, in the absence of protective agents such as sodium glutamate.

Looking at these scattered bits of experimental evidence we can now ask some of the questions that must be answered in order to construct a reasonable hypothesis for the mechanism of drying injury.

Dominating the list of questions are those concerning the last few per cent of residual moisture. What is this last one per cent of water? Where does it come from? Why is it the last per cent? And why is it so important to the viability of the living cell?

I would now like to itemize the various steps of the freeze-drying process and look at each step in terms of its effect upon living organisms. Such an analytical approach may help to spotlight the areas where damage is most probably done and the kind of investigations that must be carried out to increase understanding of the phenomena involved.

I. *Injury During Freezing*

Mechanical. Mechanical injury is defined as physical rupture or displacement of tissue or cell components as a result of the growth of ice crystals. Viruses and many bacteria can be frozen and thawed without substantial loss of activity so that we can presume that mechanical injury from external ice crystals is not important during the freezing of these organisms. Higher forms of life, such as protozoa, are more variable in their response to freezing and thawing. In general, mammalian tissues will not withstand freezing and thawing in the absence of a protective substance such as glycerin which protects from chemical injury and is discussed subsequently. However, even here there is still substantial ice crystal formation, and one is forced to conclude that the physical presence of external ice crystals is not in itself inevitably lethal. In tissues that have rigid cell membranes such as plant tissues, mechanical injury is undoubtedly a vital factor, but it is apparent that for many cells irreversible injury does not result from the physical presence of extracellular ice crystals. Very rapid freezing is tolerated only by erythrocytes and, under unique conditions, spermatozoa—two very special cell forms. It is quite probable that the formation of intracellular ice crystals by rapid freezing is mechanically destructive to most if not all cells.

Chemical. When water is removed from solution to form ice, a high concentration of solutes remains. Much of the injury done to living cells by freezing is chemical in nature, resulting from this concentration of solutes. Since any chemical reaction is time and temperature dependent, the rate at which injury from concentration will take place will depend upon specimen temperature. The total amount of injury will in turn depend upon the time available for reaction. Many cells that are otherwise injured by freezing can survive low temperatures if they are frozen in the presence of a protective additive such as glycerin. The mechanism of glycerin protection is presumed to be related to its ability to prevent water from freezing, thereby reducing the total amount of concentration to a tolerable level. It would thus appear that susceptible cells, such as those from mammalian tissue, cannot be frozen rapidly without suffering mechanical injury from intracellular crystal formation, but neither can they be frozen slowly without suffering chemical injury from the concentration of solutes. Of course, one must also bear in mind that observations of freezing injury cannot be divorced from the thawing process and that the possibility of thawing injury as an independent event has not been satisfactorily evaluated.

When freezing is the initial step in a freeze-drying procedure, one would like to have some assurance that the freezing alone will not produce irreversible injury. At the moment this assurance is not easy to obtain since, with the exception of viruses and bacteria, most living cells are quite susceptible to freezing and thawing unless pretreated with glycerin. Unfortunately one cannot dry from the glycerinated state since the glycerin does not sublime during the drying, and one is left with a concentrated glycerin solution which is highly toxic. So far, the only mammalian cells that can survive freezing without glycerin are erythrocytes and spermatozoa. Whether the fact that these two are also the only mammalian cells that have been successfully dried merely reflects their resistance to freezing is unproved, but the circumstances are certainly suspicious.

II. *Injury During Drying*

Removal of Free Water. It is generally true that one can remove somewhat more than 75% of the water from a mammalian cell without creating rapid irreversible injury. In addition, somewhere between 5 and 10% of cell water is sufficiently involved chemically so that it does not freeze. When a cell is frozen prior to drying, all free water is removed on completion of freezing and the maximum concentration of solutes is created. Subsequent drying will be carried out on a specimen with maximum potential for chemical injury. On the other hand, when drying occurs from the liquid state, the degree of concentration is a function of the extent of drying. Exposure of the specimen to highly injurious concentrations will not occur until the drying is nearly complete. Thus, the opportunity for chemical injury from concentration can be less when drying is carried out from the liquid state than from the frozen state. In actual practice, of course, the temperature at which drying is carried out can be so much lower for freeze-drying than for liquid drying that the reduced rate of denaturation more than compensates for the prolonged concentration.

One real virtue of drying from the frozen state lies in the increased solubility that results. During drying from the liquid, as the total water content is reduced, surface tension will exert strong inward forces tending to shrink the specimen. During freeze-drying, when the many scattered ice crystals are removed, a porous structure with large surface area and good solubility results.

Removal of Bound Water. So far we have only considered the solute concentration from dehydration during freezing or preliminary drying. This is the dehydration resulting from the removal of free water —the water that does freeze. The remaining water in the cell is chemically occupied in one way or another, bound to chemical constituents with various energies ranging from weak electrostatic forces to hydrogen bonding. This nonfreezable component is obviously much more critically involved in the nature of cell constituents than water of solvation. Bacteria may be frozen or dried to a few per cent residual moisture and remain quite unaffected, but when their moisture content is reduced to a fraction of a per cent, survival is seriously diminished. In particular, removal of the last per cent of water results in extensive loss of viability. It is useful to speculate regarding the way in which bound water might be removed during the drying process and the effects of such removal.

For purposes of speculation we will presume that proteins are among the most sensitive chemical components of a cell. Enzyme systems in particular are highly dependent upon the integrity of their configuration and relationships with other molecules. There are two principal ways in which water is vital in maintaining this integrity. The first of these depends upon water molecules electrostatically bound along the surfaces of proteins. The presence or absence of this layer of water can materially affect the configuration of the protein. Although such water is sufficiently bound to be unfreezable and to fall within our definition of bound water, the binding energy is not high. This water is probably removed during the later stages of conventional freeze-drying. Since many organisms will survive the removal of at least half their bound water, the removal of this weakly bound water is apparently not wholly irreversible.

Water engaged in hydrogen bonding is another matter. This is a relatively high energy bond compared with adsorbed water, and the rate of its release will accordingly be much lower. The last fraction of a per cent of water removed from a specimen is undoubtedly derived from this source. One must always bear in mind that the application of high vacuum or reduced water vapor pressure does in no way alter the probability that a water molecule will leave its site of binding. The sublimation rate for water molecules involved in hydrogen bonding, electrostatic adsorption, or any other state is solely a function of bond strength and the thermal energy of the molecule or, in other words, its temperature. The reduction of water vapor pressure serves simply to reduce the probability that a vacated site will be refilled. If, as would be true during the last stages of secondary drying, the water vapor pressure within a cell were virtually zero, most water molecules that were lost from the sites of chemical binding would be removed from the system without being replaced. One must expect the last stages of secondary drying to be extremely slow since the rate of net water loss from higher energy binding sites will be relatively independent of the degree of vacuum and primarily dependent upon bond strength and temperature.

Inasmuch as any structural alterations which might result from the removal of bound water would require the physical motion of large molecules, the fact that such water can be removed without irreversible destruction of proteins or even of living cells suggests that molecular motion is prevented by prior removal of the free water. This factor, of course, is the basic rationale behind the use of the dried state for preservation. We expect that the removal of water will render all cell constituents immobile and unable to complete reactions which would otherwise take place.

III. *Injury During Reconstitution*

Mechanical. When a cell is completely dried we will have, in a sense, an infinite concentration of solutes. As solvent water is reintroduced, the concentration will decrease rapidly but not uniformly. The wave of water that advances into a dried specimen will contain increasing amounts of dissolved solute, and it is conceivable that the water sweeping through a dried specimen could transport the more soluble elements from one portion of the specimen to another as it moves through. Thus extremes of concentration gradients may exist momentarily throughout the specimen. The extracellular osmotic pressure can vary from that of the pure reconstituting solution to that of approaching saturation. For this reason it is necessary to use an isotonic reconstituting solution. This solution at least prevents portions of the specimen from becoming hypotonic due to the removal of solutes by the passage of the water front. Such a procedure will not, of course, prevent extremes of hypertonicity, and one must presume that major osmotic imbalances exist at least transiently during reconstitution. The large forces involved could obviously be extremely disruptive, particularly if less soluble components of structural elements had not yet regained complete integrity. There is no evidence either for or against the adverse effect of such concentration gradients, but their existence appears almost inevitable.

The observation that purified proteins may be reduced to total dryness without any adverse effect upon their reconstitution eliminates the possibility that the removal of bound water and its replacement is necessarily injurious. Since intact cells usually do not survive extreme drying and reconstitution, this observation suggests that some of the difficulty may be structural, particularly where a cell membrane is involved. Areas of the membrane involved in metabolite transfer might be particularly vulnerable to sudden pressure differentials prior to complete restoration of their integrity, being, as it were, blown out by the pressure differential leaving a permeable membrane.

Chemical. One can visualize two general areas in which chemical effects during rehydration may be of importance. The first and more general area has to do with the relocation of soluble components as described in the preceding paragraph. As it has become increasingly evident that the physical location and distribution of soluble cell components are essential to the metabolism of the cell, the possibility of deleterious effects resulting from physical transfer of such components during reconstitution must be considered.

The second area in which reconstitution can create injury is concerned with the failure to replace bound water properly and immediately. If hydrogen bonds are broken by the removal of water, the question immediately arises whether this is an irreversible event. If we assume that the removal of free water confers mechanical immobility upon cell constituents, then one might postulate that the components which had been joined by the bond would remain fixed in position despite the removal of the glue that had once united them. If this is the case, what are the chances that this bond might be reconstructed prior to the re-introduction of free water and the restoration of mobility? The possibility that this can indeed be done is suggested by experiments with erythrocytes. It was observed that dried erythrocyte preparations which had been held at high vacuum at room temperature for thirty minutes or more could not under any circumstances be recovered intact immediately following drying. However, when such preparations were allowed to stand over a silica gel desiccant for 48 hours or more, one could recover intact cells using dextran or PVP reconstituting solutions (4).

One can speculate that when the erythrocytes are maintained in a low but appreciable water vapor atmosphere the water molecules within the specimen are in sufficient concentration to refill hydrogen bonds although not in sufficient concentration to confer mobility upon the protein. A hydrogen bond, to be remade, requires a water molecule with a certain energy range and orientation, and such bond restoration will require time for completion. If free water is returned to the specimen suddenly, mobility may be restored before all bonds are repaired with denaturation the result. The experience with erythrocytes suggests that even the removal of the last fraction of a per cent of water may not be irreversibly lethal.

Conclusion. The foregoing analysis represents merely an attempt to enumerate the various ways in which cell injury might occur during the various stages of the freeze-drying process. Specific chemical reactions occurring during freezing or drying will be discussed by a subsequent panelist. By using living cells as the specimen material, a most exacting indicator is chosen. The lesson to be learned from them is that, under the proper conditions, many can survive freezing, drying, and reconstitution. This information tells us that the taste and textural changes observed in foods of animal origin at least are not necessarily unavoidable. It would appear unwise to accept a level of quality which might become an industry standard without appreciating the fact that this was a compromise of an attainable ideal. Any compromise with an ideal should be made not only with the recognition that it is a compromise, but also with the admission that a compromise is either inevitable or desirable.

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MINIMIZING PRODUCT DAMAGE WHEN DRYING BIOLOGICAL SOLUTIONS

R. I. N. Greaves

The term "freeze-drying" is one which is used in a very loose sense. If accurately defined, it must be the drying by sublimation from a totally solid material, that is, a material frozen to a temperature at which all eutectic mixtures are solid.

In the light of present knowledge, the criteria for the perfect drying of a biological solution should be as follows:

1. The solution should be rapidly prefrozen to a very low temperature, well below its lowest eutectic, owing to the considerable supercooling exhibited by eutectic mixtures.
2. The frozen mass should then be allowed to equilibrate at a temperature just below its lowest eutectic to allow crystals to grow and so provide suitable pathways for the escape of vapor during drying.
3. Drying should be carried out at this equilibrium temperature, that is, below the lowest eutectic temperature.
4. Prolonged secondary desiccation would be necessary to achieve very low residual moisture content.
5. To remove the last traces of moisture, the material should be sealed in high vacuum in the presence of a Barium getter.

These criteria would have to be greatly modified, however, if we were trying to preserve living cells in the dried state.

To achieve these criteria, drying will be, of necessity, slow and expensive and could not be considered as an economical possibility for a cheap commodity such as food. With food we must set a different standard and dry at the highest possible temperature consistent with an acceptable speed of solubility and at not too great a loss of flavor. Flosdorf's term "lyophile" or solvent loving is a better term for such products than "freeze-dried."

The damage which is done to a product by drying above its eutectic temperature will depend both on the nature of the product, that is, whether it is cellular in nature or a solution, and on the amount and temperature of the eutectic mixture. Proteins dried from the liquid state in the eutectic mixture may be aggregated irreversibly and rendered insoluble. In cellular material the eutectic mixture tends to be greatly dispersed and the resulting insolubility tends to be less marked.

Coffee extracts which have a large amount of eutectic materials may be dried at fairly high temperature if they are at low concentration, but as their concentration is increased, it becomes necessary to dry at lower and lower temperatures if "puffing" during drying is to be eliminated and poor solubility avoided. In drying coffee extracts, therefore, a compromise must be arrived at between the advantages of preconcentration and the cost of drying at a lower temperature.

With meats, the influence of crystal size and position on final solubility appears to be more important than low temperatures for producing good solubility, so that high temperatures may be used for drying.

It is, perhaps, not generally appreciated that the damage done to a product during drying can be profoundly influenced by the method in which the latent heat of evaporation is supplied. The problem may be simply stated as the attainment of a maximum speed of drying, using the highest possible condenser temperature and, to ensure minimum damage, the lowest possible product temperature. Many methods have been tried for applying the necessary heat of sublimation in freeze-drying systems, but only two, conduction and radiation or a combination of both, have been found to be generally applicable.

An analysis of the problem of the most suitable method for applying heat is best made by considering the simple case of sublimating distilled-water ice.

Assuming a coil refrigerated to −40°C, at which temperature the vapor pressure of ice will be 0·1 mm Hg, the temperature at the surface of the drying ice will be determined by the rate of drying and the resistance to the flow of water vapor of the system. The relationship, $\frac{\text{V. P. Difference}}{\text{R}} = \text{C}$ (rate of flow), will be found to hold. This may be written as $\frac{\text{V. P. (t, drying} - \text{t, condenser)}}{\text{R}} = \text{k}$ watts heat input. In an efficient system R, which is made up by the geometry of the system and the pressure of non-condensable gases, should be very low. This factor, coupled with the shape of the V. P. curve for ice, means that quite large variations of heat input make only very small changes in the temperature of the drying surface.

Supposing we have a block of ice, two inches thick, frozen on a metallic heater plate. As melting will not occur till the temperature has risen to 0°C, it is safe to raise the temperature of this plate to −5°C. The drying temperature at the surface will be about −35°C giving a gradient of 30°C through the ice. As drying proceeds, the thickness of the ice will decrease; if the heat is kept constant, the heater temperature will fall. Alternatively, the heater temperature could be kept constant at −5°C in which case the heat input would constantly increase and with it the drying rate.

If heating was by radiation, the conditions would be very different. There would be no gradient in the ice, and if the resistance of the system was low, vast amounts of heat could be applied without significantly raising the drying temperature. But as it would be quite safe to dry at −5°C, we could raise the condenser temperature to −10°C with a vast increase in the economic efficiency of the process. An additional advantage of radiant heating would be that since one side of the block no longer need be in contact with the heater plate, drying could take place from both sides of the block, effectively halving its depth.

With distilled-water ice, there is a very obvious economic advantage for radiant as opposed to conductive heating. However, the advantages for drying a biological solution are not as great because of the limitation imposed by the danger of overheating the dried material on the surface of the block.

Suppose we wish to dry a 20% extract of coffee which has a eutectic at −20°C. With conductive heating it will not be safe to raise the heater temperature above −25°C, so that the maximum permissible gradient across the block will be 10°C. This limitation means that we must either dry slowly with a thick block or faster with a thin block. The calculation of the optimal thickness for maximum economy of operation becomes difficult. As drying proceeds, the increasing layer of dried material causes increasing resistance to the flow of vapor, and if the heat input remains constant, the drying temperature will rise. At the point of contact with the heater plate, the temperature may rise above the eutectic causing local melting. This condition will completely upset the uniformity of heating, and wet patches will probably be found in the dry product.

With radiant heating, as before with pure ice, there will be no temperature gradient across the ice so that a drying temperature of −25°C may be obtained with a condenser temperature of −28°C. The limitation of heat input will be set by the highest safe maximum to which the dried material may be raised. With coffee extract, this temperature is about 80°C. If the radiant heater plates are automatically controlled at this temperature and if radiant heat is applied to both sides of the block, it is found that a 1½″ thick block dries in 38 hours, whereas a ¾″ block dries in 11 hours.

This study was initiated in an attempt to improve the economic efficiency of a commercial freeze-drying plant used for the production of freeze-dried coffee extract and, at the same time, not to degrade the quality of the final product. Originally, heating was by conduction only, and the refrigerated condenser was at −55°C. By combining radiant and conductive heating, the output was increased by 50% with a temperature rise to −50°C on the condenser and the same drying temperature. By increasing the amount of radiant heat and decreasing the conductive heat, the capacity of the plant has been doubled with no part of the material ever rising above −25°C until it is dry. It is hoped soon to double the output once more by using only radiant heat and allowing the condenser temperature to rise to −30°C, which gives some idea of the improved efficiency of the refrigerators at this higher temperature.

The one limitation to the use of radiant heat is the danger of burning the dried product. This danger may be avoided by continuously scraping and removing the dried material from the surface. If this operation is done, as I showed it to be possible at the New York Academy of Science meeting in 1959 (1), very high speeds of drying at very economic refrigerator temperatures may be achieved, resulting in high quality products dried below their eutectic temperatures.

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SOME FACTORS INFLUENCING THE REVERSIBILITY OF FREEZE-DRYING OF FOODSTUFFS

J. Kuprianoff

Introduction

The basic statement of Dr. Meryman about the mechanism of injury of living cells during freeze-drying and especially his presentation of considering the single steps of the process separately is really an excellent introduction to our main subject. Moreover, what has been said in the preceding reports is so complete that being the last in the panel it is really difficult to say something of importance which has not been touched already. Therefore, I would like to restrict myself to some additional remarks on single points which seem to me to be worthy of discussion in a little more detail, especially in relation to foodstuffs.

According to the scheme used by Dr. Meryman, I would like to say a few words about the mechanism of freezing and drying injuries of biological material and about the consequences of the reversibility of the freeze-drying process as applied to foodstuffs. Here we will have to consider proteins and possibly carbohydrates.

The combined effects of freeze-drying which lead to irreversible changes appear to be very complex. In considering the water systems in foodstuffs, we must recognize three ways in which the different substances present may be distributed:

1. As a molecular dispersion of soluble substances, a part of which may form ions. Such dispersions give uniform distribution of solute provided diffusion is not influenced by a semipermeable membrane, and provided the solution is not saturated.
2. As a colloidal solution which is formed by diluting hydrophillic macromolecules in pure water (e.g., albumins) or in salt solutions (e.g., globulins). Depending on the water content, two different forms of structure are possible, sol

and gel, which may be changed reversibly from one form to the other.

3. As an emulsion with substances of low solubility to give a coarse dispersion.

It seems obvious when speaking about the reversibility of freeze-drying that we have in mind the changes caused by transformation of water from liquid to solid state and its removal as vapor. Here we have to take into consideration:

a. The mechanical damaging action of ice formation during freezing on susceptible components of the tissue.
b. The chemical effects of the residual concentrated solution of cell fluid during freezing and drying as well as during subsequent storage on protein substances leading to denaturation.
c. The deleterious effects of the removal of water beyond some limits during drying.
d. The reactions of the more or less damaged or modified product following the addition of water for reconstitution; here we have to think of inducing reactions in the liquid phase.

Biophysical and Chemical Aspects. Individual factors associated with the freezing and drying injuries shall now be discussed in more detail.

Ice crystals may cause denaturation of proteins by mechanical action through pressure (1) which results from the greater ice volume (having in mind specific conditions which may occur during freezing of anisotropic material). The denaturation process leads to an increased permeability of cell walls. This event results in an exchange and mixing of cell components which may now interact. If crystals are large enough a rupture may also occur. Even so, the optically observable structure of products often remain unchanged immediately after thawing (2). From the standpoint of avoiding freezing damage, quick freezing before drying appears to be advantageous for materials with structure. However, contrary to expectation, when consideration is given to the whole process of freeze-drying including reconstitution by addition of water, many products show their best reconstitution after slow freezing. By slow freezing, larger holes are produced which permit better penetration of water and faster escape of air. When foaming is desired as may be the case with certain juices, then vacuum freezing will be effective and useful. It should be kept in mind that a product frozen in the range of −10 to −20°C may contain sufficient unfrozen solution to produce bubbles when subjected to vacuum. This phenomenon has been called "puffing" by Flosdorf.

When speaking about the concentration of solutes in the remaining unfrozen cell juice, it should be kept in mind that the pH value influences denaturation of proteins. If not sufficiently buffered, the pH may reach the isoelectric point at which denaturation proceeds at a maximum rate. In products such as meat it is well known that the water holding capacity is better with higher pH values; and when the pH value is over about 6.3, dripping does not occur in frozen meat after thawing. The change of pH value in meat during freezing, however, is of the order of only 0.1 because meat is so well buffered.

In a frozen substance the denaturation effect of electrolytes is noteworthy only as long as unfrozen water is present. When all freezable water has been frozen out and the residual solution becomes solid at the eutectic point, its action on the proteins along the boundary surface can probably lead only to very slow denaturation. Pure sublimation will take place only when the temperature of drying is below the eutectic temperature of the cell liquid phase. During drying when the free water, i.e., water not bound to protein, has been withdrawn by sublimation of ice and evaporation of solution, then solid solutes may act on protein to cause denaturation only if the affinity of bound water for the solutes is sufficiently high. The quantitative result of this reaction will depend on the nature of the solutes, on temperature, and on the position and extension of the reaction surfaces.

The extension of reaction surface may be of some importance as long as a liquid phase is present as a reaction medium. This is the case in frozen substances at temperatures above −30°C. Practically, it is assumed that in foods all freezable water is frozen at about −30°C; but, recently, very carefully performed calorimetric measurements on meat in Karlsruhe (3) have shown that some small amount of cell juice remains unfrozen until the temperature reaches a range of −50 to −60°C. Besides, we have to remember that even pure water in capillaries may be supercooled down to −30°C and lower and that the supercooling effect will be still more pronounced in the presence of some solutes such as glycols and especially with colloids. Therefore, in the applied temperature range practical for freeze-drying, there is a possibility of the existence of sufficient liquid for a reaction medium; this liquid may disappear during drying.

To demonstrate the action of salts on the water system equilibrium in protein foods, I would like to mention that, according to just published measurements of sorption isotherms by Nemitz (5) on egg white and on desalted egg white (egg white with salt extracted), at 20°C (Fig. 1) the presence of salt decreases the vapor pressure in the region of low water content (between 1 and 5%) by approximately 40%; at 10% water content the pressure drop is about 20%; and at 25% water by about 10%. The amount of unfreezable water in natural egg white has been determined to be about 28.5% (0.4 lb H_2O/lb dry substance). This value decreases to about 20% if the salts are extracted, which corresponds to 0.25 lb H_2O/lb dry substance or about 1/25 of initial water content. Only this water can be considered as bound to the protein molecules (intramolecular bound water). When this limit is reached, the latent heat of water binding must be considered since it increases up to the value of about 400 kcal/kg at a water content near zero. All freezable water is frozen in desalted egg white between −25 and −30°C, although the cryohydric point of natural egg white seems to be below −55°C. Theoretically this temperature should be reached during freeze-drying of egg white to attain maximum reversibility.

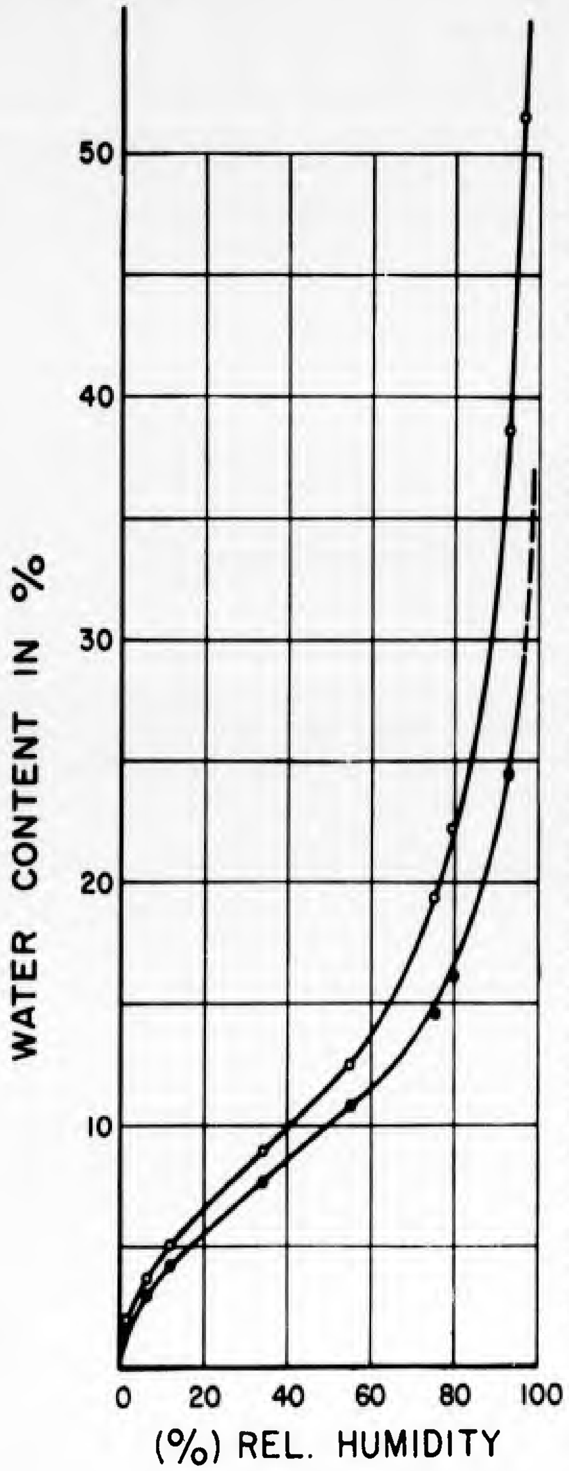

FIGURE 1. Influence of the presence of salts on the water vapor pressure of egg white: adsorption isotherm ($t = 20°C$) for egg white (—o—) and for desalted egg white (—•—). Data from Nemitz (6).

For many foodstuffs the measured amount of non-freezable water does not vary markedly. For meat, fish, and egg yolk it is of the order of 0.4 kg H_2O/kg protein (or nitrogen substance) and that again means 28.5% (Fig. 2). For white bread and other bakery

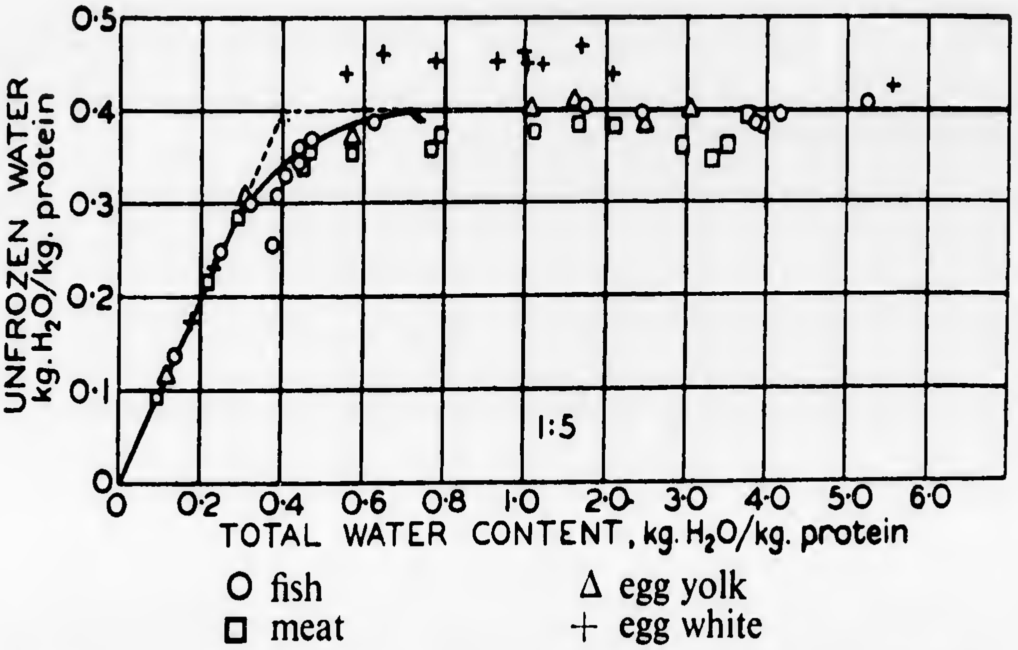

FIGURE 2. "Non-frozen" water in relation to the "total" water content for different products. Data from Riedel.

products it has been determined to be 0.3 kg H_2O/kg dry substance or about 23%.

Speaking about water content one must state (4) that up to now there is some divergency in published values. Recently, careful control measurements (5) on egg albumin have shown the following results:

	Drying Conditions			
Carl Fischer method	Time, hr.	80°C, 15 Torr	100°C	120°
11.05	2	10.98	12.18	12.73
	4	11.96	12.14	12.93
	8	12.18	12.41	13.30

Thus it is apparent that there is still need (4) to standardize the methods of determination of "total water content," otherwise results of measurements, especially at low water content, will remain incomparable.

Moreover, there may be differences in the micro-distribution of water within the dried food itself, leading to the possibility of the presence of liquid phase in very small areas (capillaries, etc.) even in a product with a very low water content as in the range of bound water.

From the above observations it seems probable that from the standpoint of the reversibility of the withdrawal of water during the drying process, some optimal limits must exist for different biological materials. Overdrying is not only uneconomical but also deleterious. In this connection a look into "freezer-burn" reveals some interesting structural changes (4). Here the progressive destruction of meat fibers leads to a complete deterioration of structure as seen from Figs. 3, 4, and 5. Here irreversible changes occur because of freeze-drying under very deleterious conditions (6).

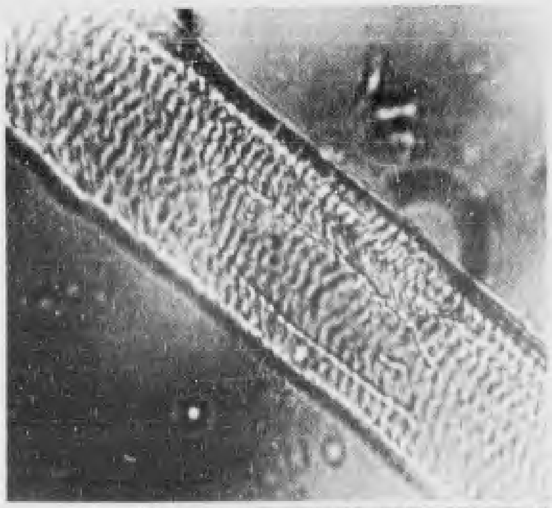

FIGURE 3. Muscle fiber fragment from the freezer-burn zone showing the beginning of damage (X590).

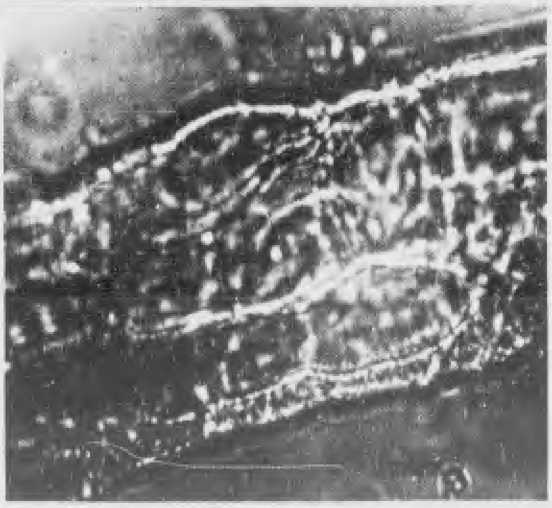

FIGURE 4. Muscle fiber fragment from the freezer-burn zone with advanced shrinkage (X590).

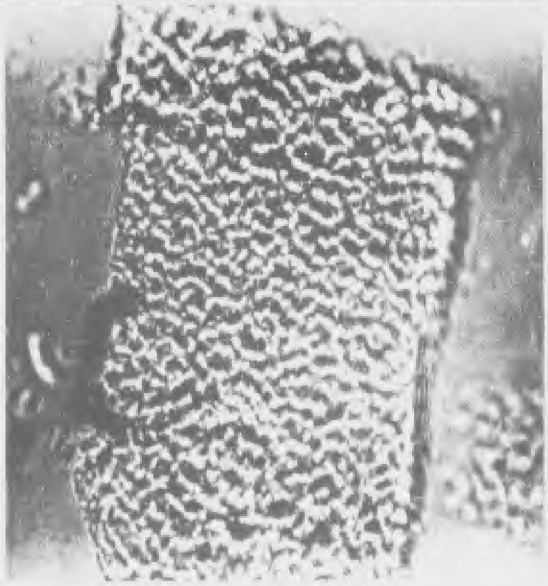

FIGURE 5. Muscle fiber fragment with heavy structural changes caused by freezer-burn (X590).

Based on analytical values shown above for foodstuffs, it can be calculated that the water content should be lowered until only water intramolecularly bound to protein molecules remains. Recently it has been shown (5) that this intramolecularly bound water is equivalent to one molecule of water for each nitrogen atom in the protein. Furthermore, studies on heat sensitivity of proteins have shown that a decrease in heat sensitivity takes place only when intramolecularly bound water is present; this means that the water content should be 20% or less.

In reality it is to be expected that no discontinuity in the different kinds of water binding exists. Therefore, besides intramolecular water also intramolecularly adsorbed water, and water held in capillaries may be present and appear to be bound. Because of this overlapping of different kinds of binding, a water content lower than 20% may be expedient, just to be more on the safe side. This may be of importance during storage when enzymic and chemical changes may occur. For enzymic reaction, capillary water must be present (5). Another factor of practical importance is that the non-enzymic reaction between amino acids and reducing sugars seem to have their maximum reaction rate at water contents near the maximum "intramolecularly bound water" content. This is another reason why the water content of a protein food of about 15% may be more advisable than the figure of 20%, calculated on the basis of protein content. Here a compromise between too high and too low a value seems to be necessary.

It is well known that drying to extremely low levels of water content may have deleterious effects on the reversibility of water removal for many products, when sufficient time passes during subsequent storage. Recent experiments on freeze-dried fish muscle brei have shown that lowering the water content down to 2 to 3% results in an unstable product (7). Storage of several months at 4°C resulted in a considerable decrease of the apyrase activity. Other experiments with the same material indicated a loss in apyrase activity of 15% occurred during freeze-drying to a water content of 3.4% (8). When stored at −24°C no further loss was found during 7 weeks of storage. Samples stored at 20°C in nitrogen showed a loss of 19%, but after 7 weeks storage at 20°C in air the activity dropped to 65% of initial value after freeze-drying (7). It is assumed that oxidative reactions are involved here.

In most cases we are not able to determine the changes in a material caused by drying and by reconstitution separately. We are lacking the exact knowledge of what happens after water is added to the dried product. It is obvious that the state of the product even after very careful freeze-drying and immediate rehydration will be different in comparison with the state of the fresh product—particularly with respect to cell wall structure and a higher permeability to transfer of single components and possibly to the presence of new substances and chemically active groups. The addition of water creates a liquid phase in which many reactions can take place. Therefore, it seems obvious that by avoiding a liquid phase a better reversibility may be achieved, as when water vapor is applied for reconstitution. Nevertheless, complete reconstitution can be expected only for molecularly dispersed soluble substances, not for colloidal systems subject to denaturation or for emulsions which generally break down.

Biochemical Aspects. During rapid freezing and subsequent drying at low temperature, enzymic reactions generally will not be of any significance because the temperature, normally in the region of −20 to −40°C, is too low to permit appreciable enzymic activity during a relatively short processing period. In most cases there is no marked inactivation of enzyme systems through freeze-drying because the process itself minimizes protein damage and dry enzymes usually have good stability at room temperatures or lower. In the case of foodstuffs, contrary to the preservation of living cells, this feature is a disadvantage of the freeze-drying process since high residual enzyme activity poses a problem during storage. During and after reconstitution of dried product the potential for increased enzymic activity is restored. The magnitude of the activity depends in the first place on the water content, processing conditions, storage temperature, and storage time (4). In some cases the preservation of enzymic activity may be of importance, e.g., baking yeast and seeds; in other cases preservation is not advantageous. In all cases, however, it is desirable to have as low enzymic activity as possible during storage in order to retard biochemical changes. When retention of enzyme activity in the reconstituted product is required, storage at a low temperature and at an optimum residual water content helps to prevent enzyme damage (Fig. 6).

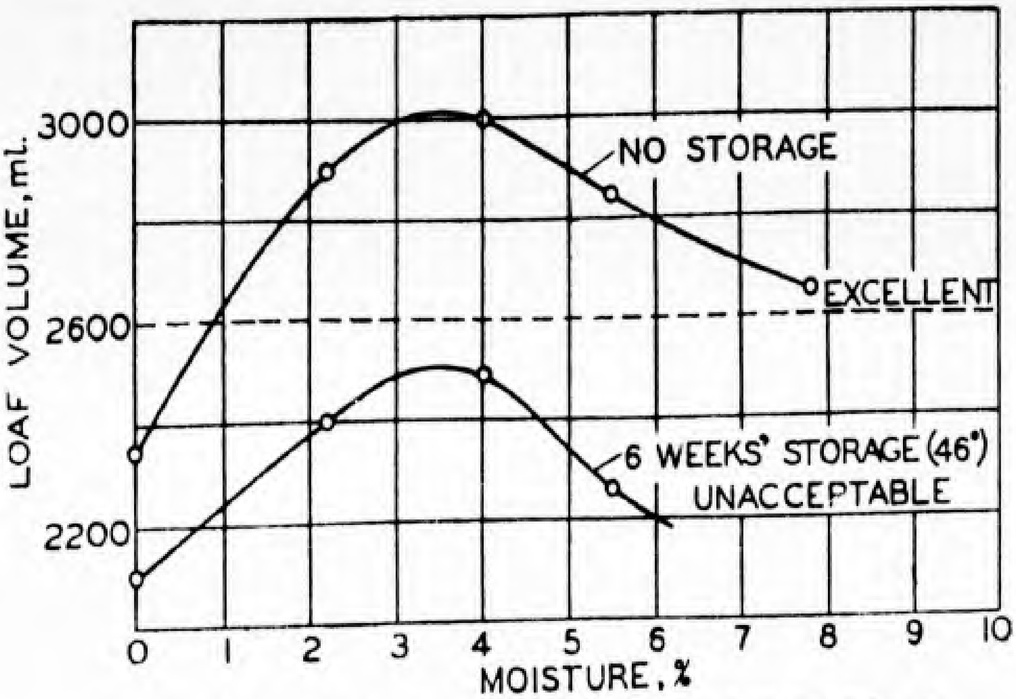

FIGURE 6. Influence of moisture content of dry yeast on loaf volume.

For adequate suppression or even elimination of biochemical changes during storage, a water content in the region of 2 to 5% or even less seems to be necessary. As an example, it may be mentioned that arrest of lipase action in stored flour has been achieved by decreasing the water content to 2% (9). Nearly the same has been found with whole egg powder, the stability of which is doubled by decreasing the water content from 4% to 2%. There is a decrease of enzyme activity during storage, probably because of partially denaturation of the protein part of the enzyme (apoferment) by the action of electrolytes. Reactions will still go on at room temperature. The aging of meat, for instance, will continue unless the water content is extremely low or unless the enzymes have been inactivated in advance. Here only a sufficient preheating (cooking of meat, blanching of vegetables) may help. Lowering of temperature during storage is not sufficiently effective.

Steps Minimizing Damage. On the basis of the biochemical and biophysical considerations here discussed, the following steps appear to be of specific importance in attempting to minimize product damage resulting from the freeze-drying process:

1. With materials having structure, freeze before starting to withdraw water vapor; if foaming is wanted, freezing should be done under vacuum.
2. Dry at a sufficiently low temperature until all free water is removed, only then may the temperature be raised carefully.
3. To decrease the denaturation, withdraw water until a concentration of less than 20%, relative to protein content, is reached. A value below 15% may be better.
4. Avoid marked differences in water content in different zones of the product. Since overdrying and insufficient drying are both deleterious, uniform drying to an optimal water content even at microscopical levels is of great importance.
5. Storage with a water content of not more than 15%; the optimal value must be determined for each item.

Finally I would like to add that it is recommended to standardize the methods for measuring water content and to define "bound water."

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BIOPHYSICAL ASPECTS OF FREEZE-DRYING

Importance of the Preliminary Freezing and Sublimation Periods

LOUIS R. REY AND MARIE-CLAUDE BASTIEN

Introduction

Usually applied to biological materials, the freeze-drying technique has quite recently known very important developments in the foodstuffs industry. The quality and reliability of most frozen and dried foods are excellent and very often well above similar products treated by conventional techniques. Actually, it looks like an unmatchable procedure for the processing of highly alterable products such as fruit juices, fish, or milk. It can even be considered as economically interesting when applied to less costly material such as chicken or beef.

Theoretically, freeze-drying could be the best technique for preservation of foods. However, it must be remembered that any industrial process must result from a happy compromise between the operating cost and the quality of the product to be marketed. The technicians, when they apply freeze-drying to the foodstuffs industry, do not aim then at getting the highest quality rank that could be obtained but to reach the level at which the product matching the good quality standards might still be processed at a competitive price.

Bearing this in mind, engineers developed large scale freeze-drying plants where, thanks to the importance of the throughput, high quality material could be obtained while processing costs were cut down in a sensible way. Thus, freeze-drying of foodstuffs requires very large units and this, of course, implies adequate means of control and automation of the whole operation. Indeed, all the different steps of the process can be of critical importance and during every period of the drying cycle, production needs must match quality requirements of the food. It is thus advisable to study with great care the particular behavior of the material to be processed to prevent drastic alterations in the course of lyophilization. It will be easily understood that there would be little benefit to try and dry chicken or beef very carefully, if an inadequate freezing technique has already destroyed most of its organoleptic properties. This is the reason why every given product has to be processed in its own particular way and, therefore, freeze-drying plants must be flexible enough to fit different cycles. Moreover, accurate laboratory experiments and small productions on pilot plants have to be conducted in every individual case. Among the different assays that proved to be necessary, we feel that the low temperature behavior of the product has to be known with sufficient precision. Indeed, we think that, among other critical steps of the process, the technique of freezing the material as well as a close control of the sublimation phase are of the utmost importance. Therefore, we shall develop that question more fully and give some results of our own experiments.

However, we think that it is useful, before going into details, to summarize briefly the different kinds of products that can be treated and the minimum requirements for a successful operation in each case.

Three main groups are to be found:

1. Products where structural integrity has to be preserved. In this group, most of the organoleptic properties of the food are bound to an adequate preservation of its original texture. For instance, in the case of beef, tenderness, plasticity, and ability to retain water are essential features of the food and have to be recovered after reconstitution from the dry state. In order to offer an agreeable taste when grilled, the meat must not be too mild and thus its original muscular structure must not have been disrupted by a crude crystallization. On the other hand, the water binding properties of myosin must be preserved so that on grilling water can be retained, as it must be, to avoid a complete shrinkage and charcoaling of the meat.

2. Products, deprived from specific structure, but where some particular compounds have to be preserved as they are responsible for the essential organoleptic properties. A good example of this class is fruit juices. A certain number of compounds, mainly esters, ketones, and essential oils, are the main roots of the taste and odor of the juice. Of course, they are not alone, but mixed with numerous substances among which salts, sugars, and acids are the most important. Accordingly, freezing will be difficult. Softening, even melting under vacuum as well as a high hygroscopicity of the dry powder are the main drawbacks of the freeze-drying of fruit juices. On the other hand, in order to avoid the loss of most of the volatile components in the course of drying, sublimation and desorption will have to be conducted with great care.

3. Products, where, independently from given organoleptic properties, special nutritional requirements have to be taken into account. Dietary products, baby food, and milk are examples. In this group, an adequate recovery of taste and structure is not sufficient. The technique must preserve vitamin activity as well as other physiological properties of the food due to the presence of given biological active compounds. The freeze-drying technique has to be elaborated so that interstitial melting does not take place. Most of these substances are highly sensitive to salt concentration, and interstitial melting will undoubtedly promote their soaking in hypertonic solutions and thus induce deleterious modifications of their structure and activity.

Experimental Results

The different experiments that we shall present here are intended to show some aspects of the above problems. For this we chose solutions which were known to be difficult to dry because of their high salt and sugar content. We first studied their low temperature behavior and then conducted controlled freeze-drying operations, the whole process being automatically regulated each time. Besides this, and in order to try and elucidate the problem of possible loss of aromas during freeze-drying, we incorporated a highly volatile compound, acetone, in the solutions. We then followed its fate in the course of freeze-drying and determined the residual amount at the end of the process. Finally, we worked out a standard technique for residual moisture determination according to Karl-Fisher's technique. Then we investigated the hygroscopicity of the dry material and found some very peculiar results.

Adsorption Phenomena: Freeze-Drying of ErGg (Earle-Glucose) Solution Containing Acetone.

In a previous paper (6) we had presented our experiments and results on the freeze-drying of Earle's balanced salt solution containing 100 parts per 1000 of extra glucose and 100 parts per 1000 of glycine. This solution was chosen as more or less representative of a complex system which can be found in biological material. Actually, its behavior is similar to that of fruit juices. For our new experiments we used the same solution and added acetone.

Experimental Solution

The solution was prepared just before use according to the following formula:

glucose	100 g
glycine	100 g
acetone	1.55 g
Earle's balance salt solution	to make 1,000 cc

The specific gravity is 1.080 at 20°C.

For certain experiments, we varied the glucose content from 50 g to 250 g.

Low Temperature Behavior

Our main study was made with the solution containing 100 g of glucose per liter.

Thermodynamic evolutions of the material at low temperature were studied by differential thermal analysis and resistivity measurements (3, 5). Figs. 1 and 2 show the following results:

> A small exothermic phenomenon can be seen between −60 and −56°C and is followed by incipient melting starting near −44°C. Accordingly, all freeze-drying experiments done at higher sublimation temperatures will result in partial evaporation due to the presence of interstitial fluids. However, these are not very important, and up to −35°C induce neither swelling nor foaming of the frozen material.
>
> Electric resistance varies regularly and in the melting zone extends between 2.5 megohms and 50,000 ohms.

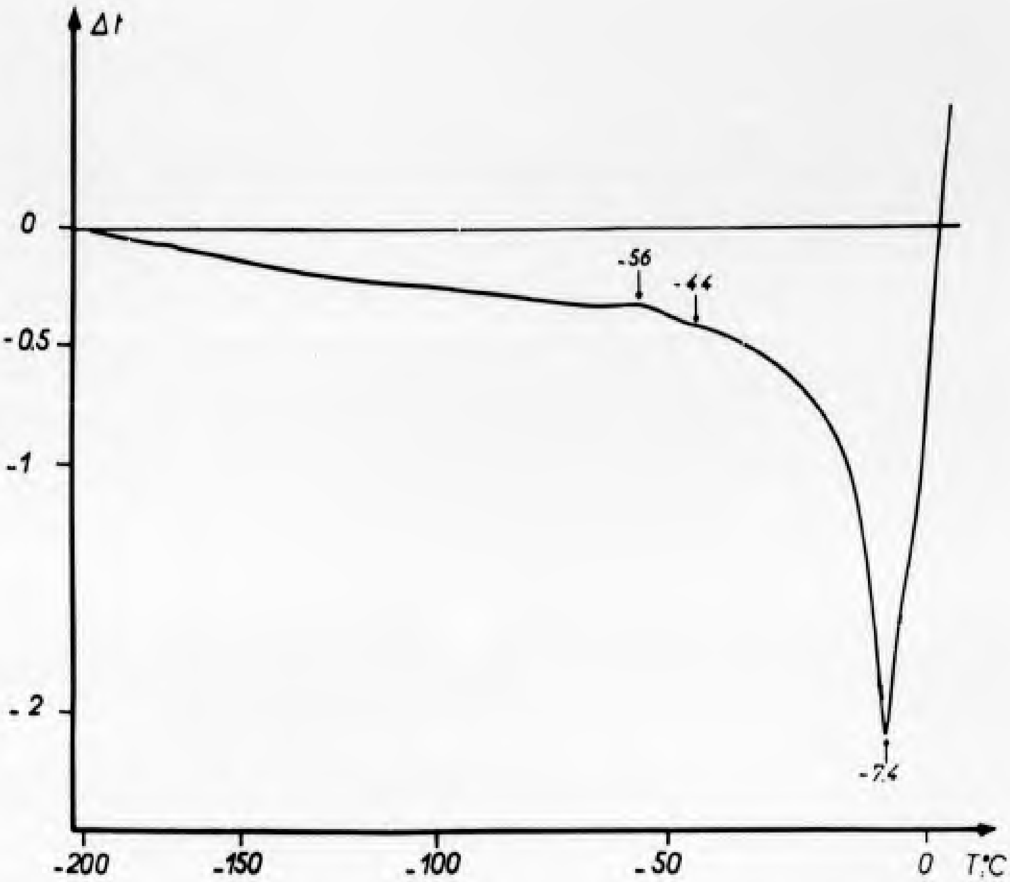

FIGURE 1. Differential thermal analysis diagram of ErGg solution containing acetone. The reference solution is distilled water.

Freeze-drying Experiments

Technical Considerations. Different freeze-drying experiments were conducted with automatic regulation, according to the electric resistance of the product. Actually, we had already shown (4, 5) that in most cases and especially for biological products such as sugars, vitamins, etc., the structure of the frozen material cannot be determined solely by temperature measurements. Supercooling, delays in crystallization, or devitrification processes very often disturb the normal freezing process. The texture and fine structure of a frozen solution depend not only upon temperature, but also upon the way it has been frozen. There is no definite structure of a given product that corresponds to any given temperature. Accordingly, all methods based upon temperature measurements alone are likely to give non-reproducible results.

On the other hand, we had established (2, 5, 6) that, in ordinary cases, variations in electric properties are a reliable index of the inner changes of the frozen specimen and that a very high resistance is always related to a state of utter rigidity. Therefore, automatic regulation based upon the electric properties of the product was expected to succeed in maintaining, during the whole process, a constant rigidity inside the product, whatever its temperature. There-

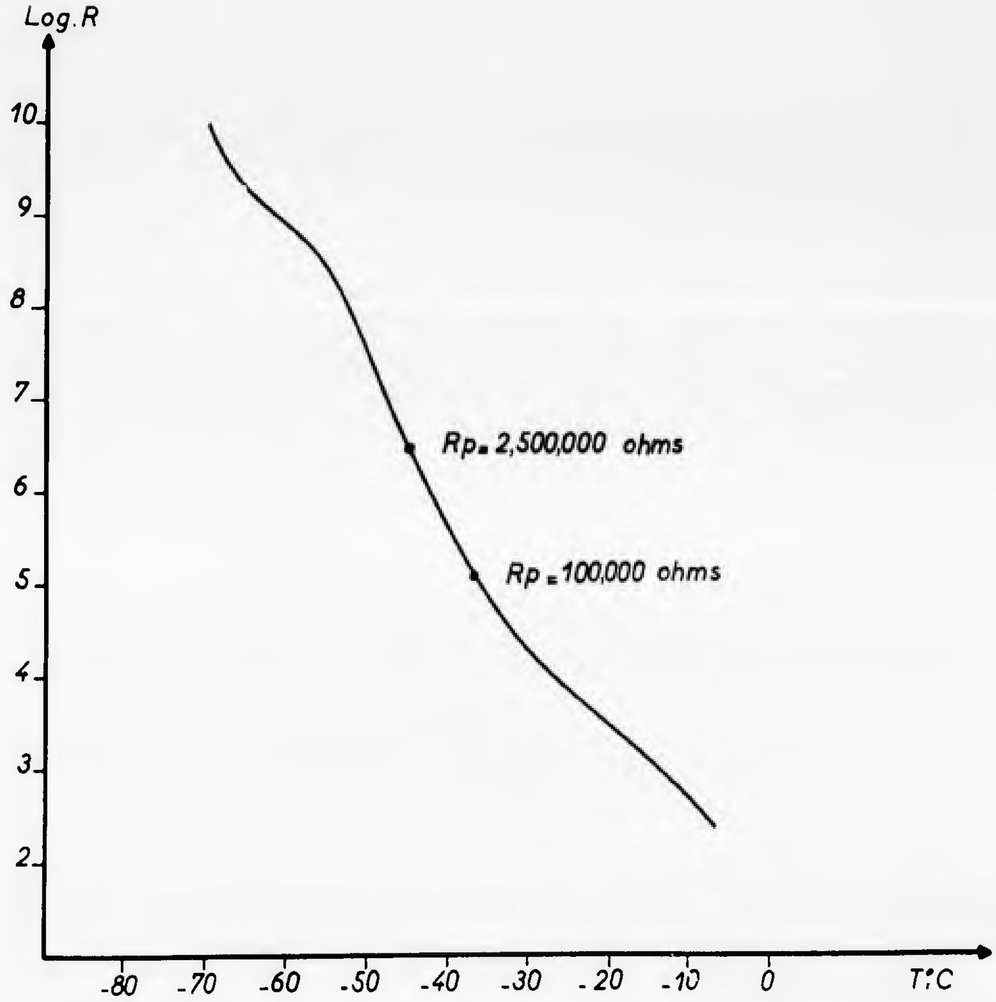

FIGURE 2. Variations of the electric resistance of ErGg solution containing acetone at low temperatures.

fore, in our method, the heating of the shelves containing the product was controlled by an electric regulator of which the sensitive element was inserted in a specimen of the product set apart as representative of the whole batch. When the electric resistance went over the "pilot value," heat was applied; when dropped below, heating was stopped and external cooling was automatically set into operation if cooling by sublimation was not sufficient (2, 6).

The solution was distributed in little glass vials (penicillin type, 10 mm in thickness) and frozen directly in the drying chamber by forced circulation of cold air. The end temperature was around −50°C and the total freezing time was 3 hours. Resistance thermometer and special electric probes (6) were placed in representative samples of the batch. After the drying chamber was sealed and evacuated, the automatic heating was started. By altering the value of the pilot resistance, we were able to change the sublimation temperature and accordingly control the interstitial melting and the length of the process. Figs. 3, 4, and 5 show examples of such regulations. Experiments were performed on an USIFROID SMJ unit.

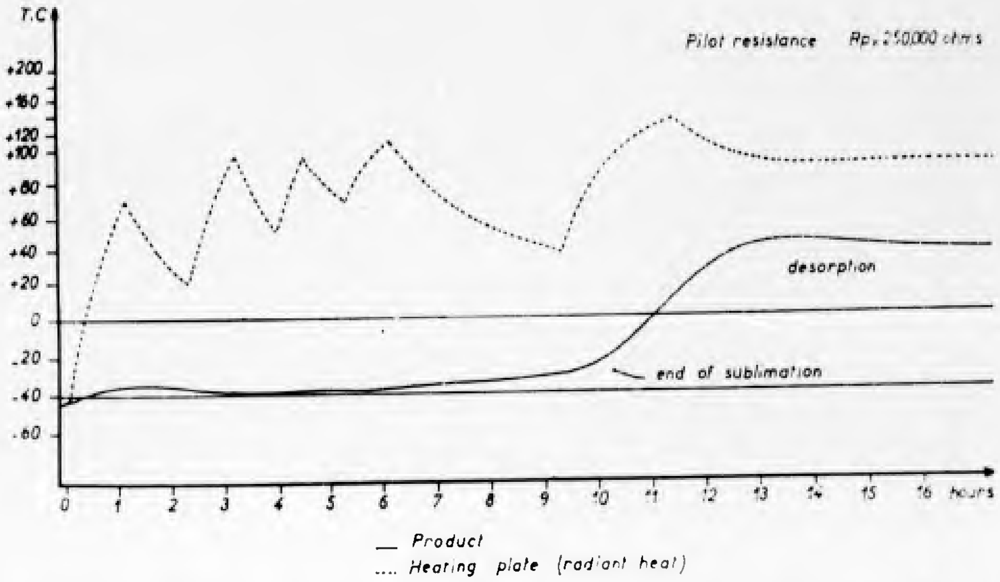

FIGURE 3. Freeze-drying curve of ErGg solution containing acetone with a pilot resistance of 250,000 ohms.

Table 1 gives some data on the freeze-drying cycles that we used.

TABLE 1

Pilot resistance, ohms	50,000	100,000	250,000	500,000	800,000	2,500,000
Sublimation temperature, °C (calculated from resistance curve in Fig. 2)	–34.5	–36.0	–38.5	–39.5	–41.0	–43.5
Sublimation temperature, °C (recorded during freeze-drying experiment)	–34.0	–36.5	–38.0	–40.0	–41.0	–43.0
Length of primary drying, hours (sublimation phase)	6	8	9	12	13	16

On the other hand, we have varied the conditions of desorption by increasing the length of secondary drying and by improving the degree of vacuum with the use of an oil-diffusion pump. (These experiments were done on a BASI BF 6/2 D unit). Table 2 gives the different working conditions.

TABLE 2

Length of secondary drying, hours (since the specimen had reached its final temperature) Isothermal desorption	Pressure in the drying chamber, Torr		Temperature of the specimen, °C
	without oil-diffusion pump USIFROID UNIT	with oil-diffusion pump BASI UNIT	
3	0.01		40
6	0.01		40
17	0.01		38
34	0.01		36
31		< 0.001	36

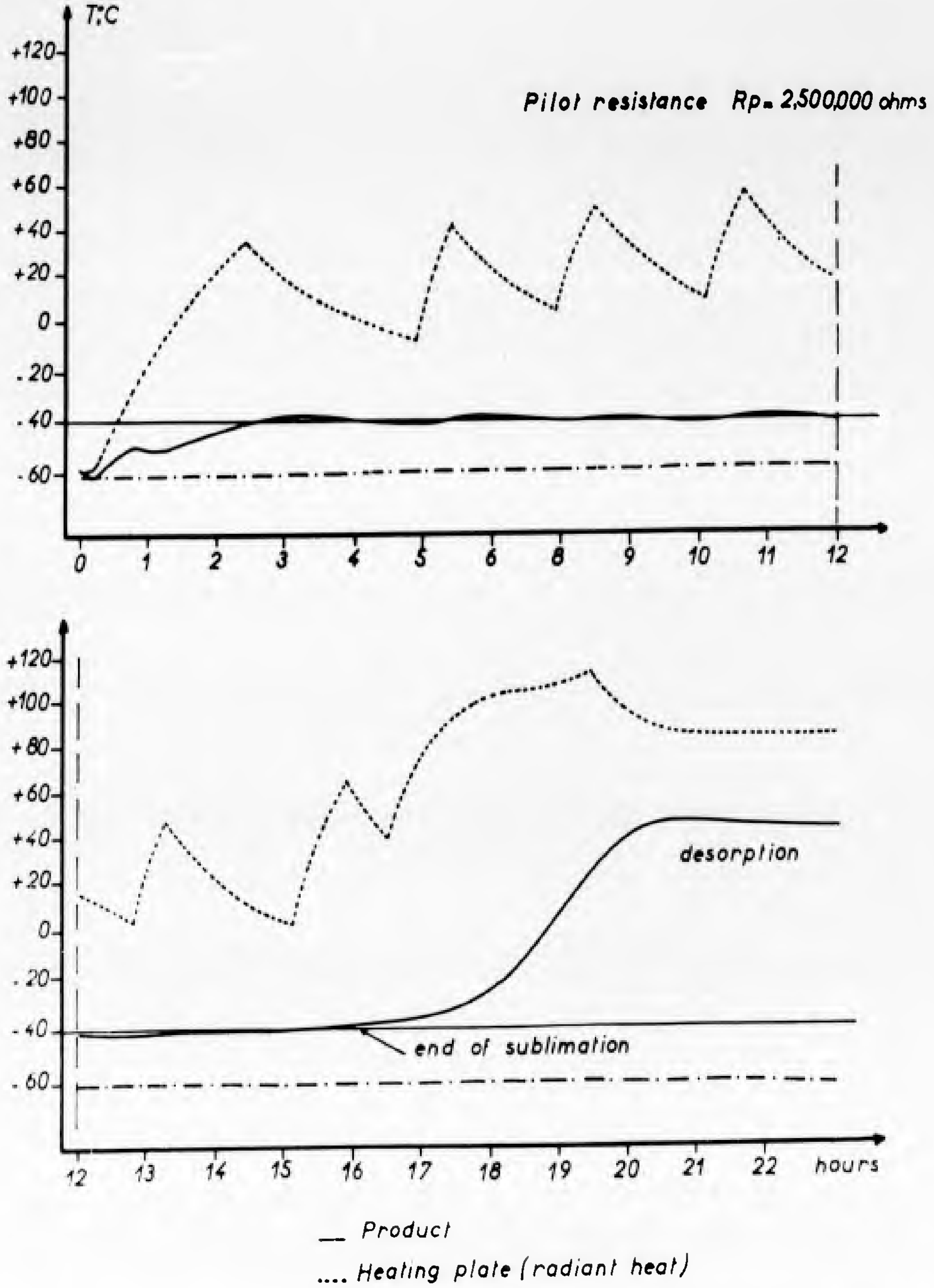

FIGURE 4. Freeze-drying curve of ErGg solution containing acetone with a pilot resistance of 2,500,000 ohms.

In all cases, the final product was correct. Of course, as we had already seen for the same solution without acetone, the structure was more vacuolar when sublimation was carried out at a high temperature. At any rate, the dry cake was very homogeneous and could be solubilized readily with water.

Titrations of Residual Acetone. Moisture and acetone determinations were done on the dry material. Moisture content, as indicated by Karl-Fisher's technique, was roughly between 1.0 to 1.2% of the dry

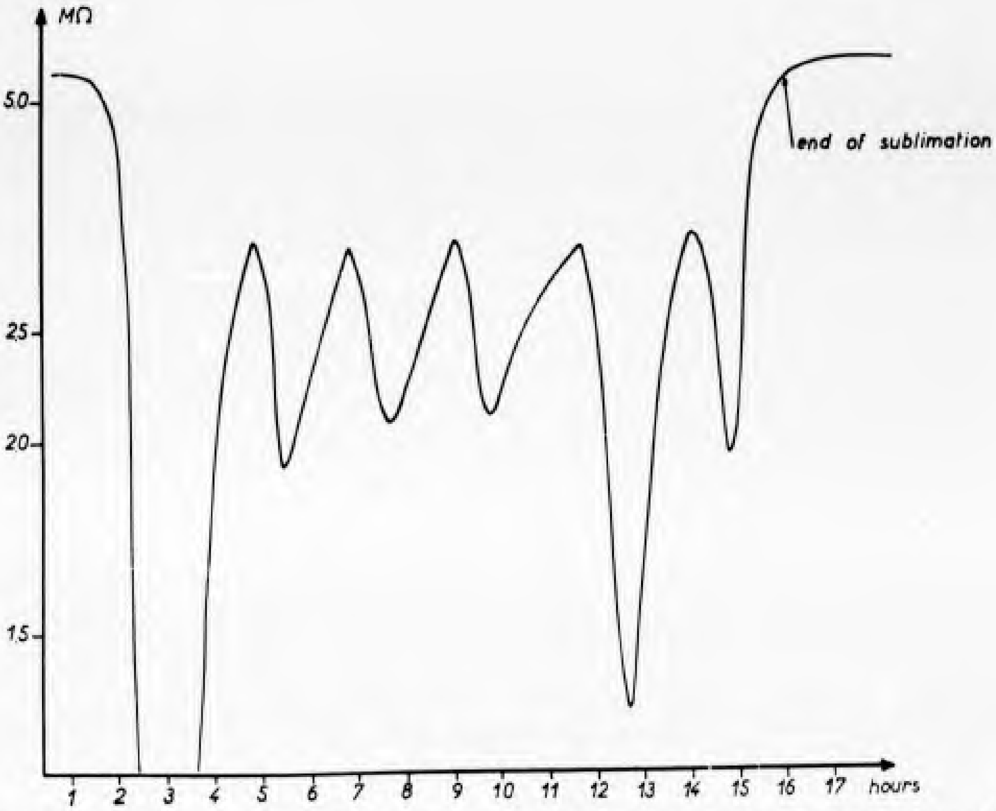

Figure 5. Recording of the resistance changes during the freeze-drying of ErGg solution containing acetone with a pilot resistance of 2,500,000 ohms.

weight. The average weight of the dry material represented 19.2% of the original weight.

Residual acetone was distilled from the reconstituted solution and titrated. Decinormal solution of iodine and sodium hydroxide solution were added to the acetone solution. Then, after formation of iodoform, residual iodine was titrated by addition of sodium hydrosulfite. Control experiments showed that the acetone content was not impaired by the freezing process. On the dry material, the main results were:

> The amount of residual acetone is the same in all the different freeze-drying experiments and, in addition, is not reduced by a long desorption period at high temperature (40°C) under high vacuum.
>
> The percentage of residual acetone is dependent only on the amount of glucose present in the solution:
>
> Data are gathered in Table 3:

TABLE 3

Glucose part per 1000 in original solution	50	75	100	125	150	200	250
Percentage of residual acetone (original solution = 100 parts per 1000)	5	20	30	35	40	45	45

These results are quite surprising if one considers that acetone is a highly volatile component. However, column 3 above shows that nearly one-third of it can be kept in a 100 parts per 1000 glucose-ErGg solution maintained for more than 30 hours at 40°C in a vacuum better than 0.001 Torr. This shows the important role played by adsorption phenomena in freeze-drying. Actually, most of the acetone is extracted at the beginning of the operation. During the initial stages it is possible to observe, at the surface of the product, numerous tiny bubbles coming out of the interstitial fluids and breaking in the drying chamber. But, as soon as the drying boundary begins to sink into the product, the number of the bubbles decreases and they finally disappear. Even if acetone is still partially dragged from the deep parts of the frozen material, along with the interstitial fluids up to the drying boundary, it is fixed on the overlapping dry material. When it is thoroughly adsorbed, it seems to be very resistant and does not follow the water vapor. In fact, at the end of the primary drying period, a large quantity of adsorbed water still remains in the substance. But the acetone content is already close to its final value. On the other hand, during the desorption period, moisture content is brought down to 1.1% and if further drying is done, the moisture content decreases. Of course, glucose has a "buffering action" on residual moisture and it cannot be brought down to very low values. At any rate, during secondary drying, water can be progressively extracted, whereas acetone cannot.

Now, if we think of the great importance of volatile components in foodstuffs, especially in fruit juices, we feel that it is fortunate for many sugars, together with other related substances, to be able to play the role of adsorbant bodies which strongly bind the aromatic components to the dry material. Freeze-drying is an adequate technique for drying these products, and it is clear that there is no other process which can be competitive with it in that particular field. Indeed it is well known that most of the atomization techniques do not keep the volatile components so well.

Determinant Role of Preliminary Freezing: Freeze-drying of Orange Juice.

Orange juice is known to be difficult to dry correctly because of its low incipient melting temperature. However, it proved to be a very interesting material and we shall present some of our experimental data:

Experimental Solution

Plain orange juice was obtained by a moderate squeezing of orange halves in a conventional fruit juice press and used immediately without any dilution or manipulation.

Low Temperature Behavior

Differential thermal analysis and resistivity experiments were done. Results shown by Figs. 6 and 7 are:

A small exothermic phenomenon can be detected around −42.5°C.

Incipient melting starts around −32°C and the corresponding electric resistance is 5 megohms.

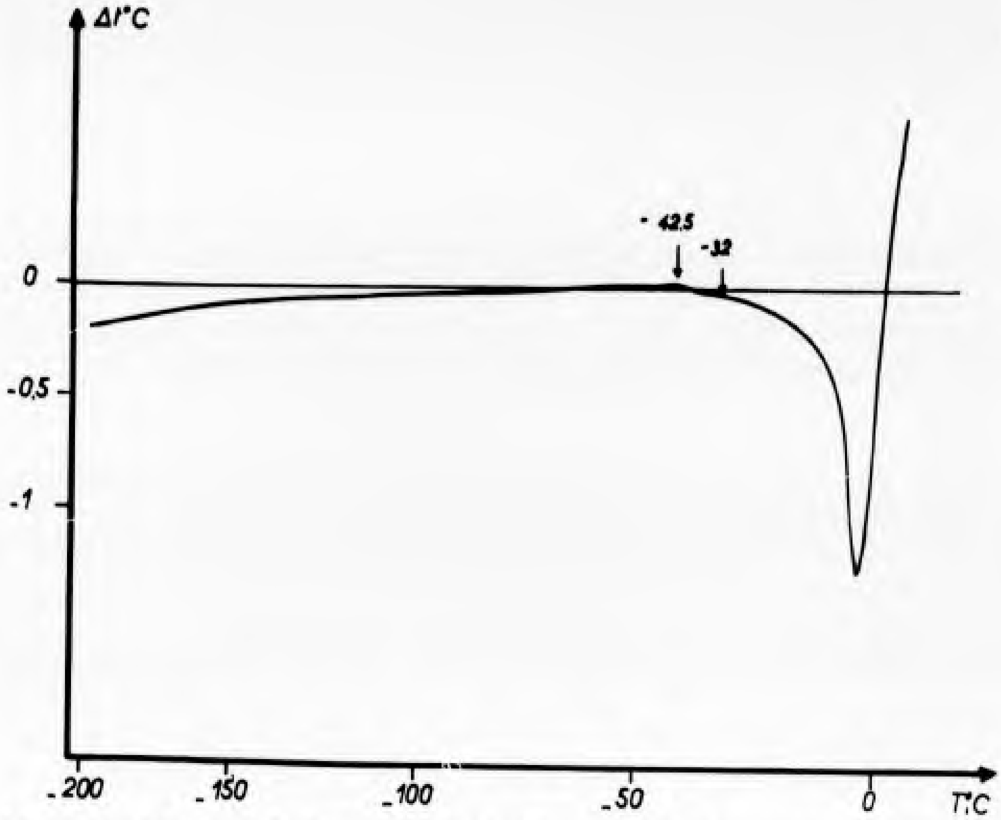

FIGURE 6. Differential thermal analysis diagram of plain orange juice. The reference solution is distilled water.

Freeze-drying Experiments

Freeze-drying experiments with automatic regulation were conducted on orange juice using 5 megohms as a pilot resistance.

In a first group of experiments, orange juice distributed in bulk (10 mm thick in metallic trays) was frozen in the conventional way (forced air down to −50°C in 2 hours). Drying was then carried on. Figs. 8 and 9 show an example of a freeze-drying operation. At the end of the process, the dry material, which gives a homogeneous cake, was taken immediately to a low humidity room (40% R.H.) and either reconstituted and checked or stored in sealed vials under a dry nitrogen atmosphere.

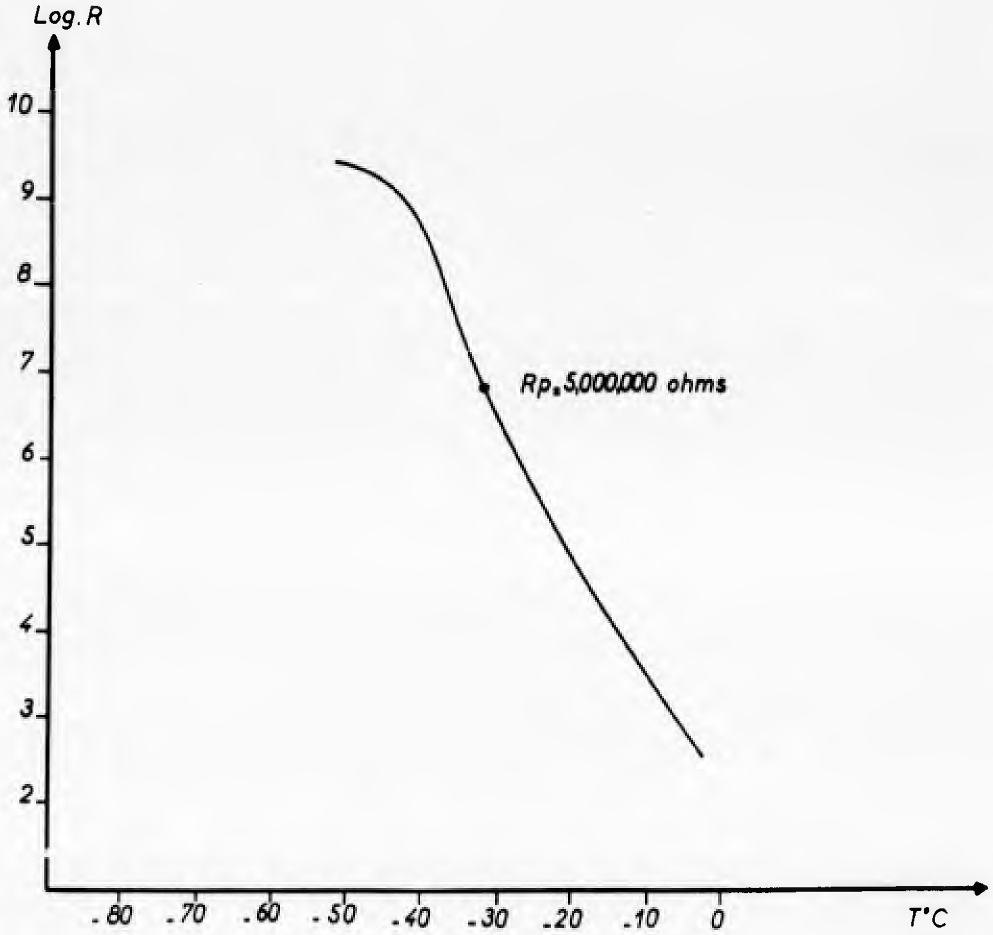

FIGURE 7. Variations of the electric resistance of plain orange juice at low temperatures.

Juice appearance was normal and the reconstituted material quite good and tasty. Initial flavor, particularly, was very well kept and it was really difficult to distinguish the original juice from the reconstituted one. However, the product was highly hygroscopic and formed agglomerates and sticky blocks upon exposure for some hours at room atmosphere.

In a second set of experiments we did try to break the metastable equilibrium which could remain in the frozen material at the end of the freezing cycle. It is our feeling that most of the irregular behavior of the juice is due to the fact that, during freezing, some of the interstitial fluids do not reach an adequate solidity. Their viscosity increases and they look like a solid, but as soon as the temperature increases, they begin to soften and give birth to a net of more or less viscous or sticky material which delays sublimation and often prevents an adequate desorption of water molecules. Actually, in the course of drying, it is frequent to see an enormous bubble all over the tray. This bubble grows during drying and is ruptured at the end of the operation when the vacuum is broken. We did try to rupture those equilibria by thermal treatment. The juice, frozen to

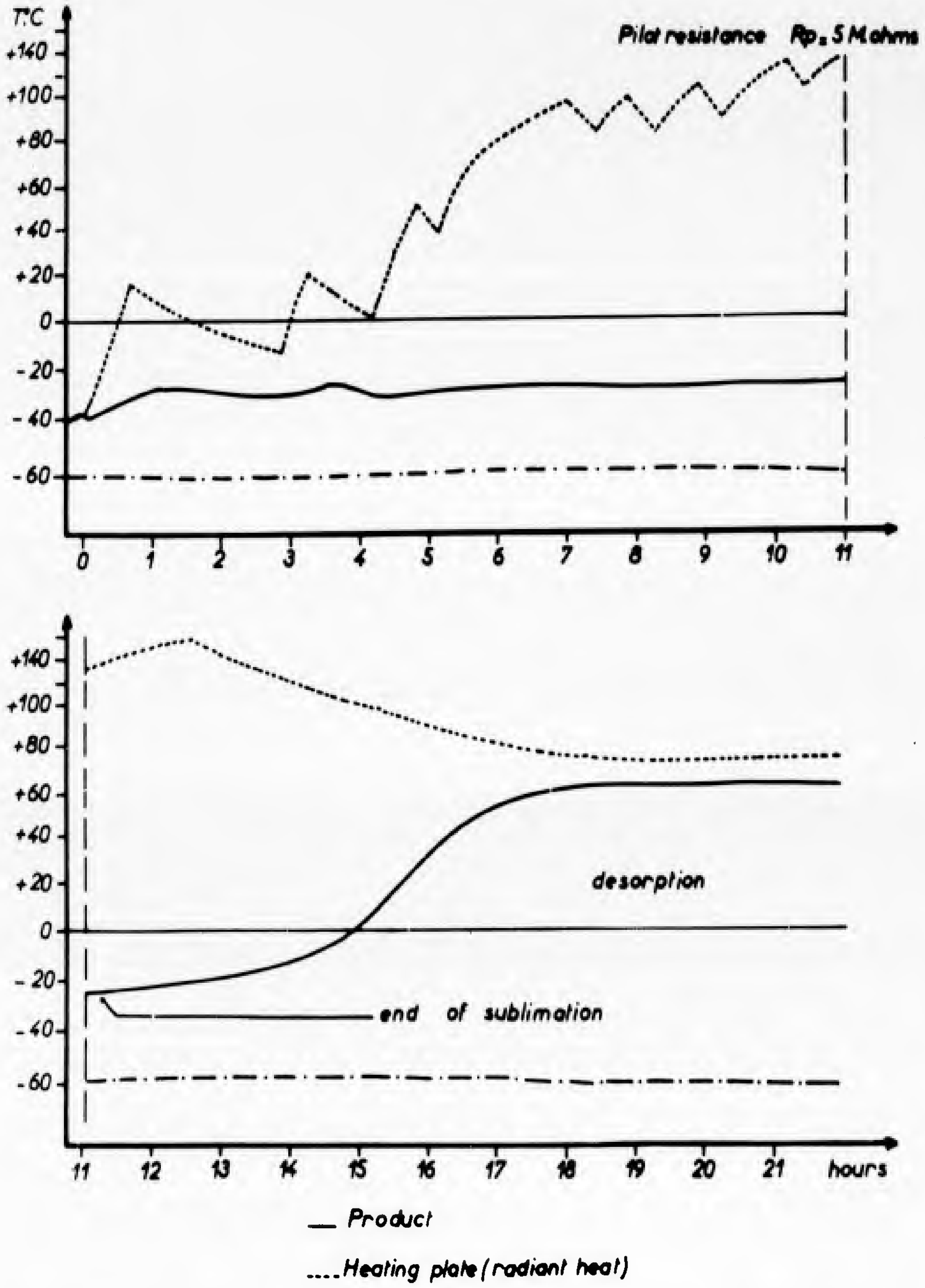

FIGURE 8. Freeze-drying curve of plain orange juice with a pilot resistance of 5,000,000 ohms.

−40°C in the conventional way, was postfrozen in liquid nitrogen down to −196°C, rewarmed back to −65°C and then placed in the drying chamber.

When vacuum was good, heating was applied. Though we did use a pilot resistance of 5 megohms, we were surprised to get a completely different drying curve (Fig. 10). Temperature rose quickly at the beginning bringing the product towards −35°C. Then sublimation rate was sufficient to maintain the drying temperature near

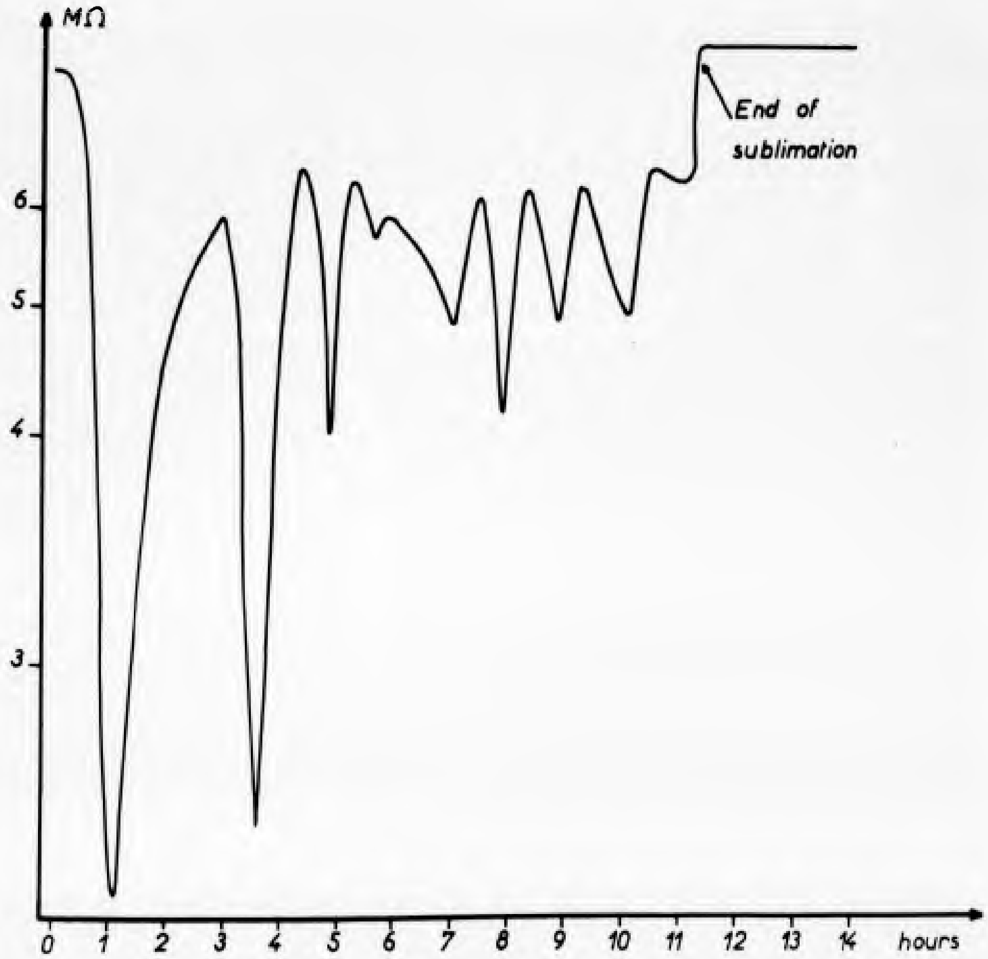

Figure 9. Recording of the resistance changes during the freeze-drying of plain orange juice with a pilot resistance of 5,000,000 ohms.

−35°C despite continuous heating. Primary drying was then very rapid and took less than 5 hours instead of 11 hours as in the ordinary procedure. During the whole time, no bubble appeared at the surface of the product which is a proof of an adequate rigidity of the frozen material. On the other hand, the absence of regulation cycles during sublimation showed that there is no interstitial melting and that the whole primary drying can be done at a temperature lower than the incipient melting point. The main effect of that thermal treatment is, as we have already underlined (4, 5), to help in crystallizing out metastable equilibria and to prevent viscous melting during sublimation. It is reasonable to assume that during postfreezing in liquid nitrogen most of these highly viscous materials are converted into glassy bodies and that those glassy structures are susceptible to devitrification in the course of rewarming. It is likely that this devitrification should correspond to the small exothermic phenomenon seen at −42.5°C (Fig. 6). Then the solid frozen material will begin to thaw only at −32°C and thus can be easily dried at a lower temperature. On the contrary, if the final temperature of freezing is not sufficiently low, those viscous bodies will remain and melt progressively when heating is applied and thus seriously impair the drying process.

A two-step freezing process with very low final temperature seems to be adequate for orange juice.[1]

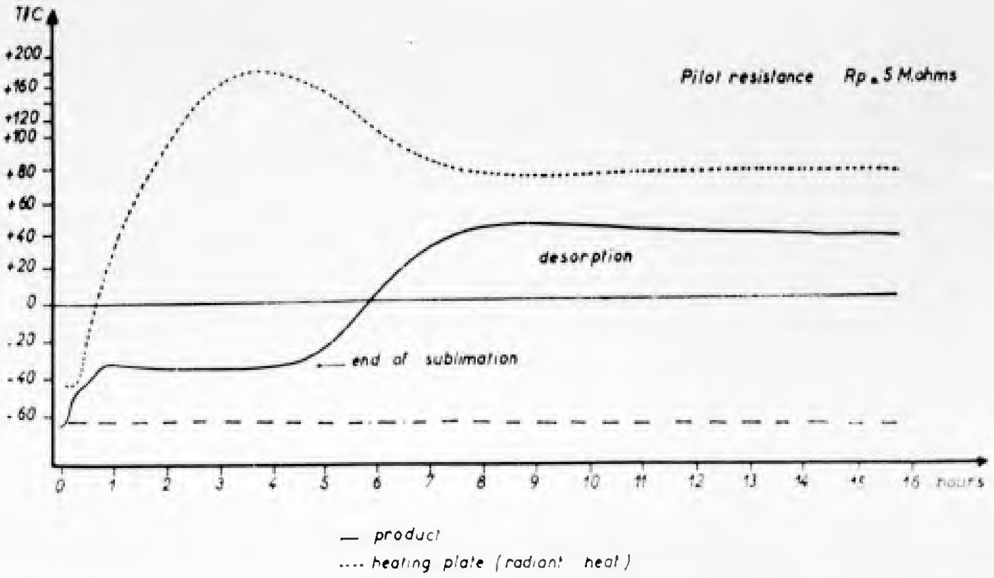

FIGURE 10. Freeze-drying curve of plain orange juice which has undergone thermal treatment with postfreezing in liquid nitrogen. Pilot resistance is 5,000,000 ohms.

Another interesting result of these experiments is that the orange juice dried after thermal treatment is less hygroscopic than the normally dried juice though it has the same initial moisture content (1.6%). This is not limited to that particular example. Actually, we have found that each time primary drying of a well frozen material was done, hygroscopicity of the dry stuff was less important than when drying was achieved from a substance which still contains some interstitial fluids or semi-viscous bodies.

Determination of Residual Moisture

Among the various difficulties that can be encountered when starting freeze-drying, the availability of an adequate method for residual moisture determination is one of the most common drawbacks. We have been working in that field for several years and though important research work is still under way, we shall present some of our experimental techniques and data. For now, we shall limit ourselves to the Karl-Fisher's technique.

Laboratory Setup for Automatic Karl-Fisher's Titration

We first designed a simple automatic recording titration bench for Karl-Fisher's titration of residual moisture. Fig. 11 shows the general setup. Automatic electrometric titration is done from a sample of the product placed in a special titration unit (TITR).

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[1] Patents applications have been made for that process by LEYBOLD Hochvakuum Anlagen GmbH Comp., in Köln, West Germany.

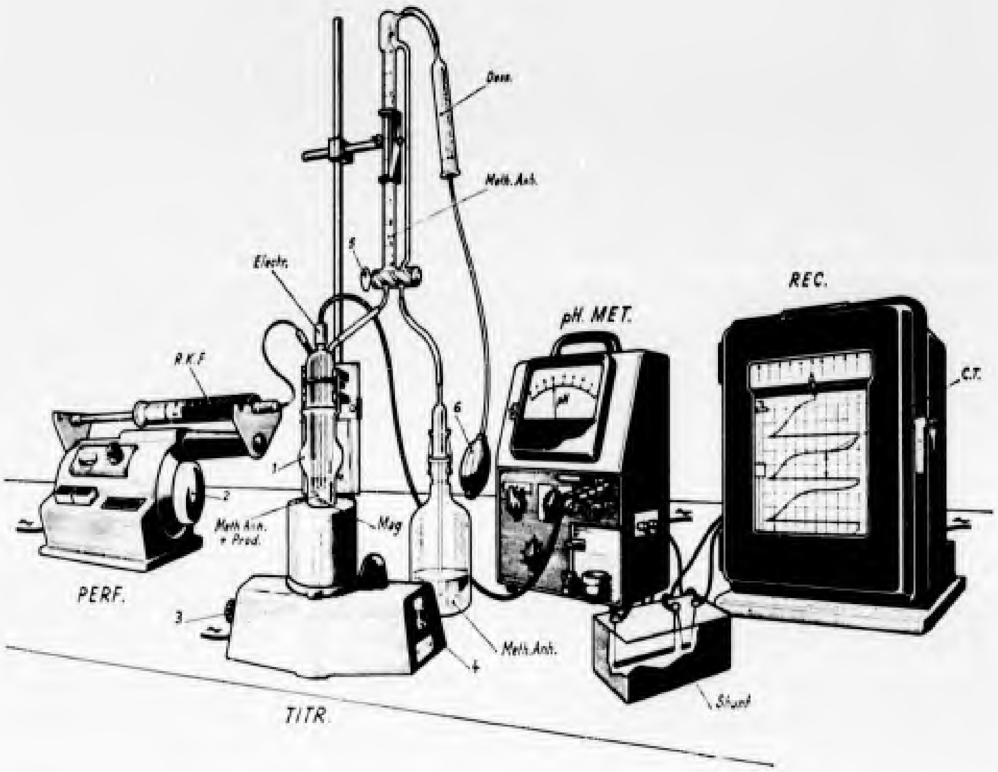

FIGURE 11. Laboratory setup for Karl-Fisher's determination of residual moisture
PERF : Braun electric perfusor
R.K.F. : Karl-Fisher's reagent
Electr. : Titrating electrode
Meth. Anh.: Anhydrous methanol
Prod. : Product to be analyzed
Mag. : Magnetic stirrer
Des. : Desiccant
TITR. : Titration assembly : 1) titration flask — 2) geer-box for speed of injection — 3) Rheostat of magnetic stirrer — 4) switch for magnetic stirrer — 5) Glass key for methanol addition — 6) rubber blower
pH Met : pH Meter
REC. : Potientometric recorder
C.T. : Titration curve.

First, the specimen is placed inside a dry pyrex glass flask containing a glass covered magnet and closed by a ground stopper. (The flask containing the magnet as well as the spatulas and forceps were previously dried over phosphorous pentoxide under vacuum.) Then the flask is weighed on a semi-automatic Mettler balance accurate to 1/100 mg. Samples of 100 mg are generally used. Afterwards the flask is fixed on the Radiometer titration unit and 10 cc of anhydrous methanol (freshly distilled) are added. The liquid is stirred with the magnet for 2 minutes. Then Karl-Fisher's reagent (R.K.F.) is slowly and continuously added to the stirred medium at a constant rate of 0.5 cc per minute, thanks to an electric Braun perfusor (Perf.). The millivolt potential of the titrating electrodes (Electr.) amplified by the pH meter is recorded on a Meci potentiometer (Rec.). The recording paper has a constant speed of 20 mm per minute. It is thus very easy to know exactly the added volume of reagent corresponding to the titration value by measuring the distance between the beginning of the addition and the inflexion point on the titration curve. Accuracy is of the order of 5% for a 100 mg sample of an average 1% residual moisture. The whole setup is placed in a controlled humidity room where a constant level of 40% R.H. is maintained. Room temperature is kept near 20°C. Two titrations are done on analogous samples from the same batch. They must agree within 10%.

One difficulty is the titration of the Karl-Fisher's reagent itself. We are doing it before any moisture determination. For that purpose, the titration curve of 10 cc of absolute methanol is done in duplicate. Then 10 mg of Codex sulfaguanidine are weighed in a titration flask and dissolved in 10 cc of absolute methanol. Two titrations are done. Out of the four curves, we can calculate the titer of Karl-Fisher's reagent and the residual moisture of absolute methanol. Actually, this can be done because Codex sulfaguanidine has a constant moisture content of 7.76% (1). This reference substance is the best one we know and we are greatly indebted to the Roussel Laboratories (Paris) for their information about it.

Experimental Results: Adsorption Curves of Dry ErGg Solution and Orange Juice

In order to evaluate the hygroscopicity of the frozen and dried material, we studied the adsorption of water on the dry sample. After a preliminary determination of the residual moisture, flasks containing the substance are left open in the still atmosphere of a 40% R.H. room. From time to time and at regular intervals, the samples are weighed and an aliquot part is taken out for Karl-Fisher's determination of the residual moisture.

Bearing in mind that the increase in weight is only due to adsorption of atmospheric moisture and knowing the initial moisture content, it is easy to calculate from the weight curve the residual moisture content after periods of adsorption of different lengths. We can then compare the "ponderal residual moisture curve" to the "Karl-Fisher's residual moisture curve."

Glucose Earle Solution. Fig. 12 shows the results. The adsorption of water vapor is very regular and almost linear during the first 5 hours. "Ponderal values" of residual moisture (●) fit very closely with Karl-Fisher's determination (X). After 5 hours, the moisture content has increased, but the material still looks very dry and is neither sticky nor denatured.

Orange Juice. Fig. 13 refers to orange juice dried after conventional freezing to −50°C. Here we can see a very big discrepancy between the two curves. Actually, in the first 30 minutes, though atmospheric moisture is picked up by the dry material as indicated by the weight curve, the chemically titrable water diminishes. We

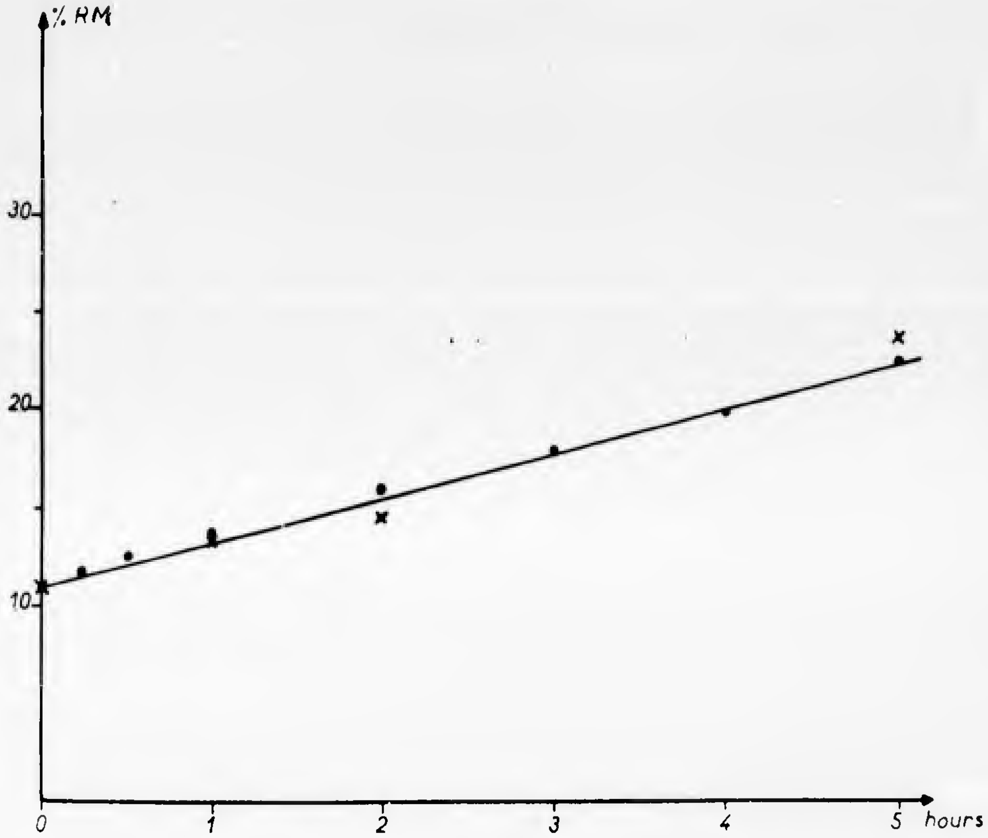

FIGURE 12. Adsorption of atmospheric water vapor by frozen and dried ErGg solution containing acetone in a controlled humidity room. (40% of relative humidity).
x = Residual moisture (R.M.) according Karl-Fisher's determination (measured).
o = Residual moisture (R.M.) according ponderal curve (calculated from weight curve).

think that we can explain this phenomenon as follows. Water vapor distributed first through the substance is progressively bound to the still "humid" parts of the material and possibly to the "sugar" fraction. After one hour, when all the binding sites are saturated, water remains free and can be found in the chemical titration. Then a slow and progressive liberation of free water occurs and the chemical titration figures go over the "ponderal curve" figures. To support that explanation we made the following experiments. Frozen and dried orange juice was left in contact with controlled humidity atmosphere for a certain length of time. Karl-Fisher's determination of residual moisture was then done on an aliquot fraction. The remaining part was left in the flask which was hermetically closed. After a certain length of time, the flask was open and a second titration immediately done. Each time, when the first adsorption period was less than one hour, we found a lower figure for the second determination. For instance, a given sample of orange juice (LEU 168) after 30 minutes of exposure to atmospheric moisture (40% controlled humidity room) reached 2.60% of residual moisture. Then it was hermetically closed. After one hour, a second determination was done and gave 2.27%, though, of course, the total weight has remained unchanged.

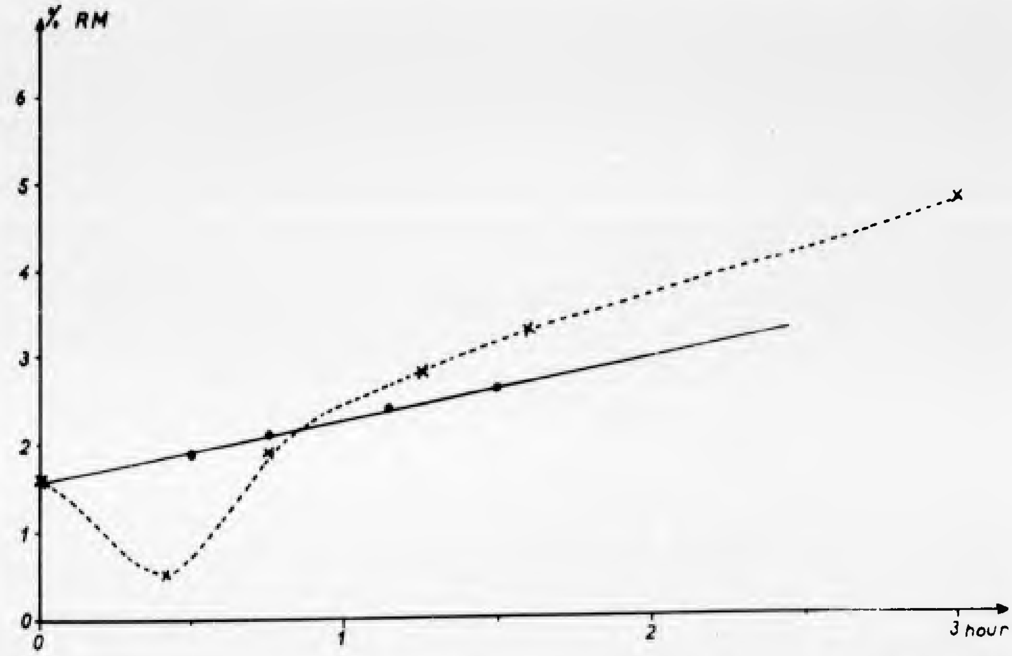

FIGURE 13. Adsorption of atmospheric water vapor by frozen and dried plain orange juice in a controlled humidity room. (40% of relative humidity).
x = Residual moisture (R.M.) according Karl-Fisher's determination (measured)
o = Residual moisture (R.M.) according ponderal curve (calculated from weight curve).

This shows quite clearly that water which was already engaged in the structure of the product was not entirely bound but was still susceptible to be bound more extensively to the dry stuff. At the same time, the binding of water is generally accompanied by a softening of the material which becomes deliquescent after a certain time of exposure to atmospheric moisture.

With orange juice which has been postfrozen in liquid nitrogen the same phenomena are much weaker. Consequently, the hygroscopicity and deliquescence of that material is markedly reduced.

Acknowledgment

The authors want to thank Mrs. J. Dumont for technical assistance.

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BIOCHEMICAL ASPECTS OF DAMAGE IN FREEZING AND FREEZE-DRYING OF BIOLOGICAL MATERIALS

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This communication discusses briefly the biochemical aspects of the damage caused to biological materials during freezing and thawing and during freezing and drying. To do this on the basis of a comprehensive body of evidence is not yet possible because the data available are limited and have often been obtained in studies incidental to some other purpose, usually preservation. A systematic study of the systems involved in the Krebs cycle, for example, or in glycolysis (as these processes are affected by various regimes of cooling and warming or freezing and drying) remains to be carried out. However, much work has been done which is biochemical in nature and which makes possible certain inferences concerning the effects of freeze-thawing and freeze-drying on materials of biological origin. Some of these inferences may have significance in terms of food preservation.

While the evidence is rather disconnected from the point of view of the biochemist, it is possible to synthesize a broad picture which, despite its present lack of detail, seems to indicate clearly enough the main reasons for biochemical change in a material during low temperature processing.

Starting first with the concept of thermal shock: It has been long known that a sudden fall in temperature in the liquid state has a damaging or lethal effect on many types of cells which have no difficulty accommodating themselves to the same temperature change when carried out more slowly. Bacteria (1), viruses (2), plants (3), and mammalian cells (4) are reported to be among the organisms susceptible to very rapid temperature change.

In addition, it is also known that cooling may be too rapid in the solid state. The work of Breedis with leukemia cells (5), Parkes with spermatozoa (6), Medawar and Billingham with skin (7), and Smith with ovarian tissue (8) is conclusive on this point. Our own studies (9) confirm those of Luyet (10) in showing that the erythrocyte is also susceptible to excessively rapid cooling.

Living cells represent complex and delicately balanced systems which, when cooled below their environmental temperature, are susceptible to disruption because of the differing temperature coefficients of their individual components. Joslyn (11) has shown that enzyme activities are progressively but differentially slowed while diffusion continues at rates slowed but little, in comparison, until much lower temperatures are reached. As Smith (12) has pointed out: "the dynamic equilibrium of cells, internally and externally, is thus upset." A sudden temperature change might be harmful to the viability of the cell, whereas, if the change were effected slowly adaptation might take place.

Nevertheless, it is reasonable to speculate that rapid freezing would reduce the amount and duration of disorganization and that rapid cooling to very low temperatures would immediately halt all physicochemical interactions of a potentially degradative nature. In practical operations, however, for a material in which cellular viability is the objective of preservation, it would appear that there exists a range of heat transfer rates during cooling and warming within which maximal yields of viable cells will be attained.

For foodstuffs, cellular viability is not an objective of preservation, but biochemical factors associated with viability, or loss of viability, may also influence qualities such as taste, texture, and, in the case of freezing and drying, food-rehydratability as well.

Intactness of membrane structure is, as Lovelock has shown (13), one of the attributes of a cell which can be seriously affected by "thermal shock." Permeability, for example can be altered by derangement of the membrane lipids. Consequently, depending on the over-all nature of the freeze-drying process, the technique of freezing itself might influence the characteristics of the product on reconstitution with water. For this supposition to be valid, however, it must be shown that damage to tissue as a consequence of "thermal shock" is not, in effect, duplicated as a consequence of the drying process. In other words, if by adequate control of the freezing operation we avoid the damaging consequences of "thermal shock," can we avoid equivalent damage during drying?

The answer to this question must depend in part on the conditions of drying, in particular, on the temperature. As Professor Rey's work (14) has shown, the eutectic temperature is an all important consideration. Clearly, if a prolonged drying operation is carried out at temperatures above the eutectic for the system under consideration, we can expect degradative interactions to proceed in the liquid phase in contact with biochemical elements of that system.

What is the nature and significance of the biochemical changes to be expected under such conditions? At the molecular level we can expect interactions between concentrated solutes (salts) with proteins, lipoproteins, and possibly with the aromatic components associated with odor and taste. Protein degradation will, of course, seriously affect the hydration characteristics of the foodstuff. At the level of structural components—membranes, mitochondria, nuclei, and so on—loss of functional integrity can be expected.

Dr. Greaves' studies (15) have indicated that human serum has a eutectic below −60°C. To prevent degradation of serum components, drying would have to be carried out at a temperature below this. Practical considerations, however, require a balancing of the various factors. Drying is actually carried out at higher temperatures because the amount of eutectic fluid is small and the amount of protein in contact with it minute. So with foodstuffs, since viability is not an objective, we can afford to sacrifice this attribute and dry at a higher temperature—a temperature, however, at which degradative interaction on proteins should proceed at the minimal rate in terms of its effect on rehydration.

Interaction between salt and protein during the freeze-dry operation may be a primary cause of alteration, but reactions leading to insoluble products are known to occur in stored preparations having relatively high moisture content. Lea and Hannan (16) showed that a frozen and dried mixture of glucose and casein was reactive, in that up to 2% of glucose reacted with the protein, without, however, obvious change in the physical properties of the bulk protein. The predominant reaction was a combination of the free amino groups of protein with a reducing group of a sugar molecule. As the residual water content increased, further changes occurred leading to loss of solubility and discoloration. Lea, Hannan, and Greaves (17) showed that similar reactions also occurred in dried blood plasma. These investigators pointed out that interaction is negligible in the complete absence of water.

Nei (18) has studied the effects of freeze-thawing as compared to freeze-drying in bacterial cells and observed a decrease in the amount of P^{32} labeled material precipitable by cold trichloracetic acid in both processes relative to controls. The increase in amount of water soluble substances was greatest in the freeze-dried material, suggesting greater breakdown of cellular components, but of a nature not leading to insolubility.

Differential effects on enzyme systems as a consequence of freezing and thawing have been observed by Nei. While most activities were depressed, *S. cerevisiae* showed increased catalase activity. He also reports increased oxygen uptake by *E. Coli.* Grieff (19) has also shown that oxidative phosphorylation could be reduced in the mitochondria of liver (rats) following freezing while over-all consumption of O_2 was not affected. These observations indicate a physiological imbalance in the cells or particulate material recovered from the process. It would be reasonable to postulate the same type of differential effect in frozen and dried materials. While the effect of freezing or freeze-drying on enzyme kinetics has been investigated only to a limited extent, the available data indicate that the imbalance of effect on interrelated enzyme systems can lead to the accumulation of metabolic intermediates with a consequent decrease in viability.

Modification of the diffusion characteristics of membranes as a consequence of freezing and thawing or freezing and drying could, in addition to reducing viability, lead to the passage of essential intermediate metabolites and cell components to the extracellular or extraparticulate environment. This situation could be expected to lead to disorganized enzymatic activity, with degradative effects occurring more rapidly, on thawing or reconstitution, than would be the case with tissue stored for short periods without low temperature processing.

Some aspects of the biochemical situation with meat tissue will be considered briefly. During freezing and drying the oxymyoglobin of meats is deoxygenated. Free myoglobin is itself labile to oxidation during storage, as a consequence of which the original pink color changes to tan. Meats become quite friable after freezing and drying which suggests extensive disruption of the original protein structure, probably as a consequence of dehydration as well as interaction of salts and other solutes with cellular components during the drying process. Some idea of the extent to which tissue disintegration can be effected by freezing alone is shown in Fig. 1. This study was carried out by Dr. S. W. Moline of our laboratory. Samples of freshly slaughtered, U. S. Grade Good beef were cooled in a −20° refrigerator (slow cooling) and by direct immersion in liquid nitrogen at −196° (rapid cooling). A non-cooled sample served as a control. All the frozen samples were thawed at ambient temperature. Microscopic examination of these samples, histologically prepared, revealed the following:

1. The fasciculi in the fresh and rapidly cooled meat samples were uniformly distributed (Figs. 1b and 1c). In the slowly cooled meat sections (Fig. 1a) the fasciculi were disrupted leaving large interfibrillar spaces.
2. The hemotoxylin and eosin staining intensity of the three different specimens was not uniform. However, the slowly cooled sample showed the most irregularity.
3. The grain or texture of the cross section of the fasciculi in each section varies with the different cooling procedures, but not sufficiently to permit adequate identification of the sample by optical inspection.
4. Identification of the slowly cooled specimen in a series of samples cooled at different rates is made readily microscopically but the rapidly cooled samples cannot be differentiated visually from non-cooled muscle tissue.

The thawed samples were fixed in 10% neutralized formalin, dehydrated with alcohol, embedded in paraffin, and sectioned at thicknesses ranging from 10 to 20 microns. The paraffin sections were mounted on slides, stained with hemotoxylin, counter-stained with eosin, and mounted with "Permount."

It might be of interest, incidentally, to study a property such as friability of tissue dried after various freezing techniques. Would differences exist, for example, between rapidly frozen and slowly frozen specimens?

In addition to oxidation of hematins in tissue which produce the color change pink to brown in about 10 hours, we are also concerned with the oxidation of lipids and the oxidation of dry protein. Harper and Tappel (20) state that 2.5 ml O_2 per gram of dried beef is absorbed per month of storage. (This consumption of O_2 is too large to be accounted for by pigments alone).

With regard to pigments and storage atmospheres, oxymyoglobin and residual oxyhemoglobin are deoxygenated in the drying process to myoglobin and hemoglobin. In storage atmospheres containing O_2


(a) (b) (c)

FIGURE 1. 30 x magnification of fresh meat subjected to various methods of freezing and then fixed.

a) Slow Frozen in an Air Environment at −4°F

b) Nonfrozen

c) Fast Frozen by Immersion in Liquid Nitrogen at −320°F

these compounds are readily oxidized to metmyoglobin and methemoglobin. In inert atmospheres this change is slowed but not halted, which indicates the existence of a second oxidative mechanism involving the transfer of an electron to an acceptor. The globin moiety of these pigments can also be modified on storage or unfavorable processing conditions so that reoxygenation of the pigments is impossible.

Oxidation of protein is the main source (50% or more) of O_2 utilization in freeze-dried tissue. The most susceptible amino acids are cystine, histidine, methionine, tyrosine, and tryptophane. This would probably account for part of the loss of functional and biological properties such as hormonal activity (pituitary proteins), enzymatic activity, and various activities associated with frozen and dried blood plasma.

In the case of frozen and dried beef, Regier (21) has shown that deterioration during storage is mainly due to active carbonyl-amine browning. Scott (22) has done the same for suspensions of bacteria and virus. During storage under adverse conditions (5 to 8% water and high temp. →130°F) the proteins become nonextractable and rehydratability is lessened. At lower H_2O contents degradation is slower. The evidence for the existence of active carbonyl-amine browning includes the following:

1. Yellow-green fluorescence under ultraviolet light.
2. Glucose levels decline in the stored tissue.
3. Amino groups decline (as shown by formal titration).
4. Increase in reducing groups (as shown by ferric ion reduction).

5. If glucose or phosphorylated sugars are added to meat before freezing and drying, an increase in development of brown color is observed after drying.
6. Reaction rate (color development) increases with pH.
7. Amines and NH_3 as well as carbonyl compounds have been detected in stored freeze-dried beef and relatively high water content.
8. Activation energy (apparent) of 25 Kcal/mole.

To summarize, then, the picture of reaction by and interaction with biochemical entities as a consequence of freezing and thawing or freezing and drying of materials of biological origin is as follows:

With organized tissues and cells the act of cooling, itself, can be expected to induce dislocations in interrelated enzyme systems due to the differing temperature coefficients of these systems. Such dislocations are not necessarily associated with a phase change and may be intrinsically lethal before the freezing point is reached. Studies with bacteria, spermatozoa, and erythrocytes strongly suggest that the rate of cooling is the critical factor rather than low temperature per se. For some cells the greater the rate, the greater the degree of dislocation.

The structural components of the membrane, lipids and proteins, may also be affected in their relationship to each other, leading to alteration in membrane permeability. In this case the cooling rate may not permit spatial readjustments necessitated by differing coefficients of expansion. Consequent molecular disorientation may lead to the irreversible severance of weak bonds with loss of ability to maintain cation concentration gradients and possible actual loss of other intracellular contents.

These consequences of "thermal shock" may be reduced or eliminated by proper control of cooling conditions in the liquid state, but remain to be dealt with along with additional factors, after the phase transition has been completed. The removal of water as a solvent will result in solute concentration, in pH change as buffering salts exceed their solubility limits, in protein precipitation, in possible gelation followed by syneresis, and in the elevation to toxic levels of substances such as urea and dissolved gases, normally present in harmless amounts. The concentration of extra- and intracellular salt may lead to degradation of membranous structures including nuclei and mitochondria. That preservation of viability is maintained in substances subjected to freezing indicates, of course, that these difficulties can be circumvented.

Where cellular viability is not desired, as in the freeze-drying of foodstuffs, storage stability of the qualities of taste, odor, texture, and appearance depends on the extent of interaction of biochemical substances among themselves and with the oxygen of their environment. The principal reactions leading to the degradation of frozen and dried foodstuffs appear to be the oxidation of pigment, the oxidation of proteins and lipid, and the interaction of compounds containing carbonyl groups with those containing free amino groups, the so-called "browning reaction." The rate at which this last reaction proceeds is directly related to the residual water content of the dried material and the storage temperature.

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SESSION NO. 2
INCREASING PRODUCT STORAGE LIFE—BASIC DETERIORATIVE REACTIONS

D. M. DOTY, *Moderator*

THE EFFECTS OF FREEZE-DRYING AND SUBSEQUENT STORAGE ON THE PROTEINS OF FLESH FOODS

J. J. CONNELL

Introduction

A great deal of work has been done on the effects of freezing and freeze-drying on cells, organisms, foods, and so forth, and striking advances have been made in this field. However, one may be forgiven perhaps for taking the view that much of this success on the biological side, anyway, has been achieved in the face of an almost complete lack of understanding of the purely chemical or biochemical reactions involved in freezing and freeze-drying. It is necessary to confine this view to chemical and biochemical reactions because following upon the work of Luyet, Lovelock, Meryman, Rey, and others we now have a fairly clear picture of the physical events occurring during freezing and freeze-drying. That is, we have some rational principles with which to guide improvements in the engineering aspects of the freeze-drying of foods but much fewer to guide the chemical or biochemical aspects.

In particular, I want to direct attention to our lack of knowledge about the effects of freeze-drying and storage on those labile and almost ubiquitous constituents of foods, the proteins. The foods I shall be discussing are meat and fish. This is an apt selection because the freeze-drying of these foods has received special attention in recent years. Their high initial cost makes them attractive propositions for the freeze-drying process. Unfortunately, they are among the most difficult foods to freeze-dry successfully.

Although this Session is primarily concerned with the deterioration in quality of freeze-dried foods during storage, I believe that an understanding of the reactions underlying this deterioration would be facilitated if we had a clear picture of the nature of the starting material, i.e., of the freshly-prepared freeze-dried food. One cannot completely define a reaction without first defining the state of the reactants and their end products. At the present time we have only a partial picture of the state of the proteins in freshly-prepared freeze-dried beef or fish. I will first review what is known of the proteins of freeze-dried meat and fish and then deal with reactions of the proteins during storage. In both instances possible relationships between the proteins and the texture or properties of the food will be discussed.

The Proteins of Freeze-Dried Flesh in Relation to Texture

Freeze-dried raw meat and fish (at least those products that I have encountered) are, after reconstitution and cooking, distinctly tougher and less juicy than untreated cooked controls. This defect, which is probably the main cause of loss of quality in these products, is particularly noticeable with fish. Fish is very soft and glutinous in texture, and changes in these characteristics occur readily and are easily noticed. In fact, fish makes a useful experimental subject; I believe that if one could freeze-dry round, white fish and store it successfully, one could do the same with any flesh food.

It is easy enough to conclude that these textural changes are the result of changes in the flesh proteins, but which proteins are involved and what is the exact nature of the changes?

It is well known that the gross amount and condition of the connective tissue in flesh has an important influence on tenderness. However, in view of their relative inertness, it seems unlikely that freeze-drying would affect much the major connective tissue proteins. On the other hand, we are still uncertain of the part played by structures such as the sarcolemma and sarcoplasmic reticulum in influencing the psychorheological concept we call texture. Both structures just mentioned are probably lipoprotein in nature, and certain lipoproteins are definitely damaged by freeze-drying. If changes do occur in the connective tissue or other scaffolding elements of the muscle, it is unlikely that they can account for the pronounced loss of water-holding capacity shown by freeze-dried flesh.

There is good evidence that the sarcoplasmic proteins survive freeze-drying virtually intact. The electrophoretic and ultracentrifuge properties of extracts of this group of proteins prepared from freeze-dried beef and cod are very similar to those of frozen controls. In addition, the amounts of these proteins extractable from freeze-dried beef and cod are also unchanged. Dr. Matheson has demonstrated the presence in freeze-dried beef and cod of several of the typical sarcoplasmic enzymes. All these results are not surprising when we consider that these proteins are rather like the serum proteins in character, and it is well-known that the latter (with the exception of the serum lipoproteins) can be freeze-dried with impunity.

The toughness developing during freeze-drying always appears to be associated with a loss of the true water-holding capacity of the muscle. In fact, I would go so far as to say that the development of toughness in processed cod is always associated with a loss of true water-holding capacity. By true water-holding capacity I mean, of course, the ability of the muscle protein to hold water firmly in the form of a gel. Now, it is unquestionable that the water-holding capacity of muscle resides in the ability of the principle myofibrillar proteins, i.e., actin and myosin, to form a network gel. What is more, certain of the viscoelastic characteristics of the muscle, e.g., elastic modulus, are determined by the state of these proteins. Finally, these proteins are very susceptible to denaturation. In other words, it is evident that changes in actin and myosin or their association with one another are prime causes of textural deterioration following freeze-drying.

Results reported by us some years ago showed that, judged from the amount of protein extractable from freeze-dried cod with neutral 0.5 M KCl, either or both of these proteins, actin and myosin, were denatured by the freeze-drying process. That is, no actomyosin could be extracted from the product. In this connection, denaturation is defined as the development of insolubility in a solvent that normally dissolves the protein near its isoelectric point. As will be shown, this criterion has its pitfalls if not used with care. Practically all the actomyosin in fresh or frozen and thawed cod muscle can be extracted into neutral 0.5 M KCl, but it has since been confirmed that only traces of protein having the properties of actomyosin can be extracted from freeze-dried cod. On the other hand, Cole and Smithies have shown, and we have confirmed, that the amount of actomyosin extractable from freeze-dried beef by normal procedures is only slightly less than that extractable from fresh beef. However, the actomyosin complex extracted from freeze-dried beef appears to sediment faster in the ultracentrifuge than that extracted from fresh beef.

These observations left unanswered the question of the separate roles played by actin and myosin. In recent experiments we have found that the actin of cod flesh appears to survive *in situ* freeze-drying. I say "appears" because actin is a very queer protein, somewhat difficult to characterize. As much actin is extractable from freeze-dried cod as from a frozen control, and the protein that is extracted possesses in full the characteristic reactions of actin, i.e., it polymerizes and combines with myosin. What about the myosin? A roughly quantitative estimate of the amount of myosin can be obtained by extracting muscle with a solution of slightly acid 0.5 – 0.6 M KCl containing magnesium ions and pyrophosphate, i.e., Hasselbach and Schneider's Solution. Using this extractant we have found that about 75% of the myosin is extractable from freeze-dried beef as compared with a frozen control. In the case of cod the proportion is about 50%. Freezing and thawing alone do not reduce the amount of myosin extractable from cod. The properties so far examined of the myosins prepared from freeze-dried beef and cod are identical to those of normal myosin, i.e., their sedimentation behavior is the same and they combine characteristically with F-actin.

As far as freeze-dried cod is concerned, pyrophosphate appears to be a specific reagent for extracting myosin because very little, if any, protein is extracted from this material by a variety of neutral solutions including some (metaphosphate, tripolyphosphate, citrate) designed to complex with calcium ions.

Therefore we have a situation where although no more than about 1-3% of the actomyosin can be extracted from freeze-dried cod, all the actin and half the myosin can be. By measuring only the extractable actomyosin we have obviously been given a false impression of how much myofibrillar protein had been denatured. Similar observations to those just described have been made with cod stored in the frozen state until it has become tough and dry.

In attempting to account for these phenomena, we have considered them in relation to the Hanson-Huxley picture of muscle ultrastructure. This picture shows that the numbers of actin-myosin bonds in normal, relaxed muscle is small and confined to the area of contact between the two interdigiting arrays of myofilaments. Yet small alterations in the numbers or state of these bonds are crucial in determining the over-all physical state of the muscle, e.g., whether it is relaxed, contracted, or in rigor. These bonds can be dissociated under certain conditions by addition of ATP or pyrophosphate to the muscle.

It seems likely that the numbers of actin-myosin bonds will increase during freeze-drying by two processes: 1) the removal of the water normally interposed between the two arrays and 2) the tendency of the myofibrillar protein to dissolve as a result of the inevitable concentration of solutes. This increase in bonds would have two probable effects. Firstly, it would increase the size (or possibly alter the shape) of any of the actomyosin complex that is extracted by means of 0.5 M KCl, as in the case of beef, or tend to fix the actin and myosin molecular arrays in the muscle, as in the case of cod. Secondly, it would make the muscle more rigid and firmer, tougher if you like, and would reduce the water holding capacity of muscle because fewer water holding sites are available.

It is necessary to add the postulate that at least some of the additional bonds formed between actin and myosin during freeze-drying are still dissociable by pyrophosphate. This postulate receives support from the observations of Hopkins on frog muscle and Matheson on beef and cod that some part of the contractile mechanism survives freeze-drying. What is more, addition of pyrophosphate slightly improves the texture of accelerated freeze-dried beef.

However, this is not the whole story. It will be recalled that not all the myosin of freeze-dried beef or cod is extractable, which admits the possibility, at least, that some of this protein is indeed denatured, i.e., rendered insoluble near the isoelectric point. Denatured myosin molecules in solution show a pronounced tendency to aggregate laterally, a process that if carried far enough and if actually proceeding in the muscle cell could obviously lead to an increase in rigidity of the whole muscle.

Some years ago we obtained evidence from measurements of the stress-strain behavior of cod muscle fiber bundles showing that toughening during freeze-drying and subsequent storage is associated with an increase in the number of bonds between the myofibrillar proteins. It was found that increases in toughness assessed by chewing resulted in increase in elastic modulus of the bundles. Thermoelastic measurements on these bundles indicated that they could be treated as quasi-rubberlike materials (as, in fact, can other dried muscle systems).

For these materials, increases in elastic modulus are the direct result of increases in the numbers of bonds between the molecular network elements which in the case of muscle are the molecules of myofibrillar protein. This process can be imagined as a change from a "fine" to a "coarse" gel. Drs. Hamm and Deatherage have proposed a somewhat related change as a result of their observations on hydration and charge of freeze-dried beef.

Types of Interprotein Bond in Freeze-Dried Beef and Cod

The exact nature of the interprotein bonds that form during freeze-drying is uncertain. If the above analysis is correct, there are two possible types of bonds to be considered: 1) Actin-myosin bonds. 2) Bonds between denatured myosin molecules. As far as the first type is concerned, it is not known what these bonds are even in normal muscle except that the sulphydryl group of myosin are necessary for the combination between actin and myosin. Calcium ions also influence the elastic properties of muscle fibers probably through modifications of the actin-myosin bond, and Hamm and Deatherage have recently drawn attention to the possible importance of calcium ions in affecting the texture of freeze-dried beef.

With regard to bonds between denatured myosin molecules, the observation that freeze-dried cod swells enormously and dissolves considerably in solutions of hydrogen-bond breaking solvents (e.g., urea) provides circumstantial evidence for the existence of intermyosin hydrogen-bonds. Reducing agents that are known to split disulphide bonds specifically enhance the solubility of freeze-dried cod in hydrogen-bond breaking solvents. From this knowledge it can be inferred that the structural proteins of freeze-dried cod are stabilized partly by disulphide bonds. However, it is difficult to actually prove the existence of either of these types of bond in a partly insoluble protein system. There is a tendency nowadays among protein chemists to emphasize the importance of hydrophobic bonding in contributing to the binding forces between different parts of protein molecules or between the molecules themselves. Such bonding arises from, for example, the tendency for aliphatic amino acid side groups to attract one another. It seems likely that the formation of this type of bond would be particularly favored in the dry or near-dry state in freeze-dried muscle.

In summary, I would say that many of the muscle proteins resist freeze-drying *in situ*, but there is some evidence that myosin is partly denatured by the process. In addition, actin-myosin bonding is modified or increased. Because of these additional bonds (or cross-links) between these thread-like proteins, the reconstituted and cooked muscle is more rigid (increase in toughness) and has less water-holding capacity (decrease in juiciness).

Changes in Freeze-Dried Beef and Cod During Storage

How does the freeze-dried beef or cod, which I have delineated, change on storage? Most observers agree that freeze-dried meat and fish become tougher and drier in texture during storage at rates which depend markedly on the temperature of storage and the moisture content of the sample. There is agreement too that products as made nowadays can be kept at least 6 months at 37°C, if the moisture content is kept at about 3% or less, before they become unacceptably tough and dry. (Oxidative changes are not considered here because it is deemed mandatory to vacuum or inert gas-pack freeze-dried meat and fish). This performance can be judged satisfactory. However, if it ever becomes possible to make products of much improved initial texture, taste-panels may become more discriminatory and the problem of textural deterioration during storage would consequently be more serious.

We can again offer the formation of cross-links between the myofibrillar proteins as a plausible explanation for the increased toughening and the loss of juiciness during storage. This view was discussed in a paper of mine some years ago. I do not wish to go into detail about it now except to say that evidence from the stress-strain behavior of muscle fiber bundles and from the swelling behavior of finely divided muscle substantiated this view.

What is the nature of the cross-links that form during storage, and can they be prevented from forming? Dr. Tappel and Dr. Regier have provided convincing evidence that the major deteriorative reaction involving the protein of stored freeze-dried beef is a carbonyl-amino browning reaction. Browning reactions of this type lead to insolubility of the proteins probably through the introduction of inter-protein bonds. They could therefore be an obvious cause of toughening, and by preventing such a reaction one should be able to reduce the amount of toughening. It has been found that by inhibiting browning reactions it is possible to reduce but not eliminate the rate of insolubilization of muscle protein. However, as far as I am aware, the few attempts that have been made to prevent development of toughness during storage of meat by chemically inhibiting browning have not met with success. What is more, reducing the moisture content of beef or cod to levels where one would expect browning to virtually cease does not dramatically reduce the rate of toughening. It may be that in all these cases the browning reaction has not been entirely inhibited and that the small amounts of cross-linking resulting from this residual reaction are sufficient to produce large textural effects. However, it also makes one suspect that other cross-linking mechanisms may be as important as browning during storage. After all, toughening can occur without any evidence of browning during the preparation of the fresh freeze-dried product, so why not also during its storage? In addition, a number of investigators have shown that when pure (at least supposedly pure) moist proteins are heated at moderate temperatures, they become insoluble at rates that are sensitively dependent upon moisture content. Presumably, thermal denaturation is the only mechanism producing insolubility in these cases.

In recent experiments, we have attempted to assess 1) the relative importance of myosin and the sarcoplasmic group of proteins in the development of insolubility and 2) the importance of browning reactions in the same connection. Neither actin nor the connective tissue proteins have been examined. The experiments were performed on freeze-dried beef and cod at moisture contents of 5%, in an atmosphere of nitrogen, and at the temperature of 50°C. It can be claimed, of course, that in a complex system like muscle, events at 50°C may not be the same as at, say, 37°C. For example, the temperature coefficients of the browning reaction and of denaturation are different so that measurements at 50°C may give a distorted impression of what would happen at 37°C.

It must be emphasized that the effects reported here concern only 50% (in the case of cod) or 75% (in the case of beef) of the myosin that remains soluble in the freshly-prepared freeze-dried material. The sarcoplasmic proteins are involved in their entirety.

It has been found that myosin becomes insoluble much more rapidly than the sarcoplasmic proteins. The myosin that does dissolve from the heated muscle appears to have normal properties. On the other hand, the electrophoretic and ultracentrifugal properties of that portion of the sarcoplasmic group of proteins which survives heating are markedly different from the normal properties of this group. The most striking change is the disappearance of the two most rapidly sedimenting components of the ultracentrifuge picture. It appears that the proteins responsible for the two fast components are becoming insoluble at a rate far in excess of that of the proteins in the slow components. These observations show that some proteins in the muscle are either more heat labile than others, i.e., are more susceptible to heat denaturation in the "dry" state, or have a greater tendency to react with carbonyl compounds, i.e., as one might say, are better "browners" than others. It seems more likely to me that the first explanation is true in the present circumstances for the following reasons: Firstly, prevention of browning reactions by washing the muscle extensively with water before freeze-drying and heating it does not, if at all, reduce the rate much at which the myosin becomes insoluble. If the rapid insolubilization of myosin were due to a more pronounced tendency to react with carbonyl compounds, removal of the latter would be expected to reduce its rate of insolubilization. Secondly, the rate of insolubilization of beef myosin is less than that of cod; a behavior that is in line with what is known of the relative stabilities of these two proteins towards denaturation.

Once the muscle proteins have become insoluble in ordinary solvents like neutral 0.5 M KCl the number of techniques for studying their interactions is limited. A rough indication of the type of bond stabilizing insoluble fibrous proteins can be obtained by measuring the solvent effect of various specific reagents. The essential point is that the reagent should initially dissolve an appreciable portion of the protein; it is obvious that the behavior of a small readily-soluble portion of the system will not be representative of the whole. The use of urea has been mentioned and we have found that 50% urea plus 0.1 M monothioglycol always dissolves more protein from freeze-dried beef than from freeze-dried cod, indicating a larger number of interprotein bonds in the latter. About 75% and 60%, respectively, of the total protein in these products is soluble in this reagent. Sodium dodecyl sulphate (5%) dissolves practically all the protein of freeze-dried beef and cod. The solubility of washed, freeze-dried, and heated muscle remains much higher than the corresponding unwashed sample, a result which emphasizes the importance of browning reactions in the formation of cross-links. The solubility curve of cod in this solvent and at pH 12 (where again the initial solubility is very high) is biphasic, showing a rapid initial fall in solubility followed by a slower fall. It is tempting to attribute the more rapid phase to the rapid insolubilization of the myosin caused by heat denaturation illustrated earlier. This kind of behavior is not clearly shown with beef because it becomes insoluble more slowly. The rapid fall in the extractability of myosin from washed muscle by Hasselbach and Schneider's Solution indicated earlier is not reflected in the solubility results for washed muscle in sodium dodecyl sulphate. Part of the explanation for this difference must be that the detergent initially dissolves practically all of the protein in the muscle and not just a portion as in the case of Hasselbach and Schneider's Solution. Nevertheless, it must be acknowledged that the interpretation of solubility effects of this kind is hazardous and discrepancies remain.

The Enzymic Activity of Myosin in Relation to Denaturation

Matheson and Cole and Smithies have shown that much or all of the ATP-ase activity of muscle myosin *in situ* survives freeze-drying. By this enzymic-activity criterion, myosin is not denatured; however, by the solubility criterion, it is. These observations become reconcilable when it is remembered that myosin is one of the thinnest and longest of proteins. By means of proteolytic enzymes, large portions of this molecule can be lopped off without much harm to its ATP-ase activity. It thus seems probable, as Partmann has hinted, that one could denature one portion of such a molecule, say at the end, without affecting the enzymic active center located at the other end. The denaturation of one part could then lead to insolubility of the protein or, in the case of whole muscle, to cross-linking, without it necessarily losing its enzymic activity.

Storage of freeze-dried beef progressively reduces its ATP-ase activity, measurement of which has actually been proposed as a criterion of quality. It has been shown recently that the blocking of a very small number of the amino groups of myosin suffices to eliminate its enzymic activity, therefore one cannot rule out the possibility that the loss in activity observed on storing freeze-dried beef is the result of browning reactions involving these groups.

Practical Implications

The most important practical implication of this work is that the damage to the proteins caused by freeze-drying (to beef and cod, at least) is much less than was at first believed. In particular, a large proportion of the important myofibrillar protein fraction survives *in situ* freeze-drying apparently unchanged. As far as damage to the proteins is concerned—and for our purposes this means damage to the texture—freeze-drying of muscle is not an all-or-none process. This holds out the hope that, with suitable modifications, protein damage during freeze-drying could be eliminated and thus make possible the production of materials which when reconstituted would be indistinguishable from an untreated control.

As far as storage is concerned, the likely involvement of browning reactions in textural deterioration suggests, in principle anyway, that such reactions should be minimized. This will, of course, bring its rewards with respect to improvements in other qualities as well. The prevention of denaturation, and the cross-linking resulting from it, is likely to be a more difficult task. Very few instances of stabilization against denaturation are known, and they depend upon specific properties of individual protein (for example, addition of haem to globin; binding of long chain fatty acids to serum albumin). In this field empiricism, unfortunately, is still the best philosophy.

THE ROLE OF MOISTURE IN DETERIORATIVE REACTIONS OF DEHYDRATED FOODS

Harold Salwin

The Quartermaster Food and Container Institute for the Armed Forces is developing a new Military feeding system based on dehydrated foods. Questions pertaining to processing and packaging arise frequently in the course of this development. Because of the new and unique nature of the program, the guidance provided by precedent and experience is frequently inadequate. Extensive storage and performance studies could not be completed within the time schedule allowed for achieving the objectives. The participation of the Institute's research laboratories in this program has therefore been directed toward the formulation of general principles for application to specific problems as they arise.

"Quick-Serve" rations are formulated by combining foods which have been cooked and dried individually. The assembled meal is packaged in moisture-vapor proof, plastic- and foil-laminate contained in a paperboard carton. Some of the ingredients exert a high moisture-vapor pressure while others have a low moisture-vapor pressure. In this discussion, the moisture-vapor pressure of a food is expressed in terms of equivalent relative humidity, i.e., the relative humidity of the atmosphere in equilibrium with it. For example, in the ground beef and potato hash in Fig. 1, the relative humidity of the beef is approximately 2% at room temperature while the relative humidities of the onions, potato dice, and soup and gravy base range from 20% to 35%. These differences in relative humidity are responsible for migration of moisture vapor from one ingredient to another. When the transfer is excessive, the stability benefits of dehydration may be sacrificed.

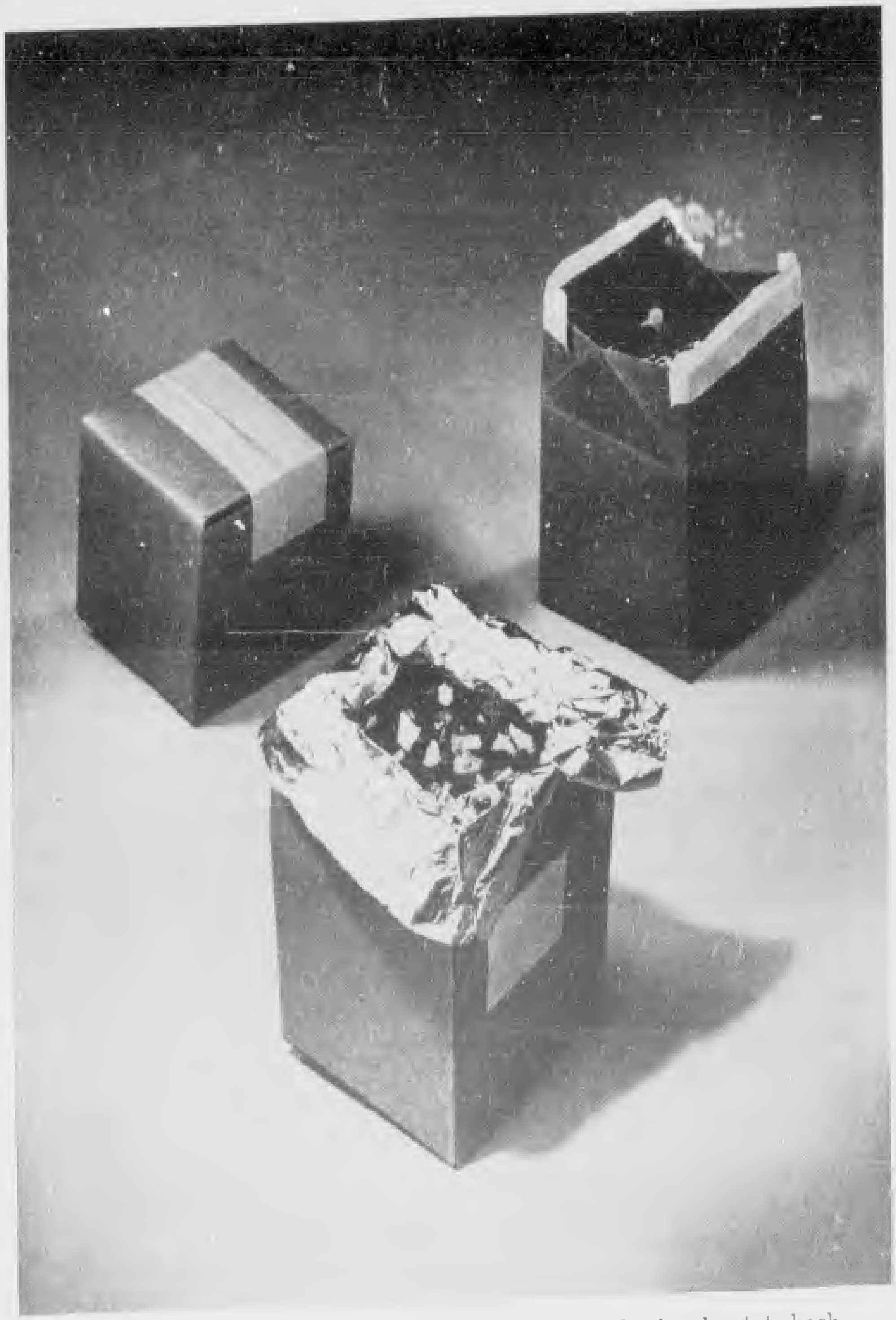

FIGURE 1. Precooked dehydrated ration of ground beef and potato hash.

By determining the moisture-sorption isotherms for each component, it is possible to compute the equilibrium moisture distribution in the combinations.

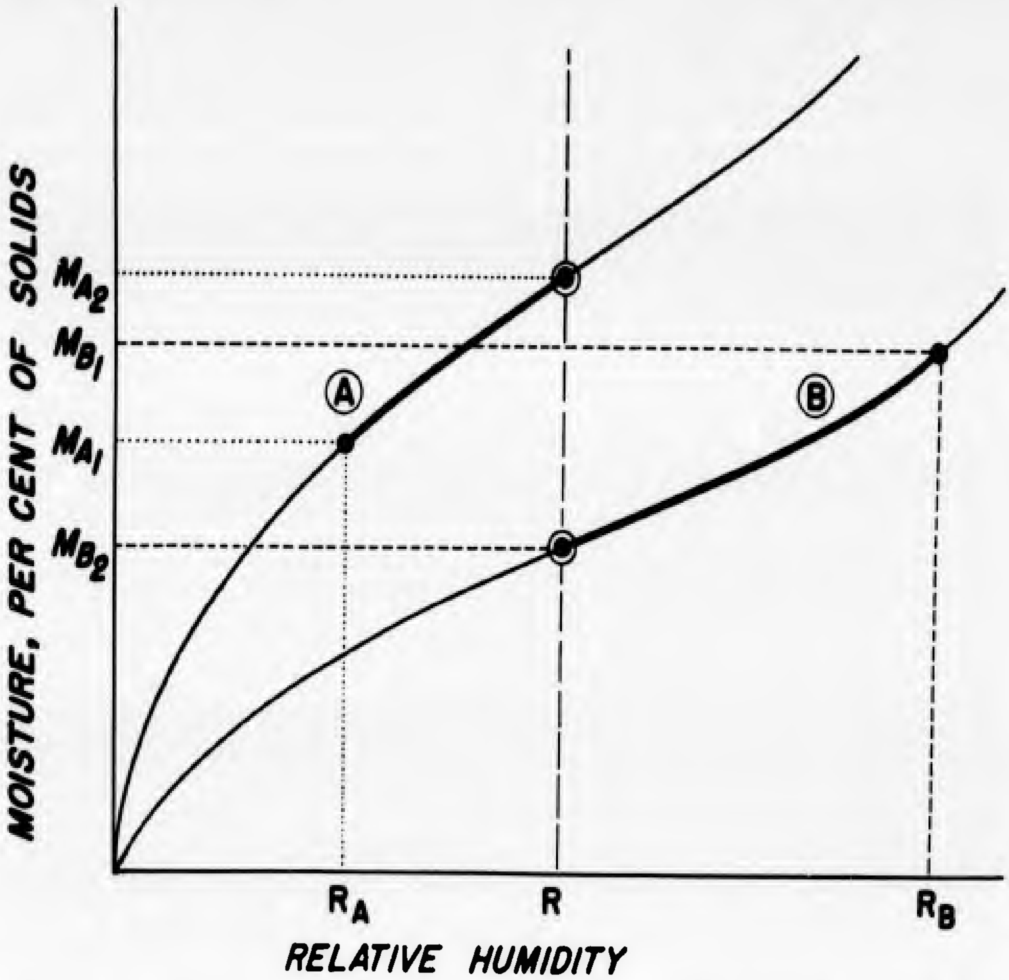

FIGURE 2. Graphical representation of changes in moisture during equilibration.

Fig. 2 shows typical moisture-sorption isotherms for food A with moisture-vapor pressure equivalent to R_A and food B with moisture-vapor pressure equivalent to R_B. When they are combined in a package, B, which has the higher vapor pressure, will lose moisture to A until pressure equilibrium is established at point R. The circled points show the new moisture contents of A and B. With a few simplifying assumptions, the following equation was derived for computing the equilibrium point R:

$$R = \frac{R_A\,S_A\,W_A + R_B\,S_B\,W_B}{S_A\,W_A + S_B\,W_B}$$

The required data are the dry weights of the components, W_A and W_B; the initial relative humidity for each component, R_A and R_B; and the slopes of the isotherms between the initial and final points, S_A and S_B. The method was reported in detail in Food Technology (1). A typical application of the equation is shown in Table 1.

TABLE 1

Predicted and Observed Moisture Transfer
in Precooked Dehydrated Chicken Stew Mixture at 72°F

Ingredient	Weight, Grams	Relative Humidity, %		Moisture, % of Solids		
		Initial	Final	Initial	Final	
			Predicted		Predicted	Observed
Chicken	39.9	1.1*	7.6	1.07	3.17	3.18
Potato Dice	30.1	28.4	"	5.88	3.45	3.64
Lima Beans	15.2	7.7	"	3.68	3.68	3.90
Cream Sauce Base	5.0	18.8	"	3.49	2.60	2.54
Chicken Soup and Gravy Base	4.0	37.0	"	2.49	1.17	1.19
Mixture,	Calculated Total Moisture, Grams:			2.90	2.94	3.03

* Extrapolated value.

The first column shows the five ingredients of a chicken stew mixture. The equation predicted that the moisture content of the chicken would increase from 1.07% to 3.17%. By analysis, the final moisture content was 3.18%. In a similar manner, the equation indicated that the potato dice would decrease from 5.88% to 3.45% moisture; observed, 3.64%; and so on with the other components.

This approach makes it possible to determine the packaging compatibility of the dehydrated foods. It is desirable to eliminate the cost, weight, bulk, and inconvenience of separate packaging of ingredients. For example, instant oatmeal and instant nonfat milk solids both require nitrogen packaging to insure keeping quality. A single packaging operation would be most economical, provided the two materials can be combined. Instant nonfat milk normally has 3.5% moisture and a relative humidity of approximately 16%. In order to avoid transfer of moisture vapor from oatmeal to milk, the oatmeal would also have to be at 16% relative humidity, which means drying the oatmeal from its normal 9% moisture to approximately 7%. In cereal products, moisture in excess of 7% is relatively free water and is relatively easy to remove. The foregoing leads to a general principle for preserving the stability of mixtures of dehydrated foods. The best relative humidity of the most sensitive component should be used as a guide to the equilibrium relative humidity of the mixture. Relative humidity, rather than moisture content, is the determining factor.

Returning to Table 1, the equilibrium moisture content of the chicken was 3.2%, considerably above the recommended maximum which at the time was 1.5%. When calculations of equilibrium moisture conditions indicated that certain ingredients would absorb moisture beyond prescribed limits, the situation was viewed with some concern. However, we began to observe with some regularity—particularly with meat products—that stability was good, or even better, at the higher moisture contents. We therefore began to examine the relationship between moisture sorption and stability. The Brunauer, Emmett, Teller adsorption theory was useful for this purpose. It seemed reasonable that the moisture content which corresponds to a theoretical monomolecular layer of adsorbed water, according to the B.E.T. equation, should represent a minimum desirable amount of water, as well as a maximum permissible amount.

The water molecules of the monolayer might be regarded as a discontinuous phase, bound by functional groups of proteins and carbohydrates, but exerting their influence over the entire surface of the food. Water attached at these sites, probably by hydrogen bonding, should protect them from reaction with oxygen possibly by excluding adsorption of oxygen directly or on adjacent surfaces, possibly by coordinating trace metals and reducing their catalytic effect, or possibly by decomposing free radicals. The adsorbed water might also inhibit interactions between adjacent polar groups, thereby preserving their hydrophilic properties and facilitating rehydration. Moisture in excess of the monolayer value represents free water which promotes browning, hydrolysis, caking, and other defects.

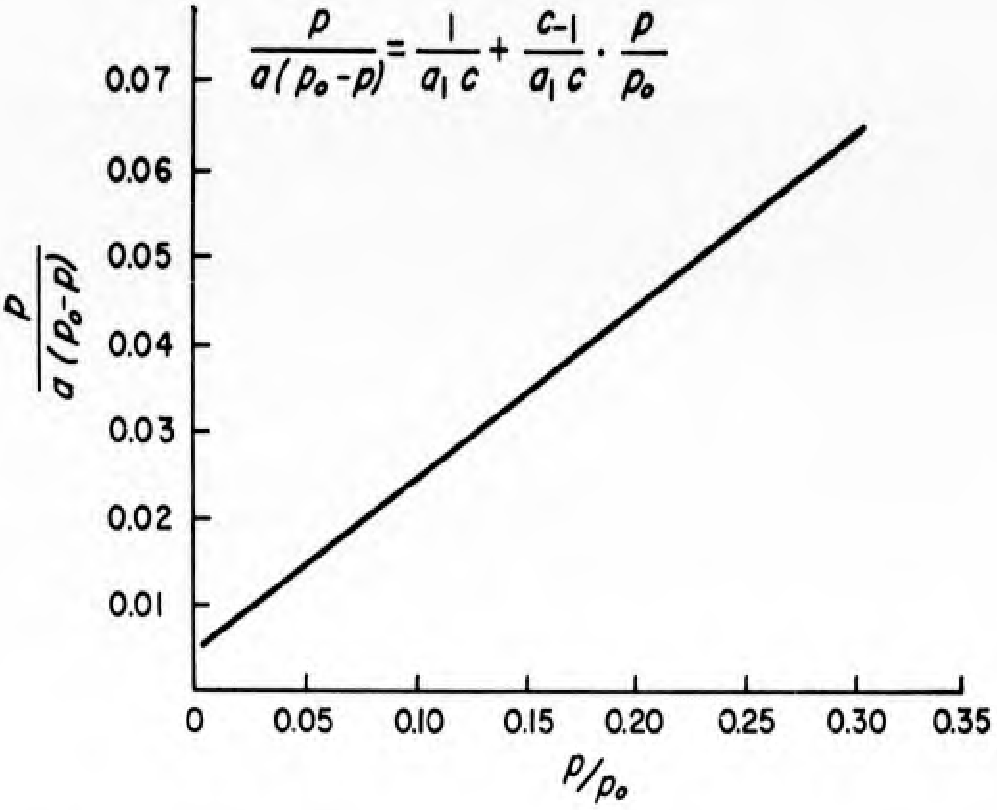

FIGURE 3. Brunauer-Emmett-Teller moisture-sorption isotherm for red beans at 72°F.

Fig. 3 shows the B.E.T. equation and plot of the moisture-sorption data for red beans at 72°F.

a = g. of water per 100 g. dry solids at moisture-vapor pressure p.
p_o = vapor pressure of pure water at the same temperature.
c = a constant related to the heat of adsorption.
a_1 = g. of water equivalent to a monomolecular layer adsorbed on 100 g. of dry solids.

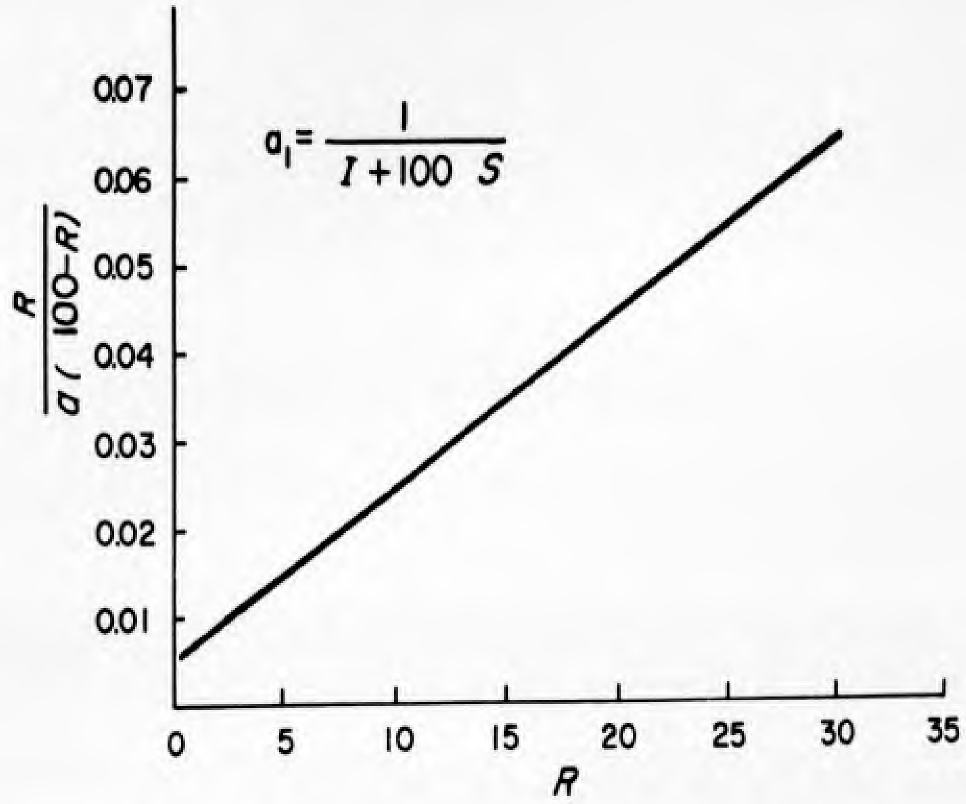

FIGURE 4. Modified B.E.T. moisture-sorption isotherm for red beans at 72°F.

The plot in Fig. 4 is another form of the B.E.T. isotherm in which vapor pressure is replaced by relative humidity (R). The equation is:

$$\frac{R}{a\ (100\text{-}R)} = I + SR$$

in which
I = Y-axis intercept
S = slope
a_1 = reciprocal of (I + 100 S)

This is a much simplified equation for calculating the monolayer moisture value (a_1) (2).

Fig. 5 shows four types of isotherms at 72°F. These four describe the moisture-sorption behavior of nearly all dehydrated foods. In the low relative humidity region with which we are concerned, starchy foods have the greatest water-holding capacity. The curve for potato is typical also of beans, corn, rice, spaghetti, flour, and other starchy

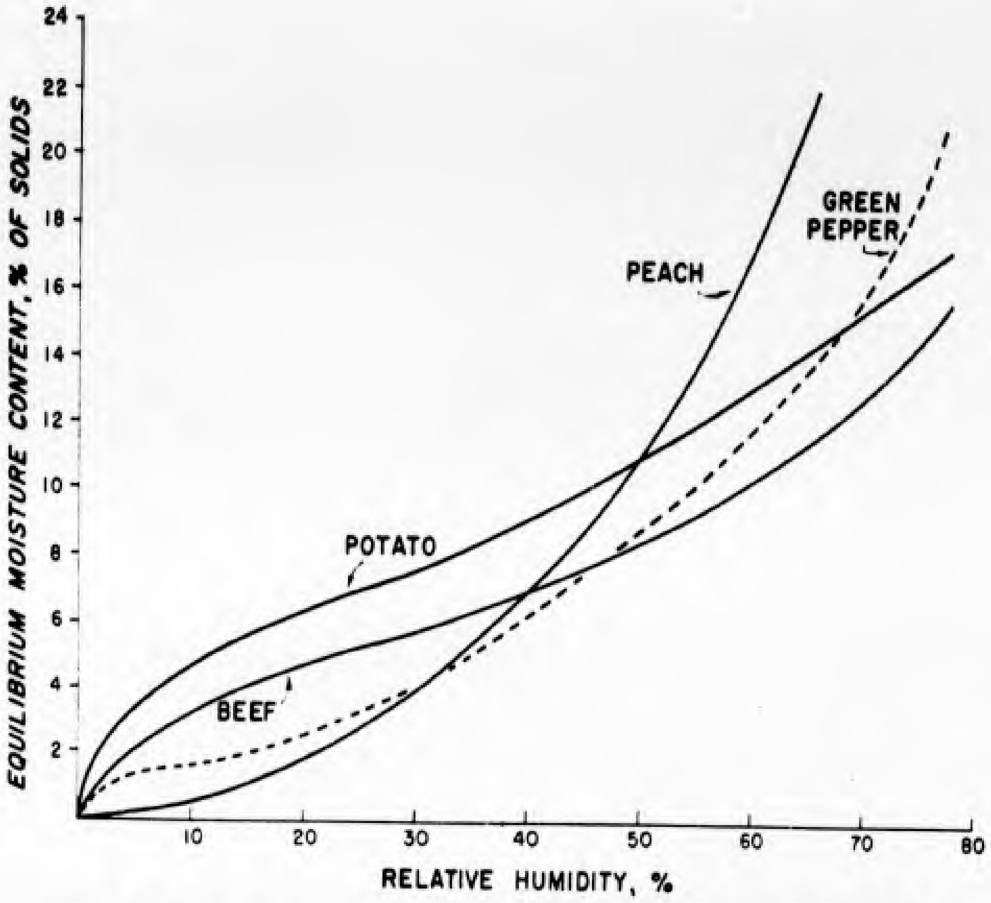

FIGURE 5. Typical moisture-sorption isotherms for dehydrated foods, 72°F.

foods. The monolayer moisture value for these products is approximately 6% (approximately 15% relative humidity).

Protein foods: meat, fowl, fish, eggs, and cheese, have a lower water-holding capacity. Beef has approximately 75% protein, 20% fat, and only 1% carbohydrate. The moisture-sorption properties change with temperature. As the temperature increases from 40° to 100°F, the isotherm becomes progressively less sigmoid and more convex toward the vapor-pressure axis. The a_1 value decreases with increasing temperature. At 72°F it is approximately 3.5% moisture on a fat-free solids basis (approximately 8% relative humidity).[1]

The curve for green peppers (broken line) is representative of a third group of foods which have high sugar content as well as high-molecular weight constituents. Green peppers have 30% sugar, 40% other carbohydrates, and 12% protein. The transition with temperature is even more pronounced than it is with high-protein foods. The behavior at 40°F is characteristic of moisture sorption by polymers (i.e., sigmoid), but at higher temperatures the solution effects of the sugars are dominant and the curve becomes more convex toward

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[1] Higher a_1 values were reported for shrimp, chicken, and beef in Food Technology, 13, 594-595 (1959). The B.E.T. plots for these products deviate from a straight line above approximately 10% r.h. The linear portion yields the lower and more reasonable a_1 values.

the vapor-pressure axis, except at very low vapor pressures. The a_1 value, approximately 2% moisture (approximately 6% relative humidity), is very low for products in this group which includes carrots, sweet potatoes, peas, cabbage, onions, and milk.

Peach is typical of the fourth group, or high-sugar items. Peach has 67% sugar, 25% other carbohydrate, and only 4% protein. At 72°F the curve is entirely convex toward the vapor-pressure axis. The moisture exerts a high vapor pressure, even at very low percentages, and is available for deteriorative reactions. The B.E.T. equation is then not applicable and complete dehydration is essential for good stability.

The results of empirical storage tests on food items and combinations were consistent with the speculation that the calculated monolayer moisture value defines the stable region for most dehydrated foods.

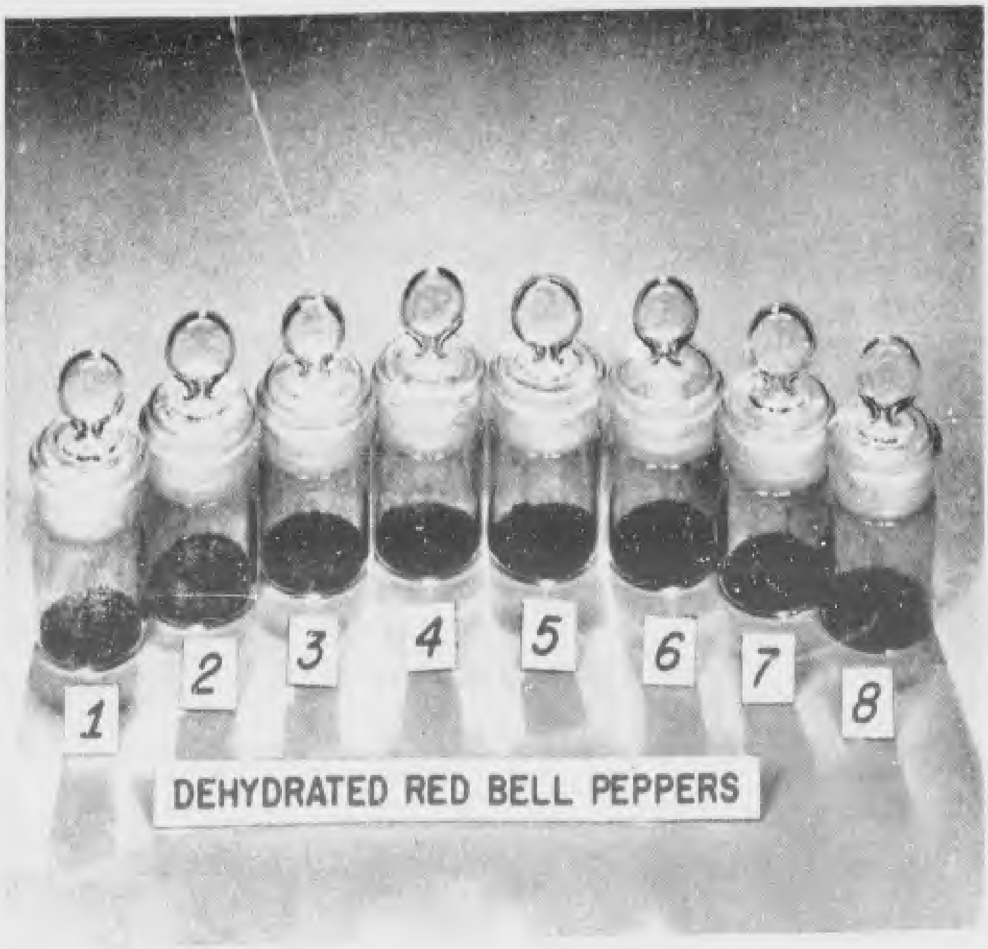

FIGURE 6. Dehydrated red bell peppers after 18 days at 72°F. Moisture contents ranged from (1) 0% to (8) 26%. Monolayer moisture content of 1.9% was between samples (3) and (4).

Fig. 6 is a photograph of ground red bell peppers adjusted to a range of moisture contents from 0 to 26%. The monolayer moisture value (a_1) was 1.9% which falls between the values for samples 3 and 4. The samples were stored in air at 72°F for 18 days. Oxidative bleaching of the carotene and lycopene was most severe in the first three samples with moisture contents below a_1. The last three samples browned and caked. Color stability was best in samples 4 and 5 with moisture contents just above a_1.

Similar results were obtained with green peppers stored in air at 100°F. The a_1 value was 1.6%. Color and odor stability was better at 1.6% and 1.9% moisture than at either lower or higher values.

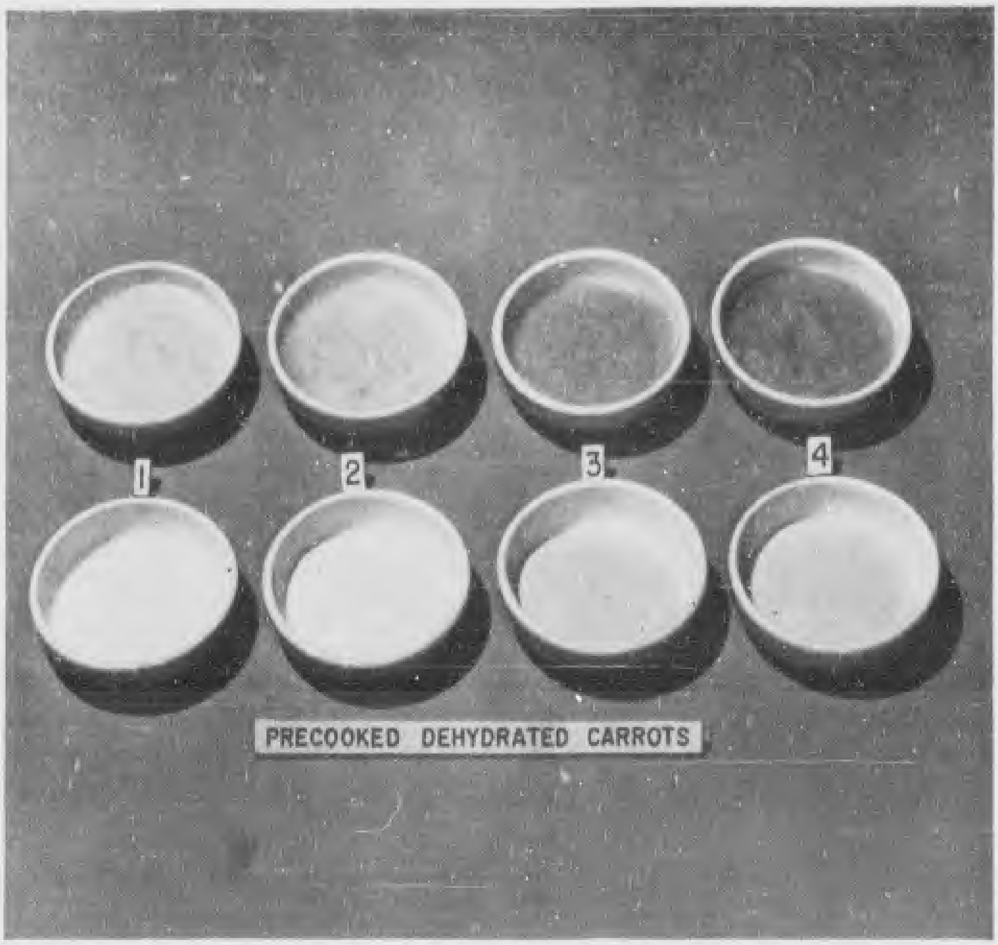

FIGURE 7. Precooked freeze-dried carrots after 37 days at 100°F. Top Row: dehydrated. Bottom row: dry. Moisture contents ranged from (1) 0.2% to (4) 5.8%. Monolayer moisture content of 1.8% was between samples (1) and (2). Color differences ranged from (1) nearly complete absence of color to (4) typical carrot color.

Precooked freeze-dried carrots were adjusted in moisture content over the range 0.2 to 6% and stored in air at 100°F for 37 days. Fig. 7 shows four of the samples in both the dry and in the rehydrated condition. The a_1 value was 1.8%, just below the moisture content of sample number 2. Color stability improved with increasing moisture content.

Fig. 8 shows the effect of relative humidity of storage on the reflectance and carotene values of the same carrots. The solid line shows

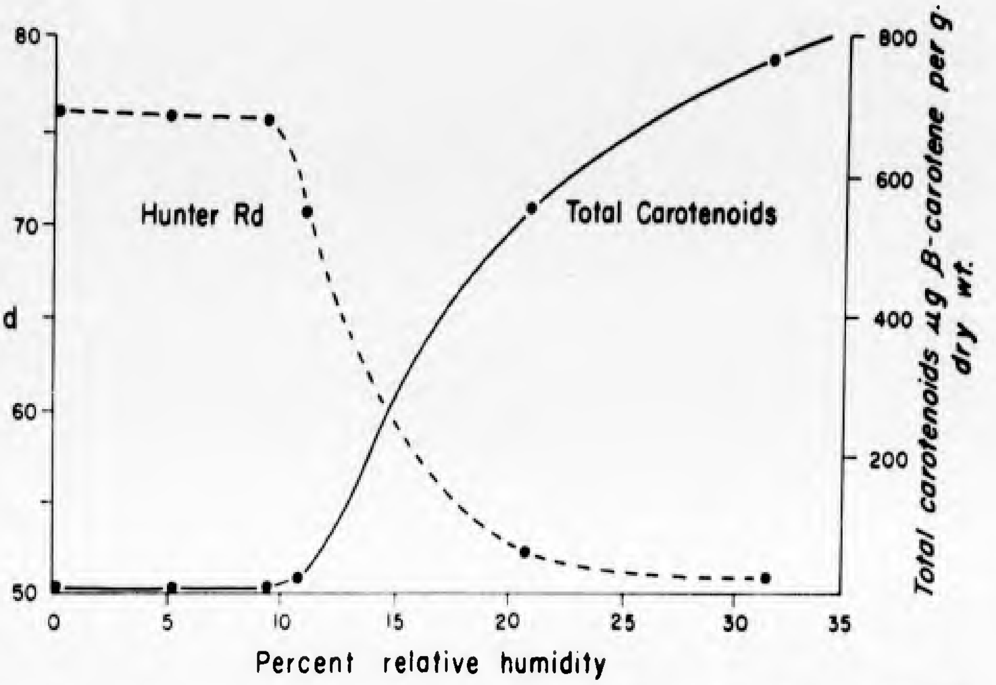

FIGURE 8. Effect of relative humidity of storage on color and carotene stability on precooked freeze-dried carrots, 100°F, 37 days.

carotene content, and the broken line shows Hunter reflectance values (R_d). Fading and destruction of carotene were most rapid at the lower relative humidities. They were complete below 8% relative humidity which corresponds to the a_1 value. With both carrots and sweet potatoes, drying below the a_1 value promoted oxidation of carotene to beta-ionone with the development of a violet odor. Moisture in excess of a_1 caused a loss of ascorbic acid, possibly by conversion to diketogulonic acid.

Raw freeze-dried pork chops containing 37% fat were equilibrated with atmospheres ranging from 0 to 32% relative humidity (0.3 to 4.1% moisture) and then stored in air at 40° and at 100°F. Fig. 9 shows the peroxide and free-fatty acid values after two weeks at 100°F. The relative humidity corresponding to the a_1 value at 100°F was 9.5% (R_1). Hydrolysis proceeded more rapidly at higher relative humidities and oxidation, more rapidly at lower values. The sample at 0% relative humidity was rancid. At 40°F, free-fatty acid values were lower, but peroxide values were higher. It appears, then, that conditions for optimum stability are low-temperature storage and moisture content near the a_1 value.

Similar results were obtained in Institute studies on other products. Chicken was always more rancid at lower moisture contents. The a_1 value for potato granules is approximately 5%. Stability at 85° and 100°F was better at that moisture content than at either higher or lower values. The a_1 value for instant nonfat milk is 3.5%. Stability was better at that moisture content than in the presence of in-package desiccant. With moisture removed, oxygen disappeared more rapidly from the headspace and panel ratings were lower.

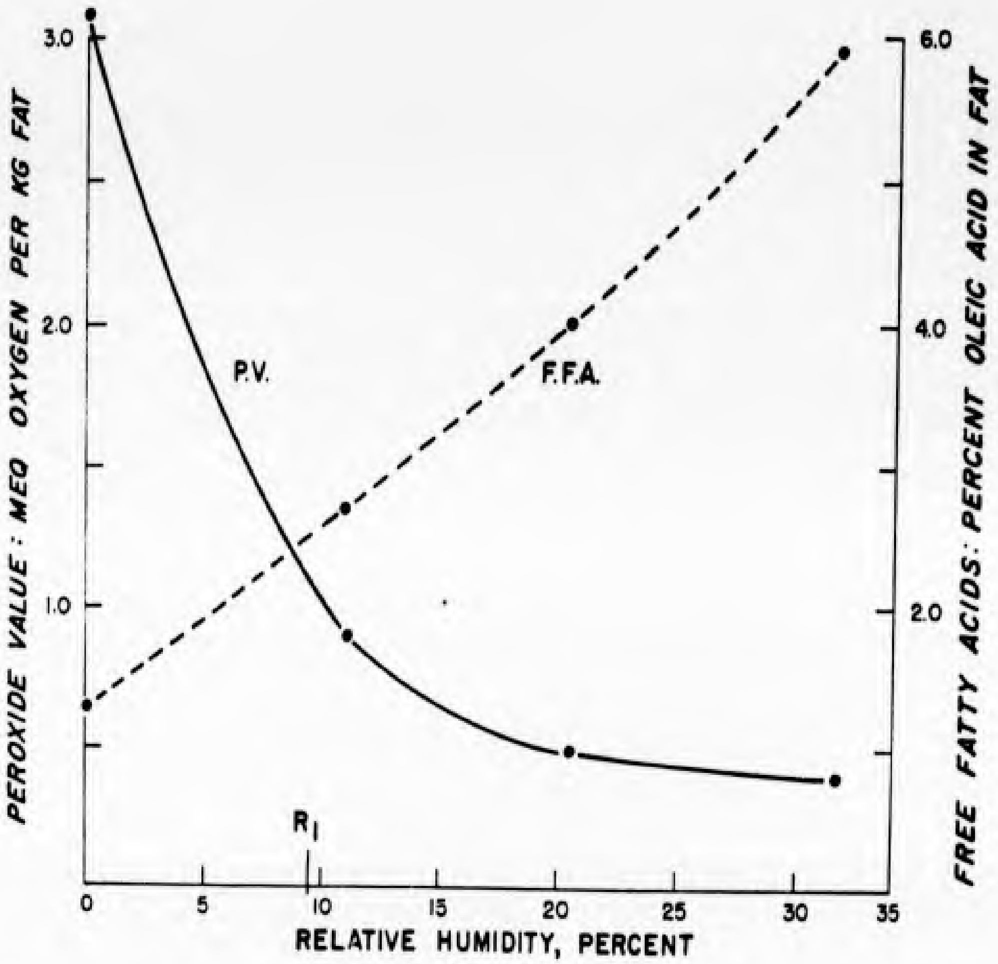

FIGURE 9. Effect of relative humidity of storage on formation of peroxides and free-fatty acids in raw freeze-dried pork chops, 100°F, 2 weeks.

Fig. 10 shows the 100°F isotherms for the seven main ingredients of a chicken stew mixture. The circles locate the initial moisture contents. They ranged from 1.3% for the chicken and for the seasoning to 5.2% for the potatoes. The individual relative humidities ranged from 3% for the chicken to 29% for the carrots. Moisture-transfer calculations, using the formula described earlier, indicated that the equilibrium relative humidity for the entire mixture at 100°F would be 10.7% (R_E in Fig. 10). At the equilibrium point of 10.7% relative humidity, each one of the seven ingredients would be closer to the relative humidity corresponding to its a_1 value than it was initially. It was therefore concluded that all of the ingredients should be combined in a single package.

A storage study was conducted at 100°F to test the hypothesis. Each ingredient was placed in a small open can; the cans were put into a single larger can which was then sealed under nitrogen. For comparison, each item was also sealed under nitrogen and stored separately.

Fig. 11 shows the results after one year. The top row shows the items which were stored separately; the bottom row shows them after

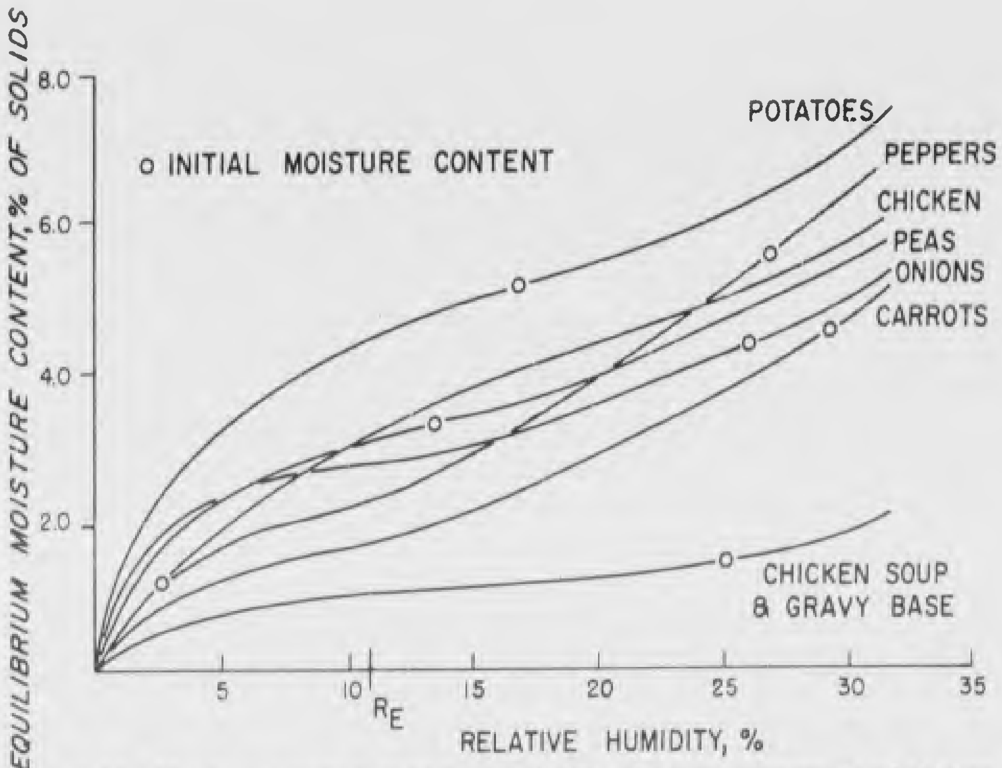

FIGURE 10. Moisture-sorption isotherms (100°F) of seven components of chicken stew.

FIGURE 11. Components of chicken stew after one year at 100°F.

being sealed in the same can. Stability was better in the combination package and the final individual moisture contents were very close to the values which had been calculated. It is interesting to note that the chicken increased in moisture from 1.3% to 3.0% and had better odor and a lighter color.

The data which I have reported lead to the single conclusion that moisture-sorption data give useful guidance for processing and packaging dehydrated foods. In most cases, the monolayer moisture content is a good first target when specific stability data are lacking. Exceptions to this rule can be identified by the shapes of the moisture-sorption isotherms.

It is in order at this point to raise a question as to what is meant by the moisture content of a food. The analytical values are determined by experimental conditions selected somewhat arbitrarily. Air ovens and vacuum ovens are operated at various temperatures for different intervals of time which depend on the material being analyzed. Distillation methods, chemical methods, electrical methods, and others give a variety of data which we call per cent moisture or which can be related to per cent moisture. The choice of method is not difficult, and frequently not critical, when dealing with stable materials such as starch or meat. But even here, a complication is introduced by the many ways used for reporting results. For example, different Military specifications for dehydrated meat products state moisture requirements on the as-is basis, on the as-is basis after trimming surface and seam fat, or on the fat-free basis.

Oven methods are not suitable for foods which are unstable or which contain volatile materials in addition to moisture. The method of drying without heat over sulfuric acid in a vacuum desiccator is an official A.O.A.C. method for some materials. It, too, gives high results when volatiles are present unless the temperature is reduced to approximately 40°F.

The hygroscopic nature of dehydrated foods adds to the difficulty of determining moisture reliably. And finally, obtaining a representative aliquot of a sample for moisture analysis is frequently difficult. Dry soup mixes, fruit mixes, and other heterogeneous materials present special problems of sampling and sample preparation.

All of these difficulties are either eliminated or minimized when moisture-vapor pressure, rather than moisture content, is determined. Large representative samples can be used, and they need not be comminuted, weighed, or heated.[1]

The determination can be made easily and rapidly with an electric hygrometer. Broader use of moisture-vapor pressure for definition of food composition should therefore be considered.

Figs. 12, 13, and 14 serve to illustrate some precautions which should be observed when interpreting moisture-sorption isotherms

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[1] If the moisture in the components of a mixture are in pressure equilibrium at a given temperature, any portion of the mixture should be a representative sample for vapor-pressure measurement at that temperature.

and oven moisture determinations on materials which contain volatiles in addition to ordinary moisture.

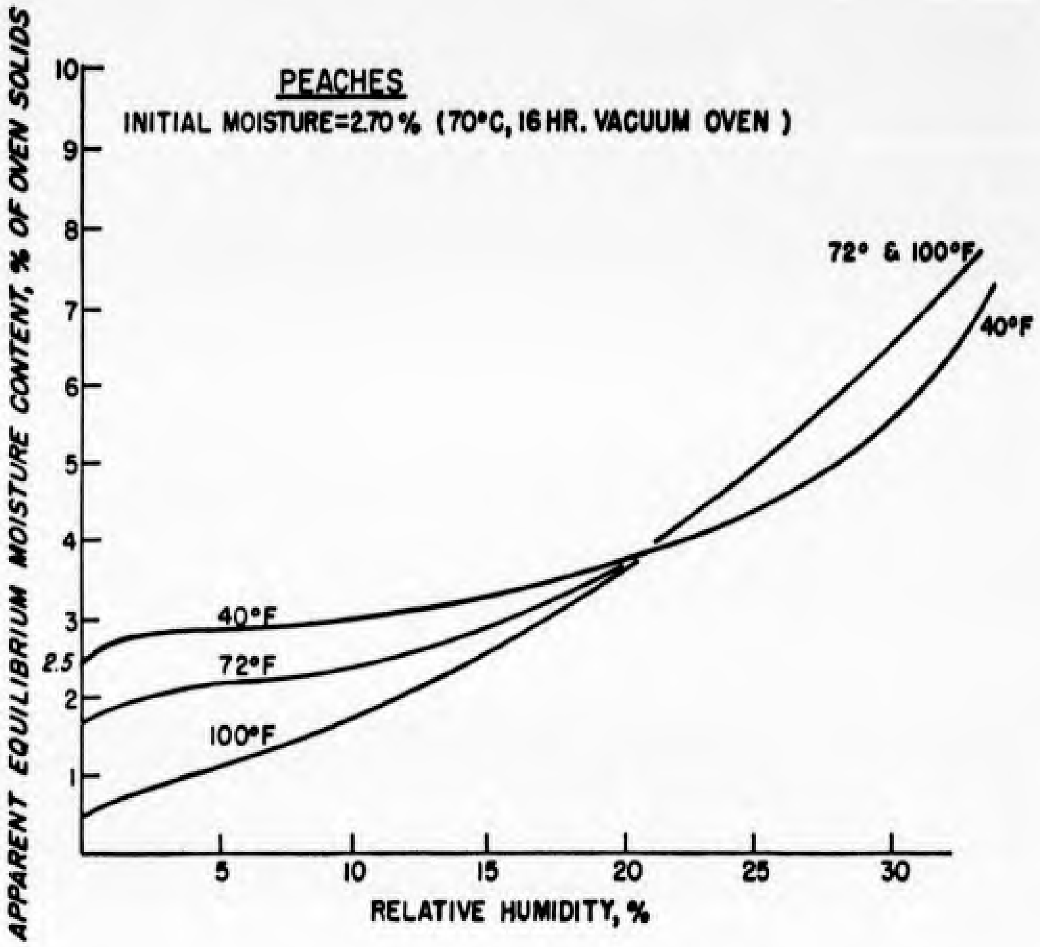

FIGURE 12. Moisture-sorption isotherms for freeze-dried peaches showing apparent equilibrium moisture contents.

Fig. 12 shows the 40°, 72°, and 100°F isotherms for freeze-dried peaches. The data were obtained by observing the changes in weight when samples were equilibrated in desiccators with relative humidities controlled by magnesium perchlorate (0% relative humidity), sulfuric acid solutions, and saturated salt solutions. The desiccators were evacuated to approximately one inch of mercury vapor pressure. The Y-axis represents apparent moisture as determined in a 70°C vacuum oven for 16 hours.

At 0% relative humidity (Fig. 12) the Y-axis intercepts are, respectively, 2.5%, 1.8%, and 0.6%. With stable materials, such as starch and meat, the intercepts are always at zero, indicating that magnesium perchlorate can remove as much free moisture as the 70°C vacuum oven. The intercepts in Fig. 12 therefore represent the per cent of material which is volatile in the oven but not volatile in the desiccators. They may represent non-aqueous volatiles, volatile material formed by decomposition in the oven, or possibly water of crystallization of certain components. Moisture percentages read from the isotherms cannot agree with the results of oven determinations.

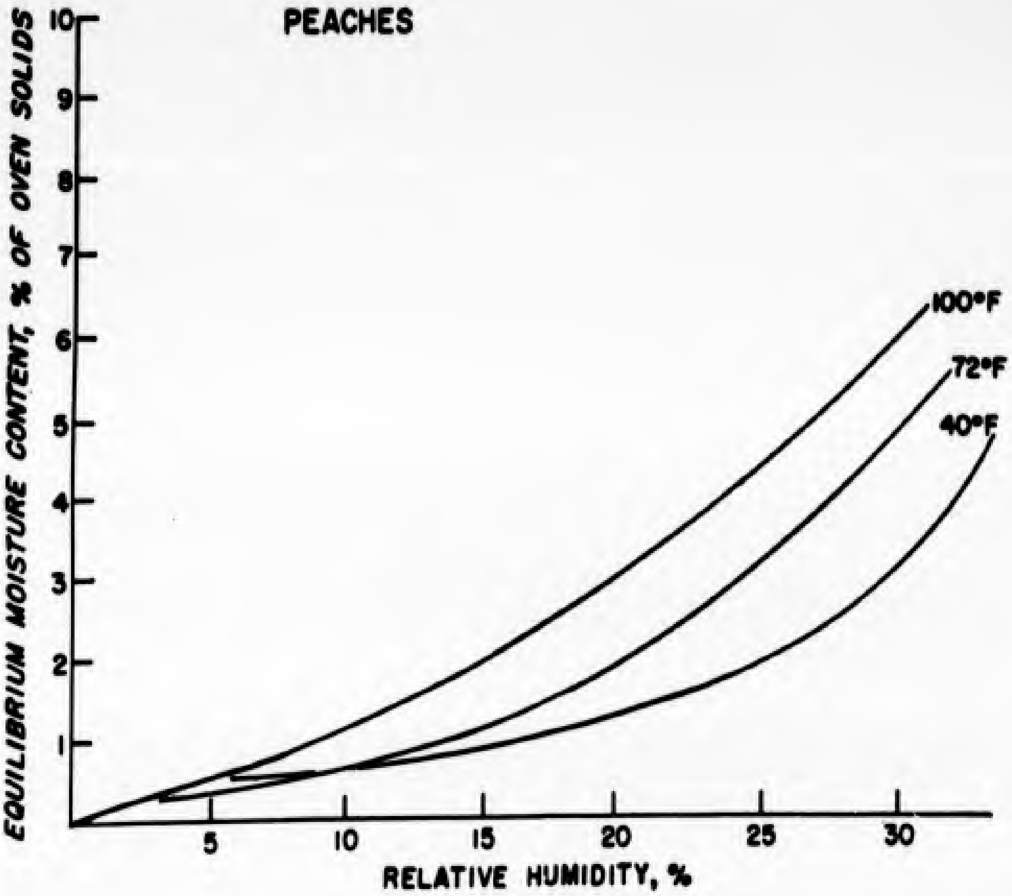

FIGURE 13. Corrected moisture-sorption isotherms for freeze-dried peaches showing equilibrium moisture contents.

Fig. 13 shows the same isotherms adjusted by subtracting the intercept values. The isotherms for green pepper and peach (Fig. 5) were adjusted in the same manner. These isotherms represent a more correct and more meaningful relationship between moisture content and relative humidity. However, they do not agree with oven-moisture determinations because they do not include volatiles.

The 40°F intercept in Fig. 12 is nearest to the per cent of total oven volatiles (other than moisture). By adding that value to the curves in Fig. 13, we arrive at the isotherms in Fig. 14. The Y-axis represents moisture and volatiles, and the values read from these curves agree closely with oven-moisture determinations.[1]

This interpretation of moisture-sorption isotherms supports the contention that the loss over magnesium perchlorate at 40°F repre-

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[1] For this discussion, all moisture values were calculated as per cent of oven solids in order to make them comparable with oven-moisture determinations. They can be converted to total solids basis by reference to the residue weight over magnesium perchlorate at 40°F.

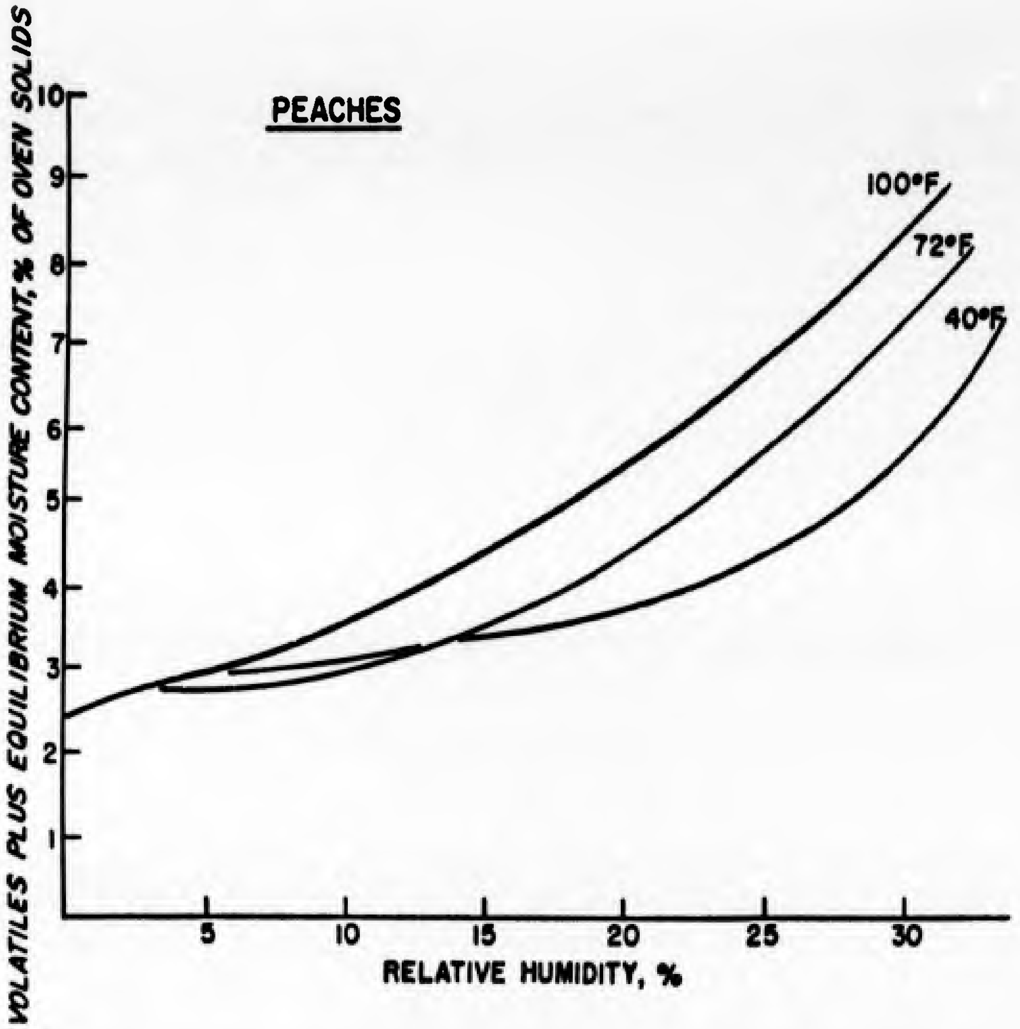

FIGURE 14. Adjusted moisture-sorption isotherms for freeze-dried peaches showing volatiles plus equilibrium moisture contents.

sents free moisture. The following are typical comparisons with oven-moisture determinations:

	Moisture + Volatiles	Moisture
	Loss in vacuum oven, 70°C, 16 hr.	Loss over magnesium perchlorate, 40°F
	% of oven solids	% of oven solids
Carrot, precooked, air dried	3.77	2.07
Apple slices, freeze-dried	2.28	1.23
Fruit cocktail, freeze-dried	2.49	0.72
Peaches, freeze-dried	2.69	0.19
Tomato powder, spray-dried	1.57	0.00

When comparing analytical results on two samples of a given food, a higher apparent moisture content by the oven method might, in fact, represent a higher content of volatiles other than moisture.

Summary

Moisture-sorption data give useful guidance for processing and packaging dehydrated foods. In most cases, the monomolecular-layer moisture content is a good first target in dehydration when specific stability data are lacking. The compatibility of items which are to be packaged together can be determined with good reliability. Consideration should be given to the broader use of moisture-vapor pressure measurements which can be more meaningful than moisture determinations on dehydrated foods.

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DETERIORATIVE REACTIONS IN STORED FREEZE-DRIED MEAT AND FISH

HAROLD S. OLCOTT

Storage of freeze-dried meat and fish in oxygen or air is deleterious. There is a rapid loss of palatability, myoglobin is converted to metmyoglobin, and oxygen is absorbed in large amounts, in part by lipids and in part by proteins. Reaction with the lipids leads to rancidity, particularly with fish in which there are highly unsaturated fatty substances. Reaction with the protein leads to insolubility; sulfhydryl groups have been particularly implicated (1, 2). On all counts, exposure to air should be avoided. The breaking of the vacuum after the end of the freeze-dry cycle with an inert gas such as nitrogen would help to avoid contact of the product with oxygen which thereafter can be removed only with difficulty. Oxidative changes usually occur more rapidly at low moisture content (1-2%) than when higher amounts of water are present.

When oxygen is absent, the most important single deteriorative reaction in freeze-dried meat or fish is the carbonyl-amine or Maillard reaction involving, on the one hand, reducing sugars or other sources of aldehydes or ketones and, on the other hand, amino compounds such as amino acids, peptides, proteins, aminophospholipids, and so forth. These two classes of substances inter-react reversibly in concentrated solution to give colorless intermediates. The intermediates subsequently decompose to yield brown, polymeric materials which are responsible for loss of palatability. The brown reaction products of reducing sugars with proteins probably account for losses of solubility and damage to rehydratability. The decomposition of the products from carbonyl compounds and low molecular weight amines contribute in addition to flavor and taste components. Under certain conditions these products improve palatability, but further long time or high temperature storage invariably leads to complete loss of acceptability.

Both decompositions are accelerated by high moisture and pH. Partial control is achieved by low moisture and low temperature storage. Methods used for other dehydrated products such as egg and potatoes are not applicable to meat and fish. These methods are: 1) acidification, 2) addition of sulfite or similar reagents, and 3) removal of glucose as by enzymes or fermentation.

Some control of browning might be achieved by handling the raw material in such manner that the reducing sugars are low. Insufficient information is available to know how to do this practically. Burt (3) has shown that the concentrations of glucose, glucose-6-phosphate, and ribose vary in a systematic manner in cod held in ice. These sugars contribute to browning but not in equal degrees. The total contents also differ depending upon the manner in which the fish were handled.

Amino compounds in fish flesh vary from species to species and also undergo *post mortem* changes in concentration. Jones (4) concluded that taurine and anserine were the amino substances most responsible for browning in cod. Anserine occurs in large amounts in some species of fish but is entirely absent from others (5).

Enzymes survive in freeze-dried fresh meat and fish products. Adenosine triphosphatase (ATP-ase), lipoxidase, and peroxidase have been identified among others. Cole and Smithies (6) have recovered practically all of the original ATP-ase of beef muscle in the freeze-dried product. Enzymatic changes are believed to be comparatively unimportant in low moisture products (less than 3% water); however, this facet has not yet been thoroughly investigated. Precooked foods would not be expected to have residual enzymes, but Olley and Lovern (7) report that the phospholipase in cod flesh is still active after 30 minutes at 100°.

The pigment changes which occur during anaerobic storage of freeze-dried meat are ascribable to the formation of metmyoglobin. Penny (8) has reported that the pigment of the freshly freeze-dried product is mostly oxymyoglobin, contrary to a previous report.

Reference has been made in this summary to a selection of recent papers. In 1958 Sharp and Rolfe (9) reviewed the deterioration of dehydrated meat during storage. Connell has summarized the available information on muscle protein changes during freezing and dehydration in these proceedings (page 50).

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SESSION NO. 3

IDEAS FOR ACCELERATING THE FREEZE-DRYING PROCESS—BASIC PHYSICAL AND ENGINEERING PRINCIPLES

EDWARD SELTZER, *Moderator*

SOME BASIC PRINCIPLES OF FREEZE-DRYING AND MOLECULAR DISTILLATION

P. C. CARMAN

Freeze-drying is a process of distillation since a vapor is formed and condensed. It is closely related to so-called molecular distillation. The exact nature of this relationship is the subject of this paper because the author was surprised some years ago to find that little thought had been given to the fundamental principles of such techniques. For this conference it may appear that the following paper deals with a hopelessly oversimplified system, in which a clean ice surface alone is considered and problems of heat transfer are neglected. This tactic is essential to produce quantitative expressions of reasonably simple character which will illustrate the relationship clearly and be subject to experimental verification. It is my view that this achievement is essential before going on to consideration of the effect of porous crusts formed during drying and of the practical problems arising from the need to supply latent heat of vaporization.

To most chemists, the process of distillation is one in which a liquid is converted by boiling to vapor which is subsequently condensed. When we turn to molecular distillation, there is a profound difference in the mechanism of converting the liquid to a vapor. The conditions generally envisaged are a pressure above the liquid which is so low that a molecule which escapes from the liquid into the vapor phase is unlikely to be reflected back by collision with other vapor molecules and so is lost permanently. The rate of evaporation is thus equal to the absolute rate of escape from the liquid phase.

In order to reconcile these modes of conversion to vapor, let us consider a liquid at temperature T, with a saturation vapor pressure Π. The pressure in the vapor space is P, due normally to presence of air. If the vapor space is pumped out till P falls a little below Π, ordinary liquids under ordinary conditions begin to boil and, as long as heat can be supplied at a rate to maintain T constant, any attempt to reduce P by pumping vapor away faster merely produces more rapid boiling.

Molecular distillation is only applicable to cases where boiling is readily suppressed (e.g., many high-boiling liquids) or is impossible (e.g., solids). In such cases, vapor can be formed only by the escape of molecules from the surface of the condensed phase, and a profound change in mechanism occurs as P decreases to Π and below. When P is very much larger than Π, the process can be described as *normal evaporation.* At the surface itself, most of the molecules which escape into the vapor collide with air or other vapor molecules and are reflected back to the surface. The fraction which escapes permanently is so small that it does not affect the equilibrium at the interface, and the partial pressure of the vapor at the interface, P_i, can be assumed to be equal to Π. As air is present to give the total pressure, P, the only way vapor molecules can escape is by diffusion. If the air layer is stagnant, true diffusion must take place through a layer of thickness x to a condenser surface. If there is convection, x represents the effective thickness of the stagnant layer adjacent to the evaporating surface. For a given value of x, the rate of evaporation, w g/cm^2/sec, will be governed by P, i.e., by the concentration of air molecules which offer hindrance to the escape of vapor molecules. Consequently, as P decreases, a stage must be reached at which w becomes an appreciable fraction of w_o, the absolute rate of escape of molecules from the interface at temperature T. The equilibrium is then disturbed and $P_i < \Pi$ replaces $P_i = \Pi$. Eventually, when $P \ll \Pi$, the rate of evaporation approaches w_o which is the limiting value, i.e., we have *molecular evaporation.*

The quantitative study of this transition is important because it should provide a sound basis for the design of large-scale equipment. A theoretical approach used by myself (1) has since been confirmed experimentally by Kramers (2), and it is therefore presented here as a basis for further development and discussion.

We start with the calculation of the limiting evaporation rate, w_o. There are two ways of approaching this. One is to calculate the rate at which molecules can escape from an interface, from calculations of the state of molecules within a liquid. Unfortunately, the theory of liquids is too incomplete for this method to be more than of academic importance. The more direct way is to assume that, at equilibrium, the rate of escape equals the rate of re-entry. Now, from the vapor pressure, Π, at equilibrium, Knudsen has shown that the rate of collision of vapor molecules with the surface, in the same units as w_o, is given by $\Pi\sqrt{\frac{M}{2\pi RT}}$. As, however, each collision may result in a reflection instead of a re-entry, we must write

$$w_o = \alpha\Pi\sqrt{\frac{M}{2\pi RT}} \qquad (1)$$

where α is a coefficient of accommodation and is only unity if there are no reflections. Until the theory of liquids has advanced sufficiently, there is no way of calculating α.

Now, on this basis, if the pressure of the vapor at the interface is P_i instead of Π,

$$\text{rate of re-entry} = \alpha_i P_i \sqrt{\frac{M}{2\pi RT}} \tag{2}$$

Consequently, if we assume $\alpha_i = \alpha$,

$$\begin{aligned} w &= \text{absolute rate of evaporation—rate of re-entry} \\ &= w_o(1 - P_i/\Pi) \end{aligned} \tag{3}$$

This relationship could be tested by pumping out the vapor space so as to keep it at a pressure $P = P_i$ and measuring the rate at which vapor is pumped off. This has not been done, but Kramers and Stemerding (2) inserted a condensing surface at temperature, T_c, under conditions where all air had been removed and assumed that the pressure in the vapor space would equal the saturation vapor at the condenser surface, i.e., $P_i = P = \Pi_c$. For ice in the region of −40° to −60°C, assuming $\alpha = 1$ in the calculation of w_o, they found good confirmation for the proposed equation.

If air is present, giving a total pressure, P, the vapor pressure at the interface is P_i, and vapor escapes by diffusion through the air layer to the condensing surface. The equation describing this diffusional process through a stagnant air layer is

$$w = \frac{MDP}{RTx} \ln \left(\frac{P - \Pi_c}{P - P_i}\right) \tag{4}$$

where D is the interdiffusion coefficient for vapor and air. More correctly, as there is a temperature gradient present, average values of D and T for the temperatures concerned should be used. Fortunately, as D/T is dependent very little upon temperature and DP is independent of pressure, we can use

$$w = \frac{k}{x} \ln \left(\frac{P - \Pi_c}{P - P_i}\right) \tag{5}$$

$$\text{where } k = \frac{M\bar{D}P}{R\bar{T}}$$

Kramers and Stemerding (2) also confirmed this equation by measuring w, calculating $P_i = \Pi(1 - \frac{w}{w_o})$, using this to obtain $\frac{P - \pi_c}{P - P_i}$, and comparing this with the value obtained from equation (3).

Theory thus leads to the relationship

$$w = w_o\,(1 - P_i/\Pi) = \frac{k}{x}\ln\left(\frac{P - \Pi_c}{P - P_i}\right) \tag{6}$$

and this relationship has experimental support. Suppose now that we follow some of the consequences. We shall take the case of ice since its physical properties are well known and removal of moisture by freeze-drying is one of the most common applications of molecular distillation. Calculations will be simplified by assuming that the condensing surface is so cold the $\Pi_c = 0$.

To follow the transition in mode of evaporation, we plot w/w_o versus P, as has been done in Figs. 1 and 2 for a distance of 1 cm between the evaporating and condensing surfaces. In order to obtain a wide range of values of Π, temperatures of −80°, −50°, and −20°C have been selected for ice. Also, a calculation has been carried out for water at 20°C. Apart from other considerations, it is doubtful that this last calculation is valid since Alty (3) and Prüger (4) have evidence that α for water is of the order of 0.05 whereas unity has been assumed for all calculations. Fortunately this does not matter since Fig. 1 shows that the curve for 20°C has the same characteristics as that for ice at −20°C. In other words, for values of Π above 1 mm, no new points for discussion arise.

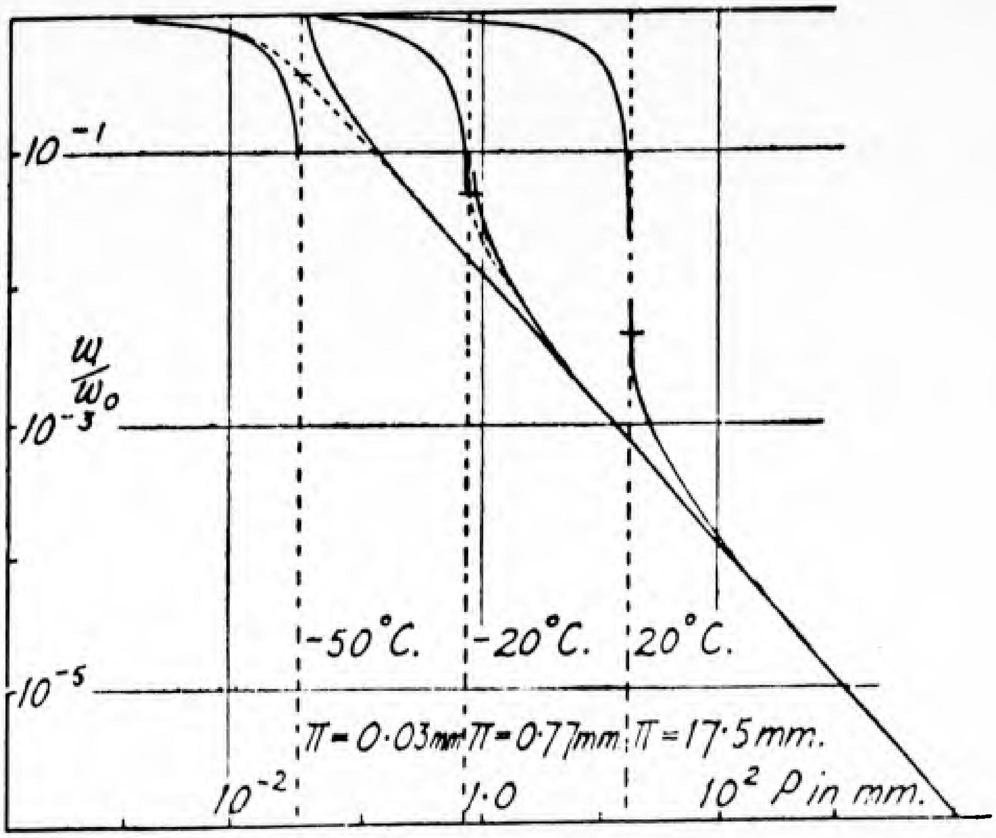

FIGURE 1. w/w_o against P at three different temperatures, $x = 1$ cm. Full curves for equations (7) and (8), broken curves for equation (6).

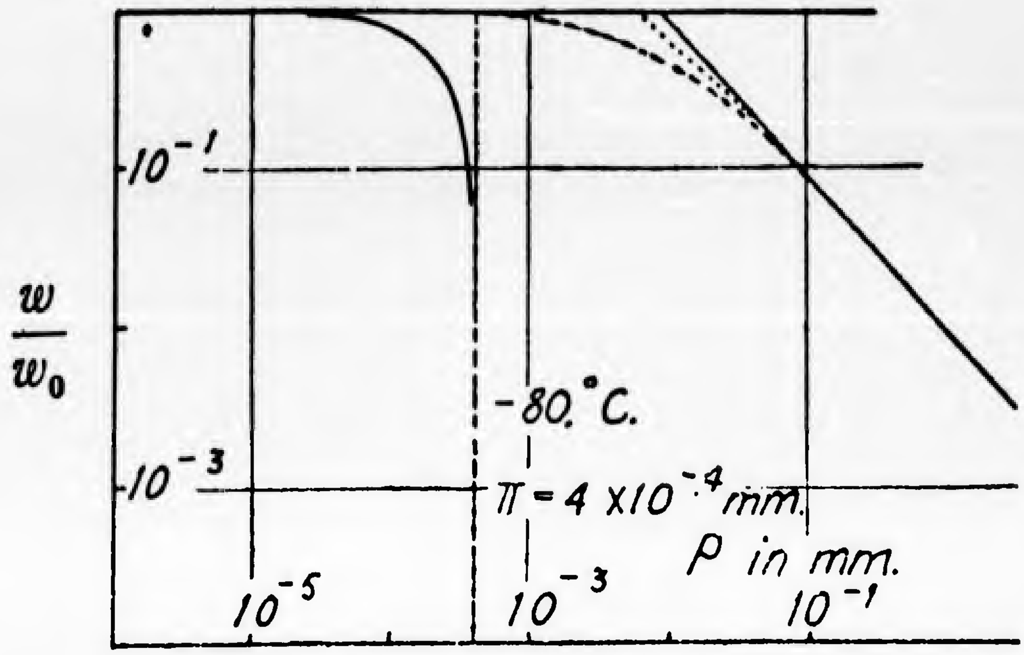

FIGURE 2. w/w_o against P at −80°C, $x = 1$ cm. Full curves for equations (7) and (8), broken curve for equation (6), dotted curve for mean free path correction.

In constructing Fig. 1, use has been made of the fact that if $P \ll \Pi$, $P_i \simeq P$ and hence

$$\frac{w}{w_o} \simeq (1 - P/\Pi) \tag{7}$$

while if $P \gg \Pi$, $P_i \simeq \Pi$ so that

$$\frac{w}{w_o} \simeq \frac{k \ln}{w_o x} \left(\frac{P}{P - \Pi}\right) \tag{8}$$

It can be seen that at −20°C and higher temperatures these two approximations cover almost the whole range and that there is a sharp change in the controlling mechanism in the vicinity of $P = \pi$ in which w rises rapidly to a value comparable to w_o. At −50°C, there is a considerable region over which the full calculation must be carried out, and the transition is not so sharp. As shown in Fig. 2, the approximations are not of much use at −80°C.

Before discussing Figs. 1 and 2, let us consider Fig. 3 where x is varied at a constant temperature of −20°C. It is sufficient to note that in effect a reduction of x is equivalent to a reduction of Π and vice versa. This result arises from the fact that Π and x appear as a product in the factor, $w_o x$.

The significance of these results is seen most clearly when we consider some of the statements which are frequently printed about molecular distillation. It is asserted that molecular distillation must

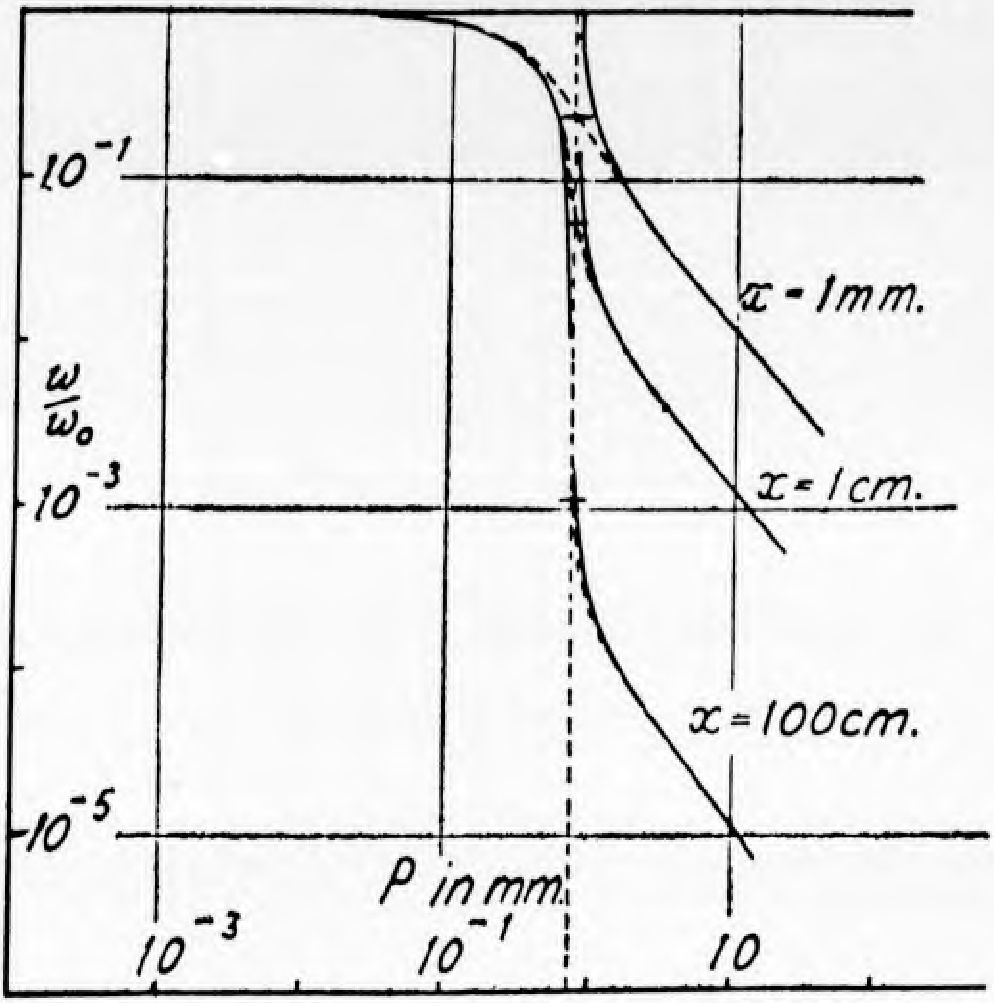

FIGURE 3. w/w_o against P at −20°C for three values of x. Full curves for equation (7) and (8), broken curves for equation (6).

be carried out under conditions such that a molecule escaping from the interface is certain to reach the condenser before it can collide with another molecule. In other words, the pressure must be so low that the mean free path is very much longer than the distance between evaporating and condensing surfaces. If this condition were applicable in the present instance, it would mean that molecular distillation could only take place when $x = 1$ cm if P is much less than 5×10^{-3} mm. If we examine the curve at −50°C, it can be seen that, while there is some justification for going down to this pressure, there is really little point in going much below $P = \Pi$. At −20°C, the sharp transition which takes place near $P = \Pi$ gives such a large increase in rate of evaporation that there is no practical point in going to lower pressures. In fact, when the enormous increase in w_o due to the higher temperature is taken into account, it can be seen that the practical difficulty lies in supplying enough heat to maintain the evaporating surface at −20°C. In the experiments of Kramers and Stemerding (2), allowance had to be made for temperature gradients through the ice layers, and their work therefore provides an ample illustration of practical factors which have been omitted in my theoretical calculations. The important point to be emphasized, however, is that, if surface temperatures can be maintained at a high level with the corresponding advantage of high evaporative rates, it is not necessary to consider going to pressures much below the corresponding value of Π.

In the case of ice at −80°C, the classical arguments are more valid. A new set of conditions now enters in. We have assumed thus far that normal diffusion takes place. It is well known, however, that when diffusion takes place in a capillary a decrease in pressure causes normal diffusion to give way to molecular diffusion as the mean free path approaches the dimensions of the capillary. To follow the transition in diffusion from an evaporating to a condensing surface in detail is not feasible, and the best that it has been possible to suggest is to apply an approximate method suggested by Fuchs (5). We assume that, up to a distance approximately equal to a mean free path from the evaporating surface, molecules leaving the surface do not collide so that it is meaningless in this region to speak of a concentration gradient along which diffusion takes place. It then follows that in a layer of thickness, Δ, approximately equal to the mean free path, λ, the vapor pressure is uniformly equal to P_i and that normal diffusion then takes place over the remaining distance $(x\text{-}\Delta)$. In other words, $(x\text{-}\Delta)$ replaces x in our equations. As the pressure decreases, Δ increases until it becomes equal to x. The effect of this calculation, with $\Delta = \lambda$, is shown by the dotted curve in Fig. 2. The effect is that $w/w_0 = 1$ when $\lambda = x$ and $P \simeq 5 \times 10^{-3}$ mm. Further reduction in P could not lead to higher evaporation rates. This value of P is considerably in excess of Π, and, furthermore, it is little affected if still lower temperatures are used for the evaporating ice. The only effect is to reduce the limiting rate of evaporation.

Approached from this point of view, the statements usually made about molecular distillation fall into their proper perspective. The statements usually apply to liquids of high molecular weight, with normal boiling points so high that they cannot be distilled by ebullition without decomposition. Owing to their large molecular size and the resultant large collision diameters, mean free paths of the vapors at a given pressure are very much less than those of water molecules, so that pressures of the order of 10^{-4} mm or less are essential for mean free paths to equal the distance between evaporating and condensing surfaces. Normally, however, their vapor pressures at practicable temperatures are very much less than this. Consequently, even if the liquids could boil, maximum distillation rates are achieved at pressures much above those required to bring about ebullition. The purpose of the present paper is to point out that the validity of such generalizations must not be stretched beyond their proper context. They are not applicable to liquids or solids with vapor pressures in excess of 10^{-2} mm. Consequently, though ice at −80°C represents a classical example of molecular distillation, this is not true of the much more normal freeze-drying temperature of −20°C and, in fact, there is little point in practice in going beyond normal evaporative distillation in freeze-drying processes.

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EVALUATION OF FREEZE-DRYING MECHANISMS USING MATHEMATICAL MODELS

WILLIAM H. MINK AND GEORGE F. SACHSEL

A number of simplified mathematical models have been developed in an attempt to determine the controlling mechanisms in freeze-drying. There are two basic considerations which determine the rate at which a material dries. These are heat transfer and mass transfer. Heat may be transferred by one or more mechanisms which are classed as 1) radiation, 2) conduction, and 3) convection. Because freeze-drying is normally carried out at low pressure, convection need not be considered. Mass transfer may occur by diffusion or by hydrodynamic flow. In freeze-drying either mechanism may become important, depending upon the operating conditions.

If we consider a piece of meat, or other material to be freeze-dried, which is being heated by dielectric energy, we can see that the factor which determines how rapidly the meat dries is the rate at which vapor can escape from the meat. If heat is applied faster than this rate permits, melting will occur. Therefore consideration of mass transfer alone gives us a limit on the drying time which applies regardless of the manner in which heat is applied. If the meat is porous so that there is essentially no restriction of the flow of vapor, drying can proceed as rapidly as heat can be applied and in this case heat transfer is the controlling factor. We can therefore consider heat and mass transfer separately in an effort to determine which mechanism places the greatest restriction on the drying time.

Heat Transfer by Conduction Only

The formulation of a heat transfer model for conduction only is relatively simple, but it illustrates both the techniques and the limitations of the development. At the beginning of freeze-drying, the side of the meat (or other material being freeze-dried) in contact with the shelf or heated plate dries most rapidly. However, as the ice phase regresses from the heat source, as shown in Fig. 1, the relatively high

thermal conductivity of the frozen portion, as compared to that of the dried portion, results in an almost uniform temperature distribution in the frozen portion. Evaporation or sublimation therefore occurs uniformly around the ice phase boundary and causes nearly uniform regression of the boundary (1).

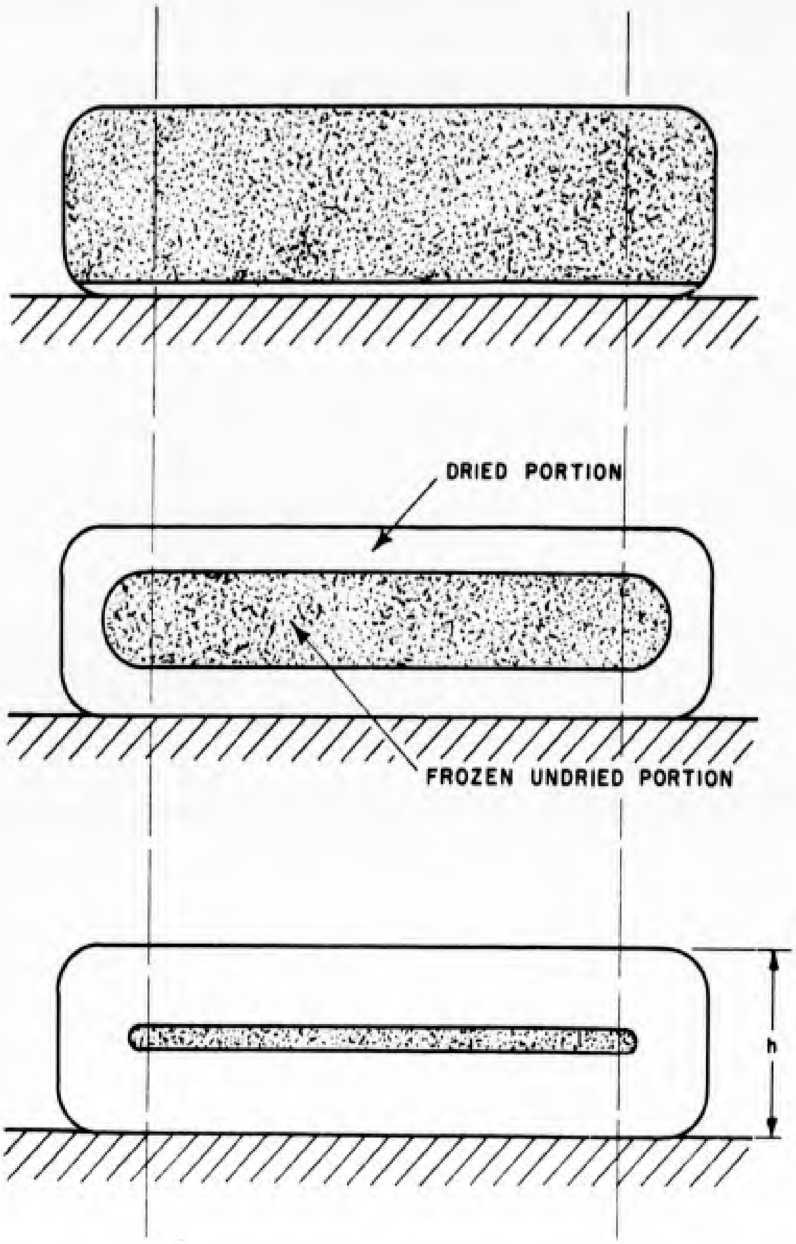

FIGURE 1. Regression of ice phase boundary during freeze-drying.

A number of simplifying assumptions have been made in formulation of each model. These are:

1. Uniform phase boundary regression. This effect has been discussed above.
2. No end effects. The meat is assumed to be large enough in length and width so that heat and mass transfer from the ends can be neglected. Thus the model consists of the portion of the meat included between the dashed lines of Fig. 1.

3. Meat is homogeneous and isotropic. This assumption is required because of the lack of quantitative data on the physical properties of meat.
4. Drying to zero moisture content. The assumption that drying will continue to zero moisture content is essentially a mathematical simplification. Due to the nature of the models, this assumption will not introduce significant errors when compared to the actual case where drying is carried out to only 2 to 4%.
5. No top-surface heat gain or loss. In the model for single-plate drying, it is assumed that heat is transferred only from the bottom plate and no heat is transferred through the top surface. In some of the other models this assumption will not be made.
6. Film coefficients are negligible. In all models where the meat contacts a surface, it is assumed that complete contact exists and the resistance of the surface film is not a factor.
7. The mechanism under consideration is assumed to be controlling throughout the entire drying period.
8. Constant temperature source.

Using these assumptions it is possible to show that the time required to dry is given by

$$t = \frac{Mh^2}{4k\Delta T} \qquad \text{Eq 1}$$

where t = Time, hr

M = Heat required to sublimate ice in one cubic foot of meat, BTU. Numerically equal to 52400 for beef.

h = Thickness of meat, ft

k = Thermal conductivity of dried meat, BTU/hr-sq ft-F/ft

ΔT = Difference in temperature between heating surface and frozen meat, F($\Delta T = T_1 - T_o$)

In the case where heat is conducted from both the top and bottom surfaces (for example where the meat is pressed between two heated plates), the time obtained from Eq 1 should be divided by 2. It should be re-emphasized here that this relationship expresses the drying time as limited by heat transfer and does not include mass transfer. If the meat is sealed between heated plates, one might expect that the drying time would be lengthened greatly because of restrictions on the mass flow. The relative importance of mass transfer and heat transfer can best be seen when the two mechanisms are evaluated separately.

Heat Transfer by Conduction and Radiation

A more practical model is one based on combined conduction and radiation heat transfer. This transfer could be expected to occur in conventional shelf freeze-drying where heat is radiated to the top of the meat from the shelf above or the sides of the dryer. The inclusion of radiant heat transfer makes the solution of the model relationships considerably more difficult. To simplify the problem, the contribution by radiation was linearized over the range of 90 to 150 F. For combined conduction and radiation, the time to dry is given by

$$t = \frac{Mb}{2k} [h - (\Delta Tb) \ln (1 + h/\Delta Tb)] \qquad \text{Eq 2}$$

Because of the complexity of Eq 2 it is difficult to visualize the relationship between the drying time, t, and the meat thickness, h. However, Eq 2 may be expanded in the following form:

$$t = \frac{Mh^2}{4k\Delta T^2 b} [1 - 2(h/\Delta Tb)/3 + 2(h/\Delta Tb)^2/4 - \ldots\ldots\ldots] \quad \text{Eq 3}$$

From this it can be seen that for small values of h, the drying time becomes proportional to h^2.

Mass Transfer

Using the same approach for the mass transfer model development that was used in the heat transfer work, a relationship was obtained for drying time as determined by mass transfer only. If vapor can escape from both sides of the meat, the drying time is given by

$$t = \frac{Bh^2}{16r} \qquad \text{Eq 4}$$

B = Weight of water per unit volume of meat, numerically equal to 43.7 lb/ft^3 for beef

r = Experimental mass transfer coefficient, lb/hr-ft^2 per ft

Here, as with heat transfer considerations, the drying time is proportional to h^2. The mass transfer coefficient, r, does not specify the mass transfer mechanism. It is a constant determined experimentally under the conditions of interest. It has been shown (2) for a sample of beef that r is a function of both temperature and pressure. Below about 0.2 mm Hg, r is constant for a constant temperature. Here the transfer mechanism is essentially hydrodynamic flow. Above about 10 mm Hg, r falls exponentially with pressure. In this area diffusional flow predominates. Between 0.2 and 10 mm Hg a transition zone exists in which both mechanisms are operating to a significant degree.

Harper and Tappel (2) give the same relationship for mass transfer and point out that the heat transfer rate is proportional to the mass transfer rate. This feature results from heat and mass transfer being analogous and from the same model being used for both. A comparison of Eqs 1 and 4 will show that for either heat or mass transfer, the drying time is proportional to the square of the meat thickness. The proportionality between heat and mass transfer no longer holds when radiation is included as shown by Eq 3 because the temperature of the surface of the meat is no longer constant but varies with time. Thus the driving force for heat transfer is changing whereas the driving force (pressure difference) for mass transfer is constant.

Heat Transfer by Radiation Only

From the standpoint of heat transfer alone, radiation will not permit faster drying times than permitted by conduction. The rate of heat transfer is limited in either case by the surface temperature of the meat; it must not exceed the point at which scorching will occur. If the surface temperature is raised to just below the scorching point, it makes little difference whether the heat is transferred by conduction or radiation for the drying times will be the same. However, radiation has an advantage in that there is no plate or shelf to retard the flow of vapor from the meat surface and in that a good contact is not required for efficient heat transfer. When heat is applied to both the top and bottom of the meat, the drying time is

$$t = \frac{0.29M(\sqrt{h})^3}{T_1\sqrt{ek}} \qquad \text{Eq 5}$$

T_1 = Temperature of heating surface, F

Heat Transfer by Spiked Plates

The obvious disadvantage of the methods of heat transfer described so far is that in all cases the heat is transferred through the dried meat which has a very low thermal conductivity. The use of spiked plates overcomes this difficulty because the heat can be conducted to the interior of the meat through the spikes. The model developed for spiked plate drying indicates that drying time is proportional to meat thickness.

$$t = \frac{3\sqrt{3MD^2h}}{4k\Pi d\Delta T} \qquad \text{Eq 6}$$

D = Distance between spike centers, ft

k = Thermal conductivity of frozen meat, BTU/hr-sq ft-F/ft

d = Diameter of spikes, ft

In this case, heat and mass transfer are no longer proportional because the methods of heat and mass transfer are no longer analogous.

In the development of this model it was assumed that heat was transferred only through the spikes and no heat was transferred through the plates. In actual practice a small amount of heat may be transferred through the plates, but the amount is probably insignificant in comparison to that transferred through the spikes. The plates that would be in contact with the meat are stripper plates not connected directly to the spikes or spike base plate, and in some cases care is taken not to squeeze the meat between the stripper plates.

Comparison of Models

On the basis of the available experimental data,[1] it is apparent that in most practical ranges mass transfer does not limit drying. Fig. 2 shows that heat transfer by conduction, or by combined conduction and radiation, limits the drying rate to about one-fifth of that permitted by mass transfer considerations. The deviation of Curve B from the straight line of Curve A is a result of the contribution of radiation. For meat thicknesses below ½ to ¾", the effect is negligible. For thicknesses above one inch, radiation becomes increasingly significant.[2] Good correlation is obtained between experimental data and the theoretical curves when the meat thickness, h, is large. For low values of h, the model gives drying times that are too short.

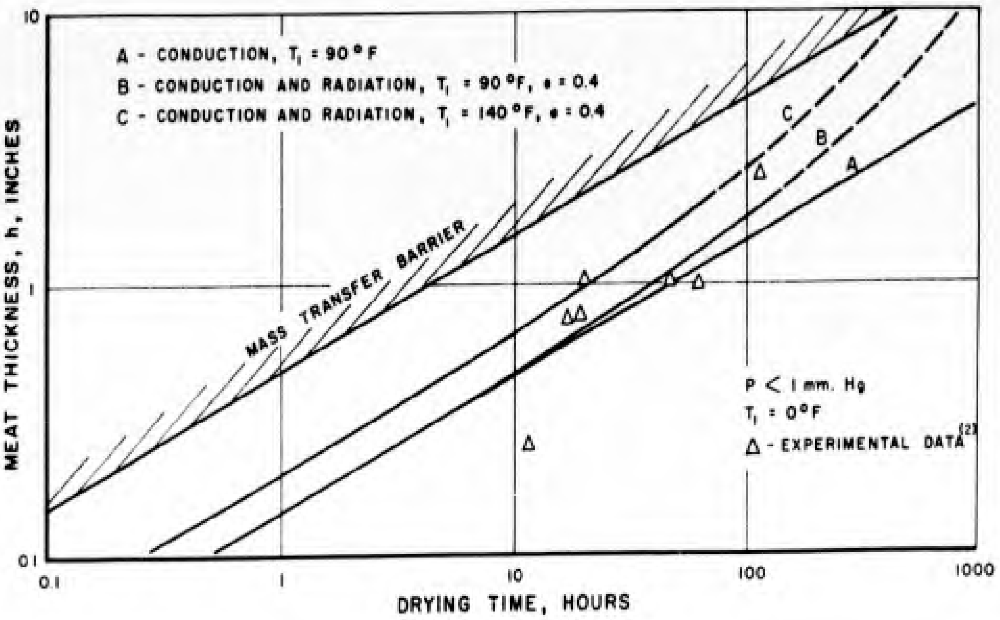

FIGURE 2. Comparison of theoretical and actual conduction—radiation drying time.

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[1] Because thermal conductivity data are available for Biceps femoris muscle, these data are used in quantitative evaluations of the models. The thermal conductivity is reported to be about 1.3 Btu/hr-ft-F for the frozen meat and about 0.02 Btu/hr-ft-F for the dried meat (1). Experimental values for the mass transfer coefficient, r, (below 0.2 mm Hg) are 0.4 g/hr-cm^2 per cm (0.027 lb/hr-ft^2 per ft) at 32 F, and 0.06 g/hr-cm^2 per cm (0.004 lb/hr-ft^2 per ft) at 0 F (3).

[2] Because of the linearizations used in the development, the Curves B and C of Fig. 2 are subject to error in the dashed region.

There is some difficulty in placing experimental points on Fig. 2 (and the Figs. following) because the temperature history is usually not well defined. All of the models are based on a constant-temperature heating source. Normally, in freeze-drying, the plate temperature is far from constant. For this reason, a temperature range of 90 to 140 F is shown in Fig. 2. This range includes the average temperature range of the points indicated. An additional difficulty is encountered with some of the data. Published drying times may include a period near the beginning of freeze-drying in which heat is not applied or is applied very slowly. Wherever possible, the data in the figures in this report have been corrected to place them on the same basis.

When heat is transferred by radiation to both sides of the meat, as shown in Fig. 3, mass transfer is not limiting for meat thicknesses less than 2 to 3 inches. In radiation heat transfer, as in surface conduction heat transfer, the surface temperature of the meat is the limiting factor.

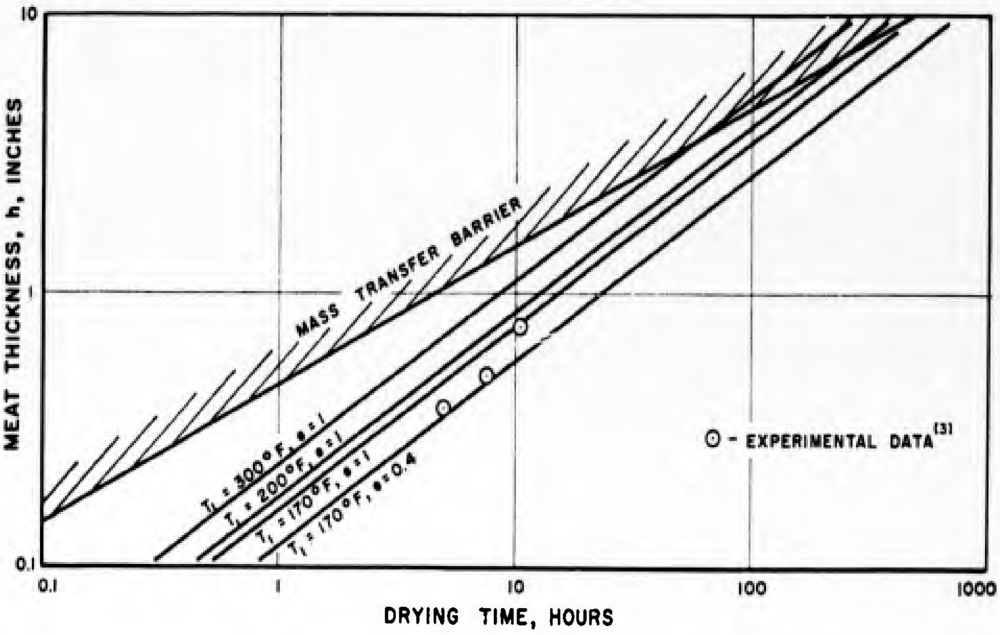

FIGURE 3. Comparison of theoretical and actual radiation drying time.

The use of spiked plates to transfer the heat avoids the problem of excessive surface temperature to some extent. Fig. 4 shows the correlation obtained between data for the model and data using spiked plates, as a function of the spiked plate design parameters. Fig. 5 shows the same data on the usual plot of meat thickness versus time. The mass transfer limit, or barrier, is shown for two meat temperatures. The meat temperature is a function of pressure and rate of heat transfer, and therefore for higher heat transfer rates the mass transfer barrier can be expected to shift so as to permit even higher drying rates.

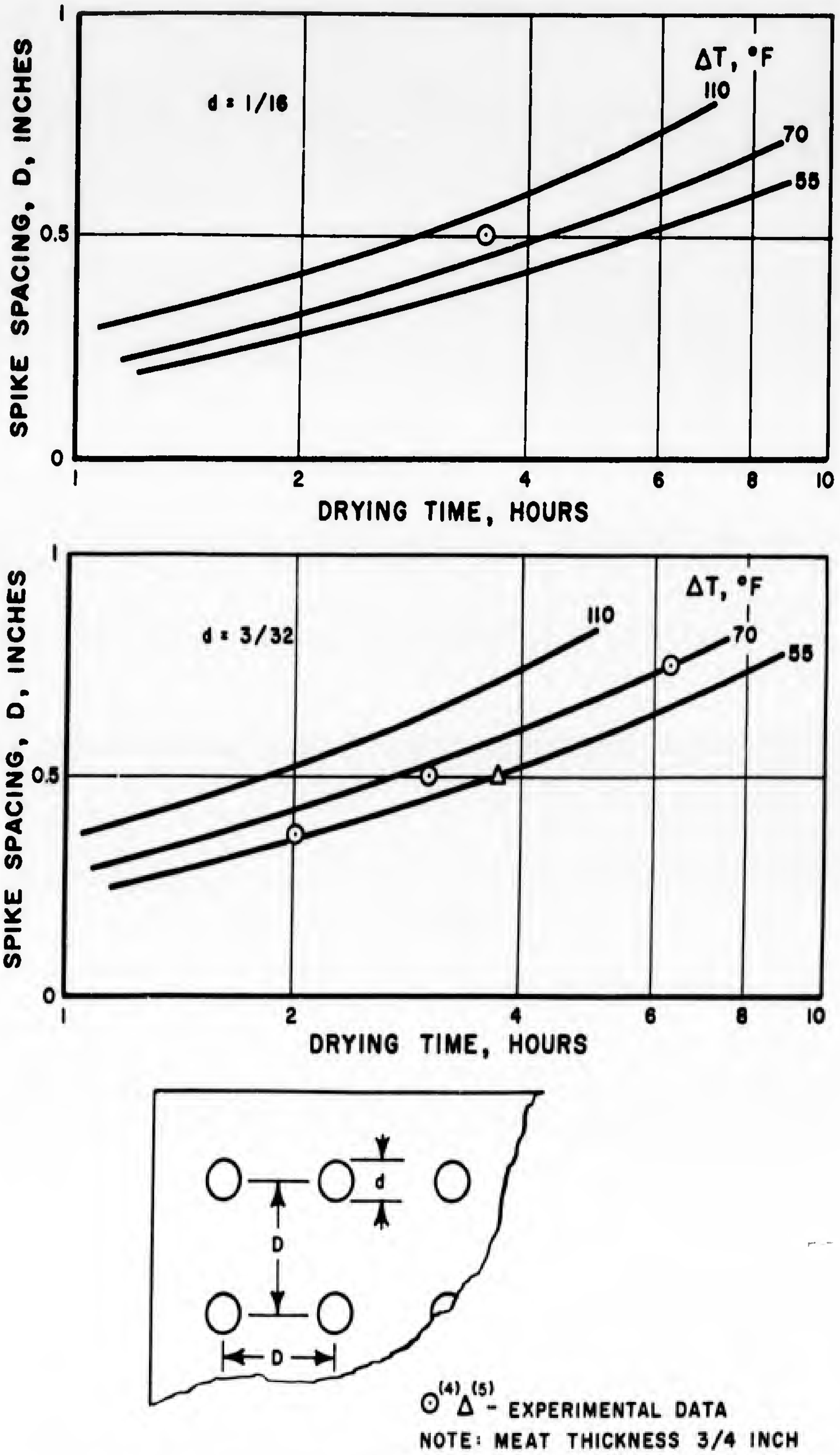

FIGURE 4. Comparison of theoretical and actual spiked plate drying time.

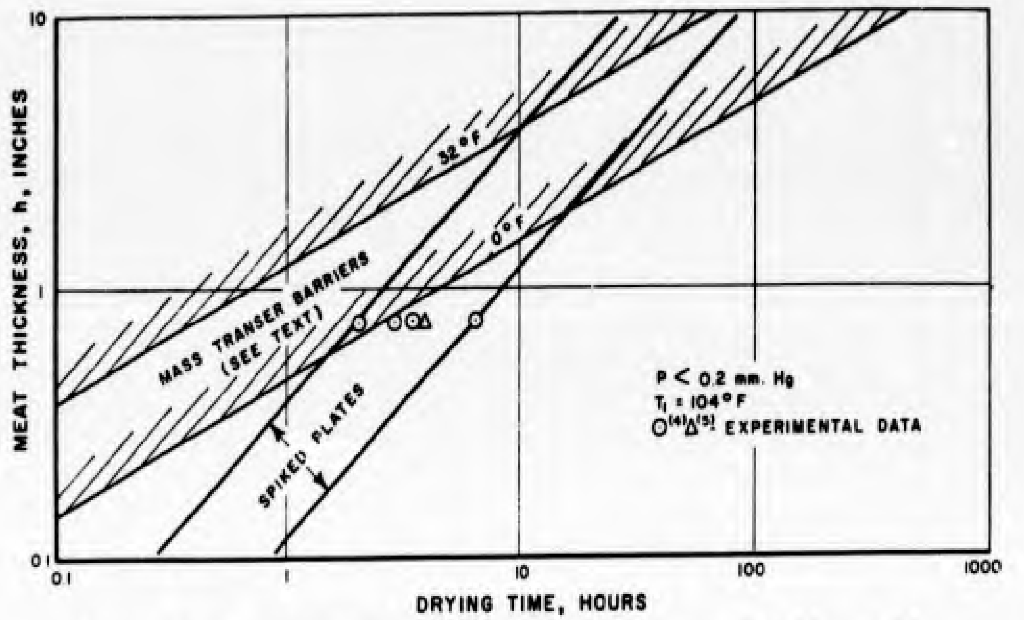

FIGURE 5. Drying time range for spiked plates as predicted by model.

Conclusions

The numerical results of the study of models were limited to one beef muscle because other data were not available. With this restriction in mind, it can be concluded that for the radiation and conduction freeze-drying systems considered, heat transfer, not mass transfer, is the principal factor limiting the drying rate. If heat transfer could be improved to the extent that mass transfer were controlling, drying times of the order of 5 to 40 minutes should be theoretically obtainable for meat of 3/8″ thickness.

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THE APPLICATION OF DIELECTRIC HEATING TO FREEZE-DRYING

ALFRED F. LEATHERMAN AND DAVID E. STUTZ

Introduction

The use of freeze-drying for the preparation of food for storage was first suggested by Flosdorf in 1945. As it is generally thought of today, the process consists of the removal of moisture from food products while in the frozen state, usually by exposing them to vacuum and heat. After removal of the moisture and enclosure of the freeze-dried product in a suitable wrapper to maintain cleanliness, storage at room temperatures for extended periods is possible with very little deterioration.

After the storage period, the addition of water restores the food material to a state offering practically no detectable difference in color, taste, or consistency compared with the original product.

For freeze-drying, the frozen food product usually is placed in a vacuum offering a pressure lower than the vapor pressure of ice at the temperature of the frozen product. The evaporation, or sublimation, of moisture from the ice to vapor state usually requires heat of sublimation to be added to the moisture. Without the addition of supplementary heat to the food product during drying, the freeze-drying process would be impractically lengthy. A variety of methods exist for supplying heat of sublimation in the practical freeze-drying process and many of these have been discussed at these conferences. Dielectric heating has been used successfully for this purpose on an experimental scale by several investigators (1, 2). The method appears particularly desirable for reducing the time required in freeze-drying because in dielectric heating the heat is generated internally, directly in the frozen portion of the material being dried. This localization takes place because those factors that determine the rate of heat generation in dielectric heating differ considerably between the dried and frozen portions of a given material, with the rate of heat generation per unit volume being perhaps several hundred times more in the frozen portion than in the dried portion (3). In conventional freeze-drying, the dried portion acts as a thermal barrier to the flow of heat into the specimen from the surface. In the dielectric method, this disadvantage is overcome because the heat is generated where it is needed, beneath the dried portion. Also, the process is self-regulating, tending to avoid scorching the dried portion and placing the heat selectively in the remaining frozen portion as the ice interface recedes. There is also a tendency, in dielectric heating at microwave frequencies, for the heat generation to be restricted to a zone within but near the surface of the undried portion. This effect helps to assure that the deep interior of the frozen portion does not become overheated and does not thaw.

In addition to the important advantages listed, dielectric freeze-drying at present apparently has at least two important limitations that will govern its rate of development. These limitations may explain why the method apparently is not yet used commercially for freeze-drying. One is an economic limitation. The most promising frequencies for dielectric freeze-drying appear to be in the microwave region. The electrical equipment that has been available to the present for application in this range apparently involves greater cost per unit of realized heat than does conventional freeze-drying equipment. However, dielectric heating equipment at lower frequencies is used widely in industry for applications such as drying and gluing. Also, microwave dielectric ovens are available for domestic cooking. Recent trends to lower equipment costs and direct savings that can be realized in accelerating the freeze-drying process may assist in speeding the commercial acceptance of microwave heating for freeze-drying. Dr. Decareau in his talk will consider some of the economic aspects of the microwave process.

A second limitation in dielectric freeze-drying involves electrical gaseous discharges that tend to occur in the vacuum chamber and tend to limit the maximum high-frequency energy that can be employed. This will be discussed later in greater detail.

We wish to point out that as yet Battelle has no experimental experience to report on dielectric freeze-drying. As intended, then, this presentation is devoted primarily to a discussion of some of the electrical engineering principles involved in dielectric freeze-drying.

Principles of Dielectric Heating

The basic requirement for dielectric heating is the establishment of a high-frequency alternating electric field within the material or load to be heated. Second, the load must be an electrical nonconductor offering sufficient dielectric loss properties to produce the required heat under the influence of the high-frequency field. Frozen food products in general offer excellent dielectric loss properties.

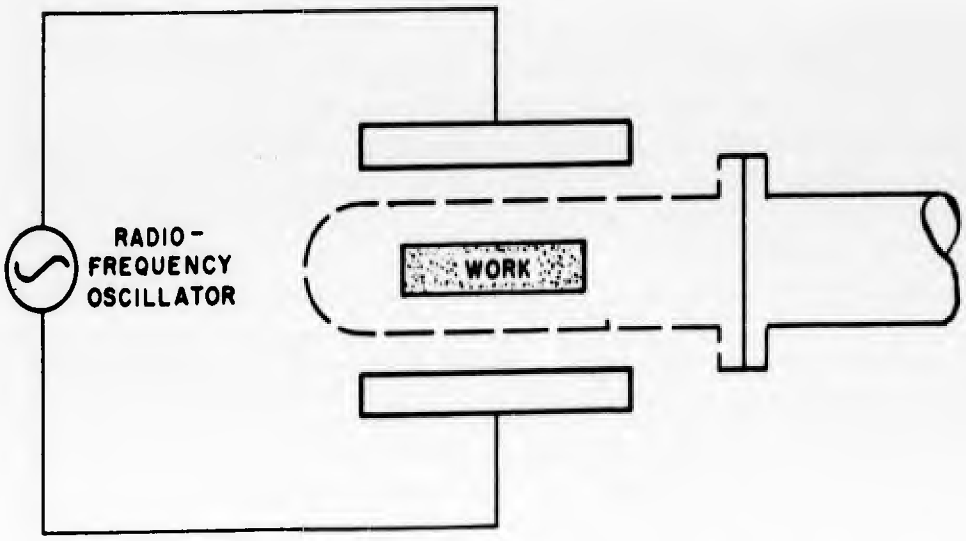
Two examples of how the required electric field can be set up are illustrated schematically in Fig. 1. In the case shown at the top of the figure, two electrodes are simply connected to the high-frequency source and, in effect, comprise the plates of a capacitor with the material to be heated placed between the plates and acting as the dielectric. The voltage appearing at the plates establishes the required alternating electric field in the space between them, including the interior of the load material. This arrangement is used for dielectric heating at lower frequencies up to around 1000 megacycles.

The second arrangement is usually used above 1000 megacycles, which includes the microwave region. In this case, the material to be heated is placed within a chamber which has electrically conductive walls. The high-frequency energy is then directed into the chamber via a waveguide transmission line from an appropriate source such as a magnetron. The energy reflects back and forth within the chamber filling much of the space within the required electric field. A rotating metal fan-like device is usually operated within the chamber to interrupt standing-wave field patterns that might otherwise cause hot and cold spots in the work.

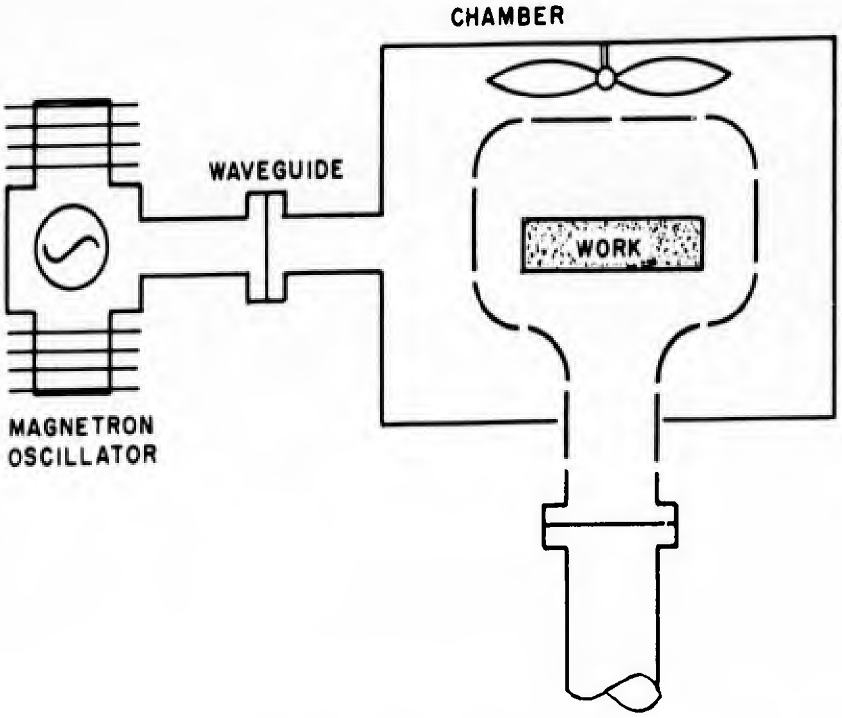
In each of these examples, the dotted lines represent the walls of a possible vacuum chamber that would be required in the application of the method to freeze-drying.

Polarization

Having established the electric field, the second requirement involves dielectric loss properties of the material to be heated. The dielectric loss property of a given material occurs as a result of elec-



a. POSSIBLE ARRANGEMENT FOR FREQUENCIES BELOW 1000 mc.



b. POSSIBLE ARRANGEMENT FOR FREQUENCIES ABOVE 1000 mc.

FIGURE 1. Arrangements for dielectric heating during freeze-drying.

trical polarization effects in the material itself. There are at least four recognized types of such polarization (4), two which occur as a result of the field itself, or induced polarization, and two which are inherent and determined by the arrangement of the component particles of the material itself. Fig. 2 illustrates these polarization effects schematically for conditions with and without the presence of an external electric field. If we assume an atomic nucleus and an orbiting electron, as shown at the top of Fig. 2, the orbit of the electron without the external field might be considered essentially as circular. When a field is applied, the electron orbit is distorted. As the polarity of the field is reversed, the distortion occurs first to the right, for example, and then to the left. In effect, this tends to stress the internal conditions of the atom and gives rise to the generation of heat. Rapid reversal of the field causes greater total stress per unit time and hence more heating. Since the electron is the primary element in this effect, it is referred to as electronic polarization.

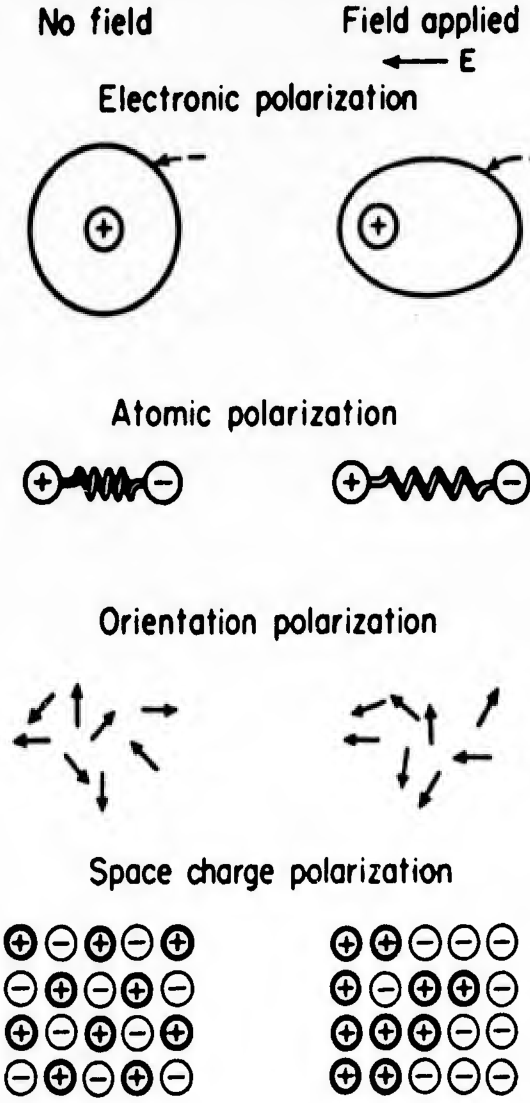


FIGURE 2. Mechanisms of polarization.

In the second example (Fig. 2) in a larger frame of reference, a larger particle such as a simple molecule is illustrated which contains atoms having net positive and negative charges. Since opposing charges attract, when a field is applied having a polarity negative to the left and positive to the right, the local charges will tend to stretch the molecule in a manner similar to stretching a spring, as illustrated schematically. When the field is reversed in polarity, the molecule will tend to be compressed. These repeated actions tend to create a sort of internal friction giving rise to heat.

In the third case (Fig. 2), again in a still larger frame of reference, the arrows each can be considered to represent more complex or "polar" molecules. As a field is applied, those molecules that are not oriented with the direction of the field experience a torque or rotational force tending to align them with the field. As the field varies, the amount and direction of torque varies, again tending to give rise to a frictional action.

In the fourth case (Fig. 2), a situation is illustrated in which particles having net over-all charges are somewhat free to migrate within a larger structure. As a field is applied, the charged particles move until they reach some restraining discontinuity or boundary where they collect. This migration is reversed upon reversal of the applied field. Except in a superconductor, electrical charges in motion experience a resistance to their movement, much as does an electric current in a resistor. Heat is generated in a resistor as a result of current flow. The generation of heat as a result of space-charge polarization can be considered as a similar effect.

As was mentioned, the various types of polarization illustrated in Fig. 2 are arranged from top to bottom in the order of increasing volume of material affected, so to speak. As might be expected, a relationship exists between the frequency of alternation of the applied field and the type of polarization that predominates in a particular case. Electronic polarization is of most importance at the highest frequencies, such as visible light. Atomic polarization is more prominent at infrared frequencies, and the mass of the particles involved is too great to respond much to the very rapid field reversals occurring at optical frequencies. Atomic polarization is largely responsible for generation of heat during radiant heating. Orientation polarization is most prominent at microwave frequencies, while space charge polarization is prominent in the lower radio frequency ranges. Of course, some overlapping of these effects occurs over the spectrum.

As would be expected, not all the atoms or molecules of a given material exhibit polarization effects. The dielectric constant of a material can be considered as an indication of the relative number of particles in a material that exhibit polarization, a larger dielectric constant meaning that more such polar or polarizable particles are present. The dielectric loss constant of a material can be considered as an indication of the average amount of friction loss effect that each polarized particle contributes. As the frequency varies, various combinations of polarization effects and resonances occur giving rise to irregularities in dielectric constant and loss values.

Power Input

Let us now consider all the factors that are involved in describing the actual amount of dielectric heat that is generated in a typical case. The volume rate of power input to the dielectric material can be expressed as

$$P_v = 1.41\, E^2 f \epsilon_r \tan \delta \times 10^{-12} \text{ watts per cubic inch } (5, 6)$$

where f = frequency of the electric field in cycles per second

ϵ_r = relative dielectric constant of the work

$\tan \delta$ = dielectric loss tangent of the work.

The voltage, frequency, and dielectric constant terms are assumed to be familiar to most of the audience. The loss tangent can best be explained with reference to Fig. 3. In the figure, E and I are vectors representing the applied voltage and resultant net current flow through the work being heated. The power factor angle, Θ, between the voltage and current is determined by the complex dielectric constant, ϵ^*. The complex dielectric constant can be resolved into a lossy component ϵ'' and a reactive, or energy-storage, component, ϵ' (or ϵ_r, the relative dielectric constant). The loss tangent, δ, is the tangent of the angle described by the projection of ϵ'' to the top of the figure, and by ϵ', and is equal to ϵ''/ϵ' and is sometimes referred to as D, the dissipation factor. (Returning to the power-input equation, we note

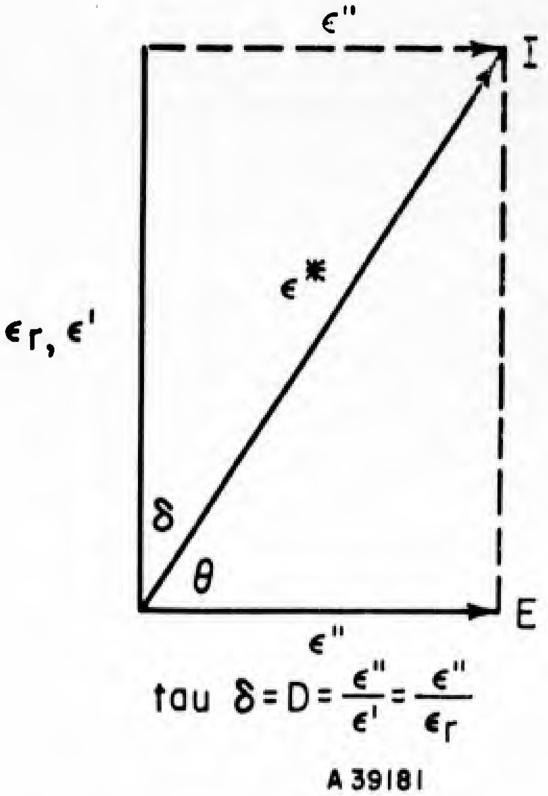


FIGURE 3. Relationship between dielectric quantities.

that if ϵ''/ϵ_r is substituted for tan δ, ϵ_r, the energy-storage component, divides out of the equation, and the rate of heating, then, is actually directly proportional only to ϵ'', the lossy component).

Some examples of typical values of dielectric constant and loss tangent reported for frozen foods are listed in Table 1 (7). Values for pure ice are given for comparison (4). Relative dielectric constants for the frozen foods vary from 2.5 for boiled peas to 13.0 for boiled spinach. Loss tangents vary from 0.15 for raw beef to 1.2 for raw pork. The wide differences in dielectric constant possibly can be attributed to differences in moisture content and to differences in the form in which the moisture is present, while differences in loss tangent probably reflect differences in fat contact and type of protein, among other factors. It goes without saying that a myriad of factors determine the dielectric properties in various specimens, and it would not be surprising to find wide differences in the measured results of various investigators. Measurements made at various frequencies also can be expected to differ somewhat. It should be pointed out that the data for the frozen foods were measured at a frequency of 1000 mc, those for ice at 3000 mc, while a frequency of 2450 mc has been used by most investigators of microwave freeze drying (1, 2). The values of Table 1, however, can be used to obtain an idea of what can be expected from the microwave process.

TABLE 1

Dielectric Properties of Frozen Foods

Frozen Material at −15°C, 2450 Mc	ϵ_r	Tan δ	Z_l cm to 37%	P_v, Power/in.^{3}, 100 v/in.
Ice (−12°C, 3000 mc)	3.2	0.00095	700 (50%)	0.105
Beef, raw	5.0	0.15	5.8	26
Pork, raw	6.8	1.2	0.66	282
Porridge	5.0	0.3	3.7	52
Peas, boiled	2.5	0.2	7.9	17
Spinach, boiled	13.0	0.5	1.42	225
Potatoes, boiled	4.5	0.2	6.1	31

As an illustration, the dielectric values of Table 1 were inserted into the power input equation assuming an applied electric field of 100 volts per inch and a frequency of 2450 mc. This resulted in the figures in the last column of Table 1. These figures may be considered as relative values of power input per cubic inch but should not be considered as quantitative.

An important condition occurs which makes the listed power input figures only qualitative. That is, the effect of attenuation of the field by the food, resulting in a limited penetration of the applied field into the interior of the specimen. The microwave field penetrates the food and creates heating action within only a relatively small depth at the surface. The amount of penetration is different for various substances and depends upon the dielectric loss properties of the material. Values calculated by others (6, 7) for the depth, Z_1, at which the applied field has been attenuated to a value of about 37% of the surface value are listed. In the case of raw pork, this point occurs at a depth of less than one centimeter, while in ice, the field has fallen only to 50% at a depth of 700 cm. The wide range of values for penetration and power input illustrate the large differences in dielectric heating performance that can be expected for different foods. As indicated by the table, the surface rate of heat generation under the conditions described differs by over 2800 to 1 between pure ice and raw pork. This difference illustrates that the heating action is not primarily attributable simply to the presence of ice.

As might be expected from the large range of values in Table 1, different portions of a given piece of food experience different rates of heating, depending on variations in composition, such as fat content. In microwave freeze-drying, care must be exercised to avoid overheating the fatty portions (3). Generally speaking, however, the dielectric properties of frozen foods are excellent for microwave heating in comparison to dielectric properties of other substances.

Referring to the power input equation, it is evident that to obtain the maximum rate of heating and, hence maximum drying speed, one should endeavor to make all terms on the right side of the equation as large as possible. Little probably can be done with the dielectric properties of food although various additions could have an effect. In working with a given food, it might be possible to select an operating frequency at which the natural dielectric values exhibit a peak, or resonance. In general, the best approach appears to be to use as high a frequency and electric field voltage gradient as possible since the rate of heat generation is directly proportional to frequency and to the square of the voltage gradient. This leads to the limitations that were referred to earlier.

Apparent Limitations of the Microwave Freeze-Drying Process

It should be pointed out that the following discussion relates only to an apparent limitation on the ultimate possible rate of heating obtainable with microwave freeze-drying. As Dr. Decareau in his talk will illustrate by actual experience, the microwave process does have practical value even at its present, we hope early, state of development.

Aside from economic considerations, which this discussion is making no attempt to evaluate, the most important condition limiting the maximum heating rate obtainable in microwave freeze-drying seems to be "ionization," or electrodeless discharge created within the freeze-drying vacuum chamber as the electric field is increased above a certain value. Difficulties with ionization as reported by Harper and Tappel (8) in the frequency range of 10 to 30 megacycles and by Jackson, Rickter, and Chichester (1) in microwave freeze-drying of peaches probably can be considered as representative of this effect. These discharges are similar to those upon which the operation of neon and fluorescent lights depend. However, the microwave equipment cannot operate properly when such discharges are present in the chamber. Also, there is some possibility of contaminating or burning the food in the presence of these discharges. Among other possibilities to be considered later, the discharges can be avoided by accepting a drying rate below that at which the microwave equipment causes the discharges to initiate.

The voltage gradient at which the discharges initiate depends upon the frequency of the field, the pressure and composition of the gases in the chamber, and probably upon the physical size of the chamber and the work. It is clear that if a good combination of these factors could be found, an increase in the maximum feasible rate of microwave freeze-drying might be realized.

A brief literature survey on the subject of electrodeless discharge resulted in practically no quantitative data and in only one study that appeared to offer a situation resembling that of the freeze-drying chamber. This was in the work of J. F. Steinhaus at the University of California, Livermore, in connection with the development of the so-called "Glo-Ball" (9). In working with particle accelerators, a method was needed by which to determine the configuration of the high-frequency electric field used in the accelerator. These investigators found that good use could be made of a device consisting of a hollow glass sphere into which a gas of known composition was sealed at a given pressure. The gas within a given sphere would become ionized and glow when the ball was placed in an alternating electric field of sufficient voltage gradient. The gradient at which glow, or ionization, would occur was reproducible and could be used to survey the configuration of the field in the accelerator.

In developing the "Glo-Ball," Steinhaus and associates catalogued the dependence of the firing voltage on pertinent factors such as ball diameter, gas composition and pressure, and the frequency of the electric field. In Fig. 4, their results are shown in which rms firing voltages were determined for spheres of one-half inch diameter containing gases at various pressures. It is seen that except for helium, the firing voltage in general decreases at lower pressures. The typical result expected would be similar to that for hydrogen in which a certain pressure exists for which the firing voltage is a minimum. Although not visible, minima are most certainly present for the other gases as well since in high vacuum, electrical discharge will occur only at very high voltage gradients. The curve for hydrogen resembles the classical Paschen's Curves relating firing voltage to gas pressure and electrode spacing.

It would be expected that the gas present during freeze-drying would be almost all water vapor. Although data for water vapor are not reported by Steinhaus, presumably the water vapor curve would resemble the other curves of Fig. 4.

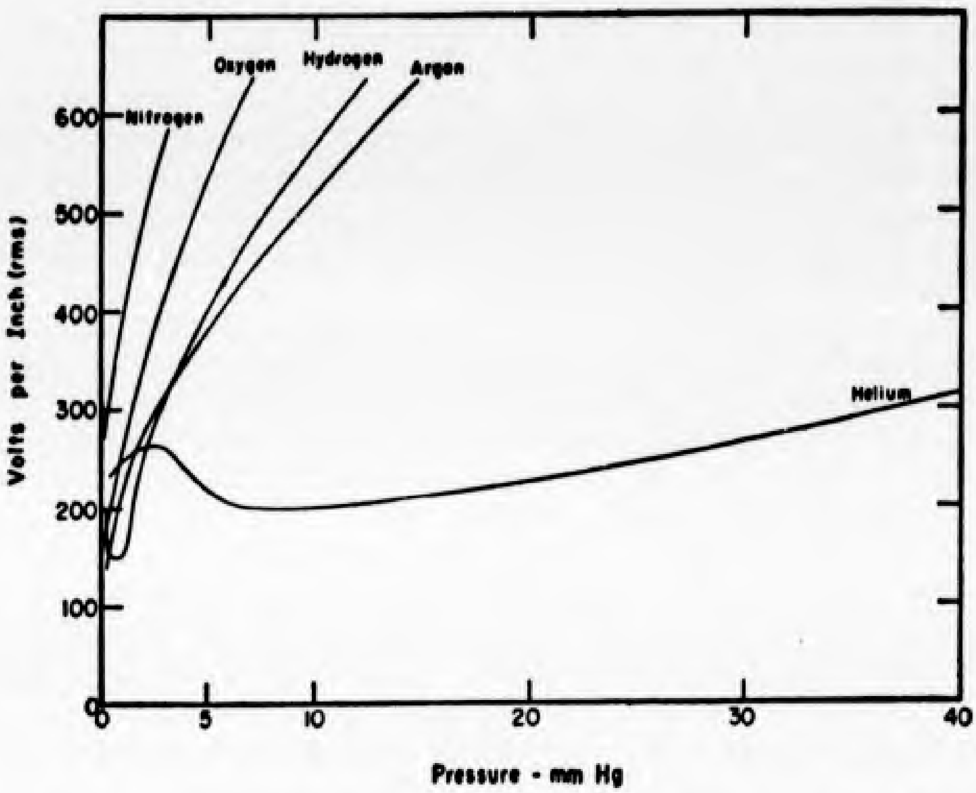


FIGURE 4. Firing voltage gradient as a function of pressure for different gases; glo-ball diameter ½ in.

It is noted that the minimum firing voltage for hydrogen, or point of easiest ionization, occurs at a pressure in the vicinity of one millimeter of mercury, a universally popular pressure for the operation of freeze-drying chambers. In this regard, Jackson *et al* (1) found in microwave freeze-drying of peaches that ionization would be reduced by operating at pressures either higher or lower than about one millimeter. At pressures of 2 or 3 millimeters, however, the difference between the chamber pressure and the partial vapor pressure of water vapor coming from the frozen specimens apparently became quite small. The combination of decreased rate of sublimation and increased level of microwave heat that could be used was sufficient to cause thawing with no appreciable gain in drying speed. As pressures of about 50 microns, greater microwave heat also could be used, and fast drying rates were obtained. As might be expected, however, the pumping requirements were considerably increased to maintain this low pressure. In conclusion, Jackson *et al* (1) claim that microwave heating permitted a 9 to 1 increase in drying speed over the single-plate type of heating and that about 74% of the power from the microwave source could be realized as heat in the specimen.

It was mentioned that the voltage gradient at which ionization occurs also depends upon the frequency of the electric field. In Fig. 5, data on firing voltages are presented for glo-balls containing a given gas, believed to be helium, at pressures of approximately 10 millimeters. The family of curves represent different diameters. The

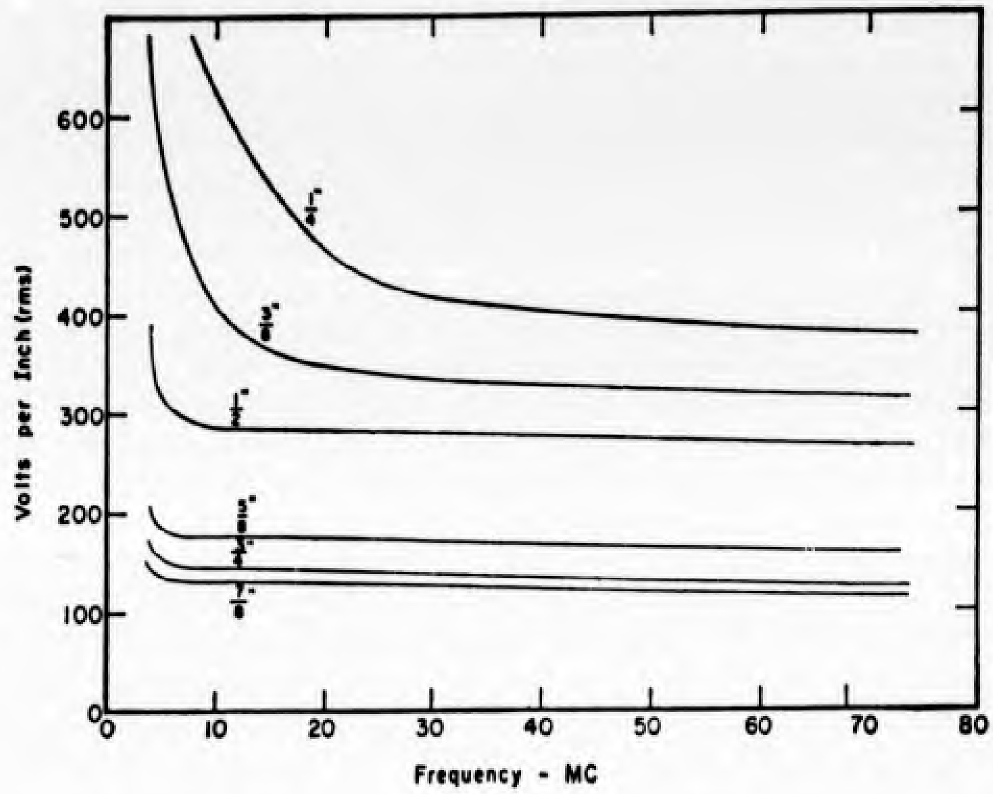


FIGURE 5. Firing voltage gradient as a function of frequency for different glo-ball diameters.

smaller-diameter balls require higher voltage gradients for ionization to occur. As the diameter is increased, firing voltages decrease and appear to approach an asymptote. Perhaps a curve just below that for the 7/8-inch ball could be assumed to represent a chamber as used for freeze-drying. For the larger balls, the firing voltage changes very little at frequencies above about 5 megacycles. Although the data are not complete for lower frequencies, it is apparent that higher ionization voltages are obtained as the frequency is decreased. This might give the impression that the ionization problem could be more easily avoided by performing dielectric freeze-drying at these lower frequencies. However, an examination of all the quantities of the power input equation reveals that because of the relatively small value of the frequency term and the fact that the dielectric properties also change with frequency, a very high voltage gradient would be required at low frequencies to realize heating rates equivalent to those easily obtained at microwave frequencies. It was concluded that the prospects for a practical low-frequency dielectric freeze-drying process were not promising and that the process is probably best performed at the highest possible frequency.

Possible Improvements in Microwave Freeze-Drying Techniques

In reviewing the literature on microwave freeze-drying, it was noticed that most investigators have used the standard domestic type microwave oven as the power source. These ovens are constructed for maximum economy and consequently usually do not employ a filtered d-c power source for the magnetron. In this case (when the power supply is not filtered) the microwave energy occurs in the form of bursts coincident with the pulsations of the 60 cycle power line. For full-wave rectified but unfiltered magnetron supply voltage, the peak voltage gradient of the microwave field would be approximately 40% higher than its rms value. Since the rms value actually describes the rate of heating, the tendency for ionization to occur when unfiltered power is used is greater than necessary. By using filtered or pure d-c supply voltage, the same heating could be obtained with a continuous supply voltage only about 70% of the peak voltage produced by an unfiltered power supply. By the same reasoning, and noting that the rate of heating depends on the square of the voltage gradient, operating with d-c supply voltage equal to the peak output of an unfiltered power supply could produce twice the heating with no increase in tendency toward ionization. In the case of the self-rectifying magnetron, and assuming only one magnetron is used, a four to one increase of heating should be realized, without additional tendency toward ionization, by changing to d-c supply voltage equal to the former peak value. Both of these suggestions assume that the design of the magnetron itself is such to permit the additional power to be generated.

A second suggestion concerns combination heating for freeze-drying. In the conventional freeze-dryer, it is reported that it sometimes takes as long to remove the last 10% of the moisture as to remove the first 90%. Since most of the heat in microwave heating is generated in the ice phase instead of the dried food material, the method should be particularly valuable at low levels of moisture. It is suggested that a conventional freeze-drying procedure might be used to remove the first 80 or 90% of the moisture, with a microwave process to finish the drying cycle.

It was mentioned that fast drying rates, with little or no ionization difficulty, could be realized by operating at low pressure in the drying chamber, for example, at 50 microns. It is suggested that if part of the drying cycle were completed at normal pressure by conventional means or by microwave heating at sufficiently low power, the last part of the process might be accelerated by using high-power microwave heating at a lower pressure. Since most of the moisture would have been removed in the first part of the cycle, possibly the pumping requirements for the last portion of moisture would be small enough to make a lower pressure feasible.

A final suggestion involves the configuration of the microwave electric field during freeze-drying. In certain types of resonant cavities, such as a cylindrical chamber operating in the TE_{11} (or H_1) mode, higher electric field gradients are found along the center axis of the chamber than anywhere near the walls. Possibly, if the specimen could be centered on the axis of the chamber, the highest voltages would be present within the body of the specimen, where ionization is assumed to be unlikely, and lower gradients would be present external to the specimen in the open part of the chamber where ionization is more likely. On the average, this might permit higher microwave power to be used.

Conclusions

Although some investigation of microwave freeze-drying has been done with promising results, the method has apparently not been accepted commercially. It is felt that much of the investigation so far has been carried out with conventional microwave equipment and conventional techniques. It is suggested that further effort aimed specifically toward the individual problems and requirements of microwave freeze-drying could very likely result in the development of a fast and efficient process of substantial commercial interest.

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HEAT AND MASS TRANSFER IN FREEZE-DRYING

JOHN B. LAMBERT AND W. R. MARSHALL, JR.

Although freeze-drying is a long established art, its commercial application has been limited to high unit cost, heat-labile materials. The advantages of freeze-drying over conventional drying methods, the minimizing of thermal degradation or loss of biological potency, the maintenance of product dispersability, and the retention of flavor and aroma are achieved only at a cost. Commercial freeze-dryers are equipment which have a) low capacity and b) high operating and investment costs. Until recently, freeze-drying has been a production technique used almost only as a last resort.

A basic problem has been the inadequacy of theory and the lack of test data to permit rational engineering design. The work of Dr. Carman, described in a preceding paper of this conference, has been important in describing the physical parameters controlling the sublimation process.

Three objectives of this study were: 1) to extend the theory to the drying by sublimation of materials containing solids; 2) to attempt to characterize such materials so that the theory could be used to predict drying times and rates; and 3) to compile performance data to serve as the basis for improved engineering design. It should be added that in this paper we have been concerned primarily with the effect of variables on drying rate, not on product quality. When considering the effect of the freeze-drying process on quality, it is important to determine the effect on quality of each process step, i.e., freezing (and thawing), sublimation, and reconstitution, if the product must be rewet before use.

Theoretical Development

Heat Transfer in Freeze-Drying

The rate of sublimation in freeze-drying equals the heat transfer rate divided by the heat of sublimation. Thus,

$$w = \frac{q}{\lambda_s} \qquad \text{Eq 1}$$

The fundamentals of both conduction and radiant heating in freeze-drying have been discussed previously (2, 10). In conduction drying, a heating fluid is circulated under or around the frozen material, and energy for sublimation is transferred across the ice thickness to the subliming interface. The rate of sublimation for the drying of a slab frozen in a tray is given by

$$w = \frac{q}{\lambda_s} = \frac{\dfrac{(T_m - T_s)}{\lambda_s}}{\dfrac{1}{h} + \dfrac{B}{k_b} + \dfrac{x}{k_i} + R_b} \qquad \text{Eq 2}$$

where R_b is a resistance to heat transfer caused by the imperfect thermal contact between ice sample and tray bottom. The analogous relation for the sublimation of a shell of ice frozen in an ampoule is

$$w = \frac{q}{\lambda_s} = \frac{(T_m - T_s)}{\lambda_s \left[\dfrac{1}{h} + \dfrac{B}{k_b} + \dfrac{r_b}{k_i}\left(\ln \dfrac{r_b}{r}\right) + R_b\right]} \qquad \text{Eq 3}$$

Equations 2 and 3 are not applicable for the final (or falling-rate) stages of freeze-drying after the ice zone has receded to the container wall. In these cases, sublimation occurs throughout the remaining porous "mass" and not from a well-defined interface. The treatment of this final period of freeze-drying has received little attention. This is an area for needed future work. The basic differential equations for heat and mass transfer to porous cakes, also applicable to the falling-rate period of freeze-drying, have been derived by Krischer (6).

Radiant energy freeze-drying is carried out by placing the sample in an infrared transparent container which is exposed to radiating heating elements. Practically speaking, the amount of penetration of infrared radiation is negligible beyond three millimeters in thickness so that no great loss of accuracy results if the conduction equations are applied. Of course, for radiant heating the heat transfer resistance attributed to the transparent tray bottom and the tray-ice interface can be neglected.

The Mechanism of Mass Transport in Freeze-Drying

General Considerations. In a fashion analogous to that used for deriving the rate of heat transfer, the rate of mass flux is proportional to a mass transfer driving force divided by the sum representing mass transfer resistance in series. Consider a typical tray freeze-dryer having a plate condenser parallel to the evaporator and using a mechanical vacuum pump to remove permanent gases. Preliminary considerations indicate that for this system the possible driving forces and resistances to mass transfer are:

Driving Forces	*Resistance*
1. Water vapor partial pressure difference between evaporator and condenser.	1. Resistance to mass transport at the sublimating interface.
2. Bulk flow of gas due to pumping.	2. Diffusional or flow resistance through dry cake above interface.
3. Transport by natural convection.	3. Diffusional resistance to water vapor flow caused by inert gas between evaporator and condenser.
4. Entrainment of ice particles by moving gas stream.	4. Resistance to interphase transfer at the condenser.
5. Thermal diffusion.	

In Fig. 1 the geometry of the system under consideration is depicted, and the various mass transfer variables are noted. Although this treatment deals with the freeze-drying of a slab, the equations are applicable to other geometries if suitable coordinate transformations are made.

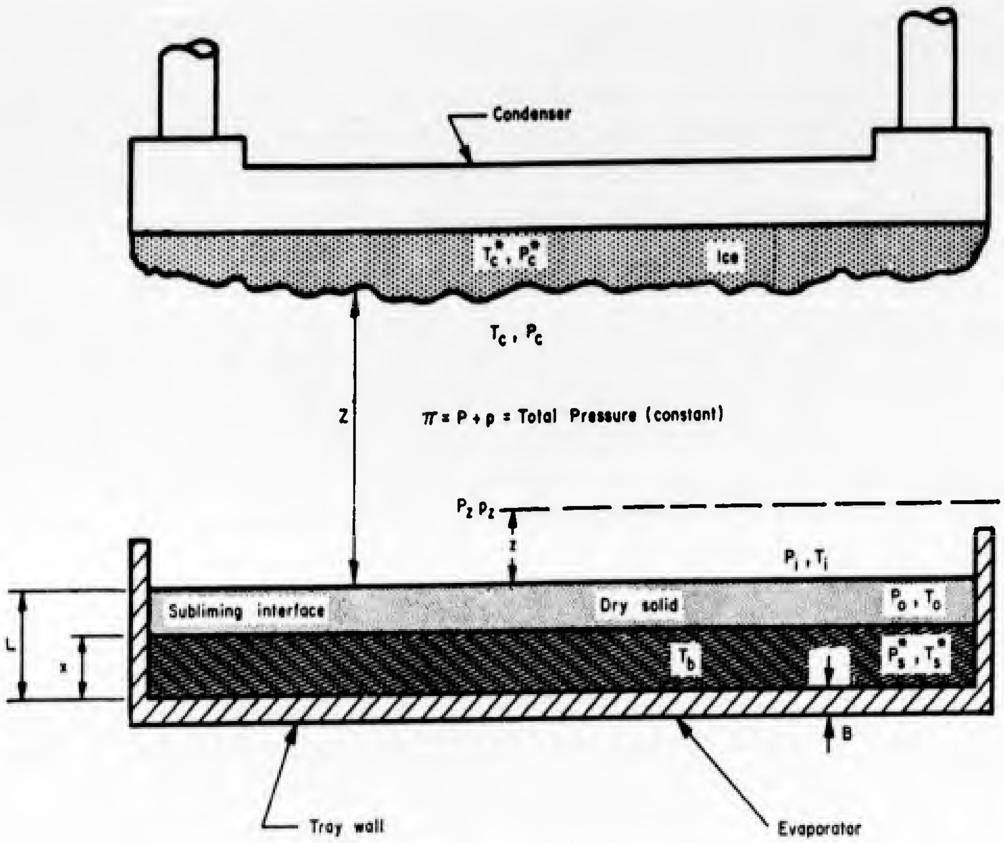


FIGURE 1. Diagrammatic view of mass transfer system.

Estimates of the magnitudes of natural convection, entrainment, and thermal diffusion under the most extreme conditions encountered in freeze-drying indicate these effects have, at most, only a minor role in the mass transfer and may be neglected. Under most conditions, the water vapor partial pressure driving force is the chief one. Bulk flow depends on vacuum pump capacity, total pressure, and the location of air leaks into the system. In most commercial freeze-dryers, the vapor flow from the sample cake to the condenser is laminar (turbulent conditions are rarely a consideration in high vacuum systems). On the other hand, the description of the flow through the partially dry solid above the subliming surface is free molecular or in the transition region between free molecular and laminar. Let us now consider separately the mass flux for each of the mass transfer resistances.

Interphase Mass Transfer. The analysis of molecular sublimation by Carman (2) has been used by most previous workers to describe interphase mass transfer in freeze-drying. He proposed the equation

$$w = w_s^* \left(1 - \frac{P_o}{P_s^*}\right) = \sigma\beta_s\,(P_s^* - P_o) \qquad \text{Eq 4}$$

where
w_s^* is the absolute vacuum rate of evaporation and $\beta_s = \sqrt{\dfrac{M}{2\pi RT_s^*}}$.

For water, $\beta_s = \frac{2447.5}{\sqrt{T_s}}$ lb/(mm Hg)(sq ft)(hr).

Schrage (8) recognized a serious inadequacy of Equation 4, that the molecules striking the phase interface from the gas phase were assumed to have a Maxwellian molecular velocity distribution. Since net mass transfer occurs in freeze-drying, it is clearly erroneous to use an equilibrium velocity distribution. Schrage extended Carman's treatment by assuming that the velocity distribution function was Maxwellian with respect to the molecular stream velocity rather than fixed coordinates (with the origin at the interface). The modified expression derived for sublimation was

$$w = w_s^* \left[1 - \frac{P_o}{P_s^*}\left(\frac{T_s^*}{T_o}\right)^{\frac{1}{2}} \Gamma_s\right] = \sigma\beta_s \left[P_s^* - P_o\left(\frac{T_s^*}{T_o}\right)^{\frac{1}{2}} \Gamma_s\right] \quad \text{Eq 5}$$

Similarly, for condensation,

$$w = w_s^* \left[\frac{P_c}{P_c^*}\left(\frac{T_c^*}{T_c}\right)^{\frac{1}{2}} \Gamma_c - 1\right] = \sigma\beta_c \left[P_c\left(\frac{T_c^*}{T_c}\right)^{\frac{1}{2}} \Gamma_c - P_c\right] \quad \text{Eq 6}$$

The quantity, β_c, is identical to β_s except that T_s^* is replaced by T_c^*, the condensing surface temperature. The term, Γ_s or Γ_c, is a nonequilibrium correction factor, dependent on the net mass flux. This factor is shown in Fig. 2 plotted versus the ratio, w/w_s^*, for the case of σ and (T_s^*/T_o) equal to unity. The most reliable

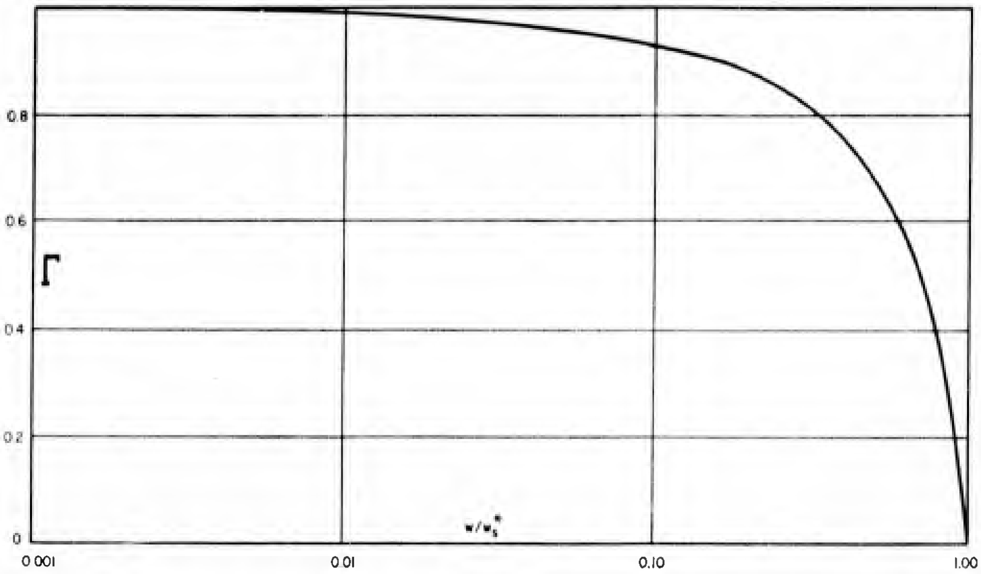


FIGURE 2. Nonequilibrium correction factor vs. reduced drying rate, w/w_s^*. σ and T_s^*/T_o assumed equal to unity.

measurements (3, 9) of the accommodation coefficient, σ, for pure ice, show it is nearly unity. Likewise, the temperature terms are present in the equations as the square roots of the ratios of absolute temperatures, and for normal freeze-drying conditions are certainly very close to unity.

Mass Transfer Through the Dried Cake. The rate of movement of water through the dry cake above the subliming interface depends upon the sample temperature, the structure of the dry material, the mean free path of the escaping gas, and the moisture content. As previously mentioned, mean free path considerations in this region indicate the vapor transport is usually free molecular or transitional. For free molecular flow, collisions between gas and solid molecules are the most frequent, and the flow of water vapor is independent of air pressure. Drying probably occurs by a process of successive evaporation and condensation. However, if it is postulated that drying occurs only at the free ice surface, the equations for free molecular flow through channels connecting two regions of different pressures may be applied. Thus,

$$w = \left(\frac{P_o - P_i}{L\text{-}X}\right)\left[K \beta d\right] \qquad \text{Eq 7}$$

The term, K, is a dimensionless factor dependent on the void fraction and flow geometry, and d is the average diameter of the flow channel (ice crystal diameter).

For transitional flow, Knudsen corrected Equation 7 by a multiplying factor, J.

$$w = \left(\frac{P_o - P_i}{L\text{-}X}\right)\left[K \beta d J\right] \qquad \text{Eq 7a}$$

This factor is a function of the dimensionless Knudsen number, defined as the ratio of the mean free path to d, the flow channel diameter. Values of J for transitional flow in circular pipes are tabulated (5), and the factor approaches unity as the Knudsen number increases above one.

At our present state of knowledge in freeze-drying, it is impossible to specify the terms, K, d, or J, except, perhaps, for the cases of carefully constructed model systems. For this reason, the term in brackets in Equation 7a is replaced throughout this paper by the single factor, C, defined as the cake conductivity.

Mass Transfer Between the Cake and the Condenser. In the region between the sample and the condenser, intermolecular collisions normally greatly outnumber collisions with the chamber walls (laminar flow regime). If there is no net air flow, water vapor movement is described by Stefan's equation for diffusion through stagnant air. This equation, assuming constant gas temperature, T, is

$$w = \frac{D_v \pi M_w (P_i - P_c)}{RTZp_{(lm)}} \qquad \text{Eq 8}$$

We may derive a general equation for combined diffusion and flow under a pressure gradient in the following manner. The general diffusion equation assuming steady-state operation and ideal gases at constant temperature and total pressure is

$$w = \frac{P}{\pi}\left(w + \frac{M_w}{M_a} w_a\right) - \frac{D_v M_w}{RT}\frac{dP}{dz} \qquad \text{Eq 9}$$

where w_a is the mass flux of air per unit time and area in the region between evaporator and condenser. The source of this air flow may be dissolved air released from the subliming ice cake or a leak introduced in the vicinity of the evaporator. An air balance around the dryer, as shown in Fig. 3, allows the estimation of w_a in terms of the speed of the vacuum pump, S, and the controlled leak rate, Q. If we assume a) that gas composition is constant at any cross-section, b) that air only is removed by the vacuum pump, and c) the total pressure drop is insignificant, then

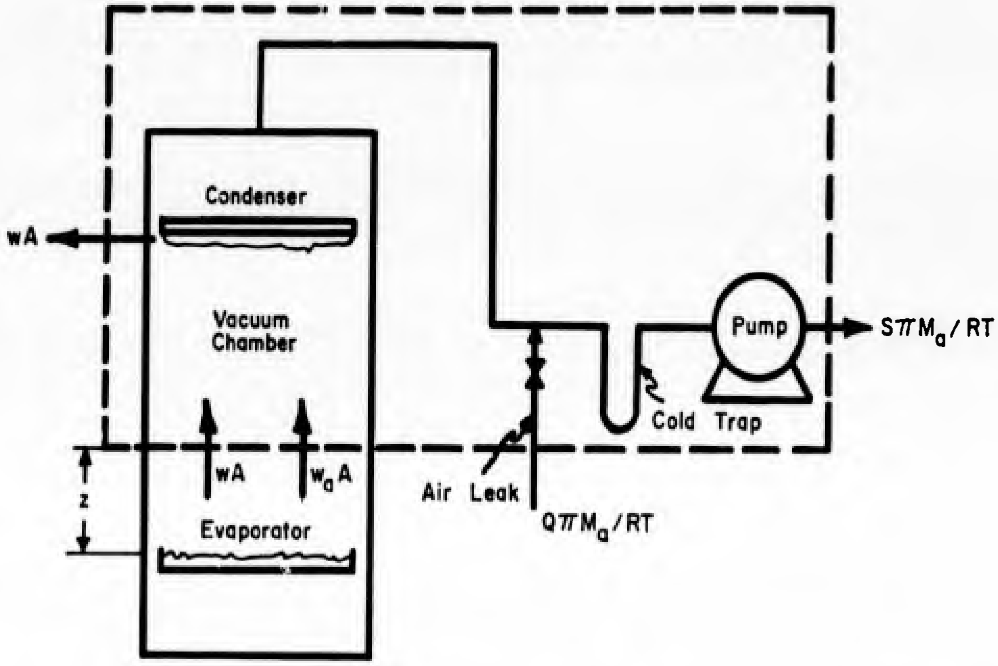


FIGURE 3. Schematic diagram for air material balance around freeze-dryer.

$$w_a = \frac{(S\text{-}Q)\,\pi M_a}{ART} \qquad \text{Eq 10}$$

When this equation is substituted in Equation 9 and the resulting expression is integrated from P_i $(z = 0)$ to P_c $(z = Z)$, we obtain

$$w = \frac{D_v M_w \pi (P_i - P_c)}{RTZp_{(lm)}} - \frac{M_w (S\text{-}Q)\pi}{ART} \qquad \text{Eq 11}$$

where the modified log mean air pressure is given by

$$p'_{(lm)} = \frac{p_c - p_i}{\ln\left[\dfrac{RTAwp_c - M_w(S\text{-}Q)\pi p_c}{RTAwp_i - M_w(S\text{-}Q)\pi p_i}\right]}$$

The expression for rate of mass transfer (Equation 11) reduces to Equation 8 when (S-Q) is zero.

Over-all Mass Transfer Equation. Separate equations have now been written for each of the mass transfer resistances namely: the interphase resistance at the subliming surface, the flow resistance through the dry cake layer, the diffusional resistance from cake to condenser, and the interphase resistance at the condenser. Under special circumstances, other resistances, such as bacterial filters, may also need to be considered. The equations for the resistances are combined by substituting the values of the boundary water vapor pressure for each successive step in the mass transfer process in the equation for the previous step. Because each of the steps is consecutive, w, the mass flux is the same for each step and may be factored. When these operations are performed, the over-all rate expression results

$$w = \frac{\dfrac{P_s^*}{\Gamma_s} - \dfrac{P_c^*}{\Gamma_c} - \dfrac{(S\text{-}Q)Zp'_{(lm)}}{D_v A}}{\dfrac{1}{(\Gamma\beta)_s} + \dfrac{1}{(\Gamma\beta)_c} + \dfrac{(L\text{-}x)}{K\beta dJ} + \dfrac{RTZp'_{(lm)}}{M_w D_v \pi}} \qquad \text{Eq 12}$$

Observe that Equation 12 has the form

$$w = \frac{\Sigma \text{ Driving Forces}}{\Sigma \text{ Resistances}}$$

The relative importance of the interphase mass transfer resistance (sum of sublimation and condensation resistances) in pure ice sublimation is shown for various total pressures and drying rates in Fig. 4. This plot is based on theoretical calculations using Equation 12 and assuming an equilibrium water vapor pressures at the condenser of 30 microns ($T_c^* = -50°C$). Since T_s (or T_s^*) and P_s^* are an equilibrium pair, Equation 12 may also be used to show the effect of the dry cake mass transfer resistance, $\frac{L\text{-}x}{K\beta dJ}$ or $\frac{L\text{-}x}{C}$, on the subliming interface temperature. The results of these calculations are shown in Fig. 5 for several total pressures and drying rates.

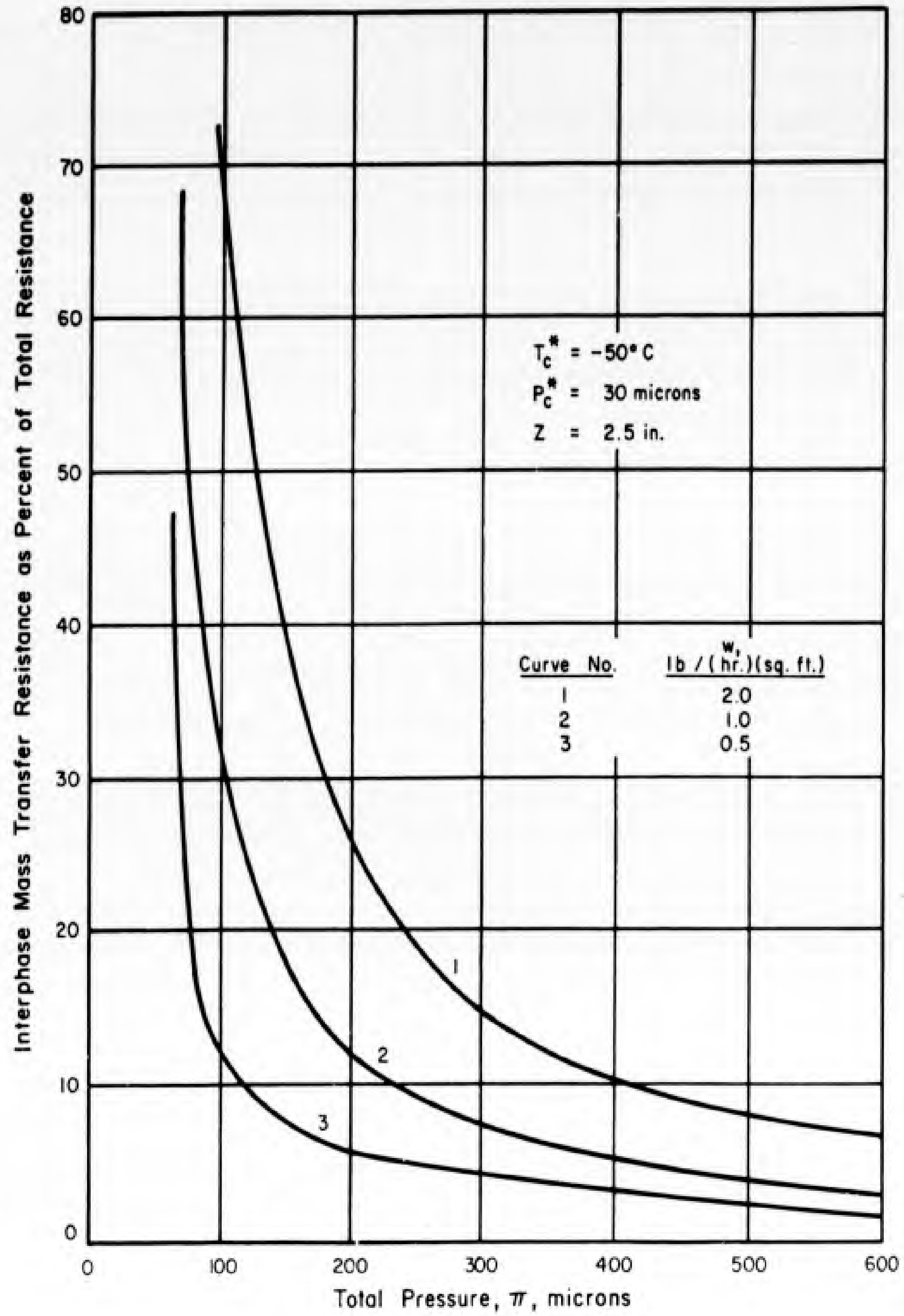
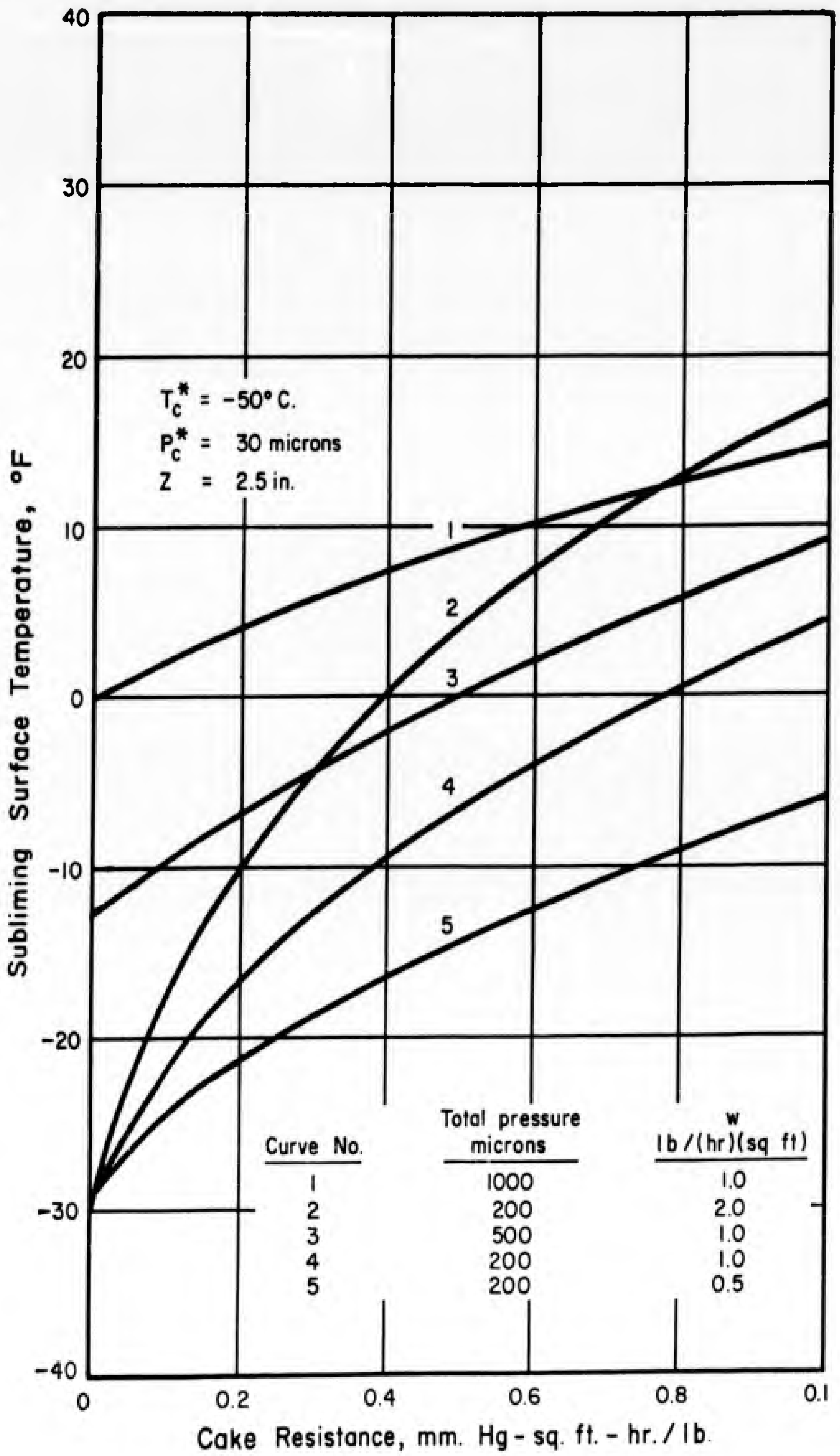


FIGURE 4. Interphase mass transfer resistance vs. pressure for ice sublimation (calculated curves).

Simultaneous Heat and Mass Transfer

The maximum temperatures for specified ice thicknesses can be calculated for the examples of Fig. 5 by substituting the values of T_s in the heat transfer equation giving the temperature drop across the ice cake. Thus,



Curve No.	Total pressure microns	w lb/(hr)(sq ft)
1	1000	1.0
2	200	2.0
3	500	1.0
4	200	1.0
5	200	0.5

FIGURE 5. Effect of cake resistance on the subliming surface temperature (calculated curves).

$$T_b = \frac{w\lambda_s k_i}{x + T_s} \qquad \text{Eq 13}$$

In practice, T_b will be specified at some maximum value. That is T_b must be less than the eutectic or melting temperature of the frozen sample or below a critical temperature for product degradation. Because in conduction freeze-drying, T_b, is much more difficult to measure than the temperature of the heating fluid, T_m, the latter is usually controlled.

If we combine the over-all mass and heat transfer equations (Equations 3 and 12), we obtain

$$T_m = T_s + \lambda_s \frac{\Sigma R_{\text{Heat Transfer}}}{\Sigma R_{\text{Mass Transfer}}} \left[\frac{P_s^*}{\Gamma_s} - \frac{P_c^*}{\Gamma_c} - \frac{(S\text{-}Q)Zp'_{(\text{lm})}}{D_v A} \right] \qquad \text{Eq 14}$$

and, assuming (S-Q) equals zero and the Γ factors are unity, the above simplifies to

$$T_m = T_s + \frac{\Sigma R_{\text{Heat Transfer}}}{\Sigma R_{\text{Mass Transfer}}} (P_s^* - P_c^*) \qquad \text{Eq 15}$$

Now let us consider that T_m is held at its maximum and that P_c^* is also specified. If the ratio of heat transfer resistances to mass transfer resistances is very small (mass transfer controlling), $T_m \sim T_s$. If the ratio is very large (heat transfer controlling), $T_m > T_s$, and as the ratio decreases, T_s approaches T_m.

Two additional points should be mentioned. First, as long as T_m is less than its specified maximum, heat transfer is always limiting. Second, when T_m is held constant in conduction freeze-drying, it is unlikely that a true constant rate period will ever be observed. If the ratio of heat to mass transfer resistances increases as drying proceeds, the rate will also increase while the reverse is true if the ratio decreases. In contrast, when a constant heat input is supplied to the frozen sample (the usual case for the initial drying period using radiant heat), the rate will be constant, but the temperature level of the sample may vary.

Description of Experimental Equipment

The freeze-dryer used in these studies was a horizontal shell dryer constructed at the University of Wisconsin. Fig. 6 is a cut-away side view of the vacuum chamber. The dryer shell was a steel cylinder two feet long and one foot in diameter. The shell was 3/8″ thick and was mounted on an angle iron frame. Two 3″ wide flanges were welded to the ends of the cylinder. Two 3/4″ thick transparent Lucite plates covered the ends of the dryer. These plates were fastened to the flanges by a number of studs. The use of large O-rings seating between the Lucite and flange surfaces assured vacuum tight seals. The Lucite allowed clear vision into the drying chamber during drying.

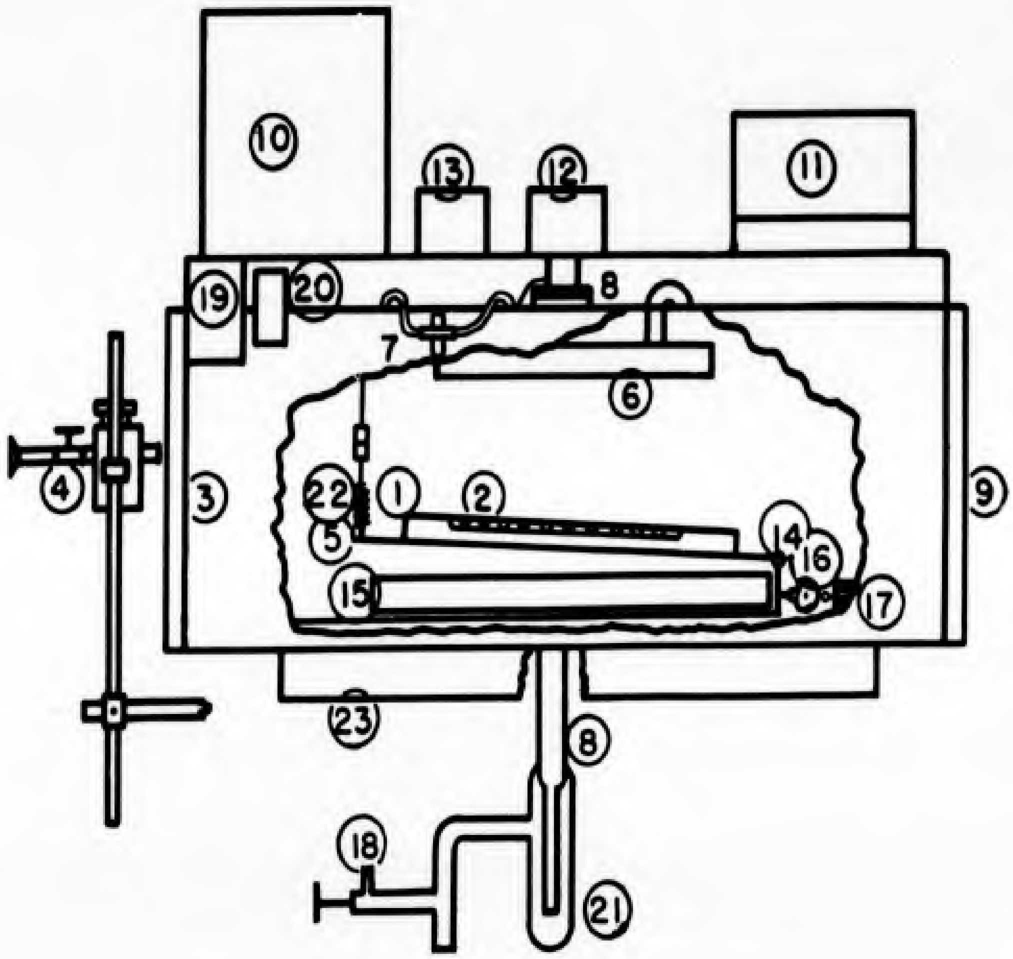


Figure 6. Diagram of experimental freeze-dryer.

1) Tray support
2) Drying tray
3) Front Lucite cover
4) Cathetometer
5) Razor
6) Condenser plate
7) Dryer shell
8) Pipe to vacuum pump
9) Rear Lucite cover
10) Potentiometer
11) Pirani gauge
12) Ammeter
13) Voltmeter
14) Tray support fulcrum
15) Heating wire holder
16) Cam
17) Synchronous motor
18) Needle valve
19) Percentage input timer
20) Main electrical switch
21) Cold trap
22) Calibrated spring
23) Control panel for heating elements

The evaporator consisted of a 5⅝″ by 11⅝″ by 1½″ deep rectangular tray containing the frozen material to be dried. The sides of the tray were aluminum, and the bottom was replaceable in order to allow a screen either transparent or opaque to infrared radiation to be used. For radiant-energy, freeze-drying runs, the screen used was a 3-mil thick Mylar sheet. A blackened aluminum plate was substituted for conduction runs. The thermocouple leads from the tray dipped into small mercury wells in the strips fastened to the holder, and thence electrical connections were made to a 30-point Brown Elektronik recording potentiometer. All electrical connections between the vacuum chamber and the exterior were made through the rear Lucite plate. Once in place, this cover rarely needed to be removed. Only the front Lucite cover was taken off for the introduction and withdrawal of samples.

Three-mil copper-constantan thermocouples, mounted in a Lucite spacer, were positioned in the sample tray so that temperature gradients could be measured during a run. When the sample tray and holder were in place for a drying run, the holder was supported at one end by a fixed pivot and at the other by a calibrated spring. The deflection of the end of the holder to which the spring was attached depended on the loading in the tray. A continuous record of the rate of ice sublimation was obtained by noting the change in position of a razor edge fixed to the movable end of the tray. A cathetometer which could be read to a deflection change of 10 microns was mounted on the dryer chassis in order to observe the razor edge.

Heat was supplied to the sample by platinum-rhodium wires stretched across the wire holder 1" below the sample. The power input was controlled by variacs and on-off, percentage input timers.

The condenser was a 5⅝" by 11⅝" flat, rectangular surface. The distance from the evaporator tray bottom to the condenser when the former was empty and mounted in position was 2.5".

Total pressure was controlled by an adjustable air leak through a needle valve located near the intake of the vacuum pump. In this fashion, pressure variation from 75-2000 microns was possible. By calibrating the rate of air leak versus valve position, we were able to estimate the term (S-Q) in Equation 11.

Pressures were measured by means of both McLeod and Pirani gauges. The majority of runs were made using a swivel-type McLeod gauge manufactured by the F. J. Stokes Machine Company having a range of 1 to 4000 microns. The pressure measuring system was modified as suggested by Ede (4). A stopcock, a 3" diameter glass bulb, and a cold trap were located in the tubing connecting the vacuum system to the pressure gauges. In order to avoid condensation errors, water and other condensable vapors were removed before taking pressure readings. A total pressure measurement was made by immersing the cold trap in liquid air and keeping the stopcock open. The condensables were quickly frozen out, and, after a short time, the inert gas pressure in the gauges was equal to the total pressure in the system. The time for the pressure reading to stabilize was observed on the Pirani gauge since this instrument read pressure continuously. The purpose of the glass bulb was to act as a thermal capacitance to minimize the cooling effect of the trap. The virtue of this arrangement was that it also enabled inert gas partial pressure to be measured. By closing the stopcock and condensing out water vapor, the pressure indicated by the gauges was the desired noncondensable pressure. Pressure readings were reproducible to better than 10 microns for the range 100 to 300 microns. Above 300 microns the reproducibility was about 5% of the reading. The pressure tap location in the apparatus was at the upper edge of the drying tray. For certain experiments a glass tube was inserted in the tap so that the pressure was measured 3/8″ directly above the sample surface.

Experimental Procedure

Prior to a drying run, the sample tray was precooled in a deep freeze held at approximately −40°C. A weighed amount of material to be freeze-dried was added to the tray. After freezing was complete, the tray was weighed, and the thickness of the ice was measured in several locations, including the region in which the thermocouples were located.

The tray was quickly transferred to the drying chamber, the thermocouple contacts were tested, and the Lucite cover plate was attached. The chamber was then evacuated and the refrigerant circulated to cool the condenser. The cathetometer was sighted on the razor's edge.

Thermocouples were calibrated in-place before power was turned on to the heating elements. When temperature readings stabilized, the measurements were recorded. The temperatures were then fit to the best least mean square line, and a correction equal to the difference between the measured temperature and that read from the line was determined for each thermocouple. The correction compensated for slight emf errors and thermocouple positioning errors.

The total pressure was then adjusted to the prescribed level and the power turned on. Readings of temperature, cathetometer setting, and pressure were taken at regular intervals. At the conclusion of the run, the sample was removed and weighed.

Results

Sublimation of Pure Ice

Temperature gradients for a typical run are shown in Fig. 7. Calculated thermal conductivities computed from slopes of temperature gradient plots were in good agreement with literature values for ice (k_I = 1.46 Btu-ft/(hr) (sq ft) (°F) at −15°F). During a run, the temperature at any point decreased, and the estimated temperature of the subliming interface increased slightly with time for constant drying conditions. Pitting of the ice surface occurred as drying proceeded, and the latter probably caused the increasing curvature of the temperature versus distance profile with time. Because of the pitting, it was impossible to tell whether some of the thermocouples indicated gas or solid temperature.

Comparison of radiant temperature gradients with the equivalent conduction gradients in which no contact resistance between tray and sample exists (see dashed lines at zero thickness on Fig. 7) showed that the tray-ice interfacial temperature, T_b, was sometimes as much as two degrees lower for radiant drying. On the basis of a

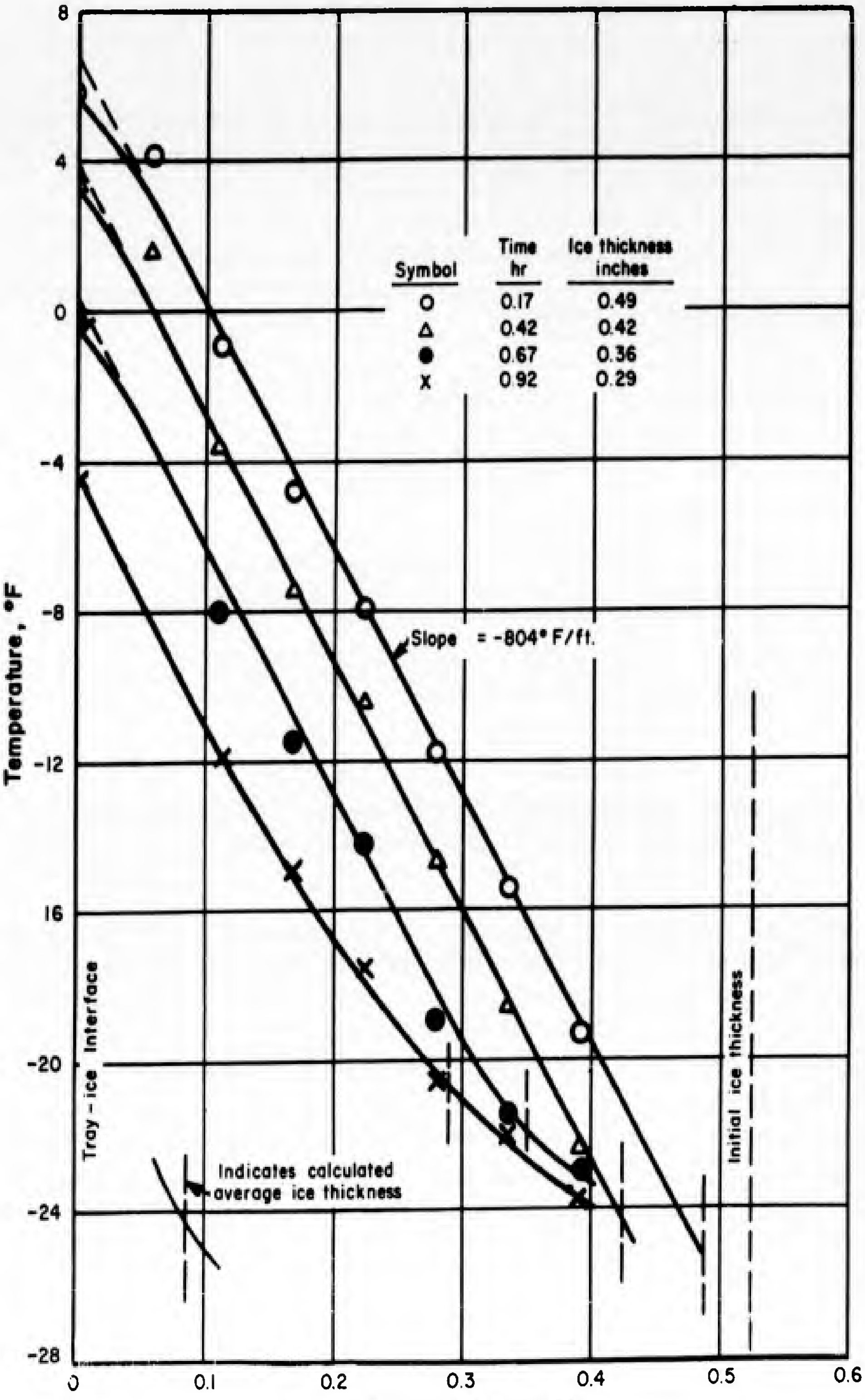


FIGURE 7. Temperature gradients in sublimation of water-ice.

0.4″ thick slab, temperature drops across the sample ranged from 4 to 15% less for radiant in contrast with conduction heating. These data were in agreement with similar measurements by Brodkey (1).

Data obtained from pure ice sublimation runs were used to test the validity of the theoretical Equation 12. Calculated and experimental sublimation rates are compared in Table 1. In the first set of calculations diffusion through stagnant air was postulated (S-Q = 0). In the second (shown in parentheses), all of the air flow differences, S-Q, was assumed to flow in the space between evaporator and condenser. Although slightly better agreement between calculated and experimental rates was obtained when the air flow term was used, the disparity between the two calculations was small enough to indicate that the major mass transfer mechanism in the experimental dryer was molecular diffusion.

TABLE 1

Calculated vs. Experimental Rates
Pure Ice Sublimation

	Rate, lb/(hr)(sq ft)							
Run	Exp.	Calc.		Calc./exp		Total press., micron	T_s^*, °F	T_c^*, °F
95	1.009	1.14	(1.182)	1.14	(1.17)	200	–24	–32
96	0.850	0.776	(0.785)	0.913	(0.92)	2150	18	–32
97	0.942	0.787	(0.830)	0.835	(0.88)	1200	6	–35
99	0.564	0.395	(0.43)	0.70	(0.76)	200	–26	–33
103	1.382	1.191	(1.364)	0.86	(0.99)	235	–24	–28
113	1.446	1.392	(1.382)	0.96	(0.96)	980	2	–24
115	0.498	0.525	(0.559)	1.06	(1.12)	630	–9	–40
	Ave. Dev. from unity			12.7%	(11.1%)			

Estimated subliming interface temperatures are plotted against total pressure in Fig. 8 for all of the ice sublimation runs of this study as well as those runs listed by Miller (7) in which values of T_s^* were reported. The points all follow closely the equilibrium vapor pressure curve of water at the same interfacial temperatures. This condition (viz., $P_s^* \approx \pi$) corresponds to that defined for Carman's "intermediate" region of sublimation. The calculations upon which Table 1 are based showed that, for a given drying rate, the diffusion resistance and partial pressure driving force both increased almost linearly with pressure. The total interfacial mass transfer resistance was relatively constant with pressure and was consistent with Fig. 4 in that it was unimportant except at low pressure and high sublimation rate.

The Freeze-Drying of Substances Containing Solids

Temperature Gradients. Typical temperature gradients at various drying times for a frozen slab of reconstituted, non-fat milk are depicted in Fig. 9. Gradients during the constant-rate period for this run are shown on an expanded temperature scale in Fig. 10. The temperature at any position normally decreased with time in the constant-rate period. Although the gradients for this run were nearly linear,

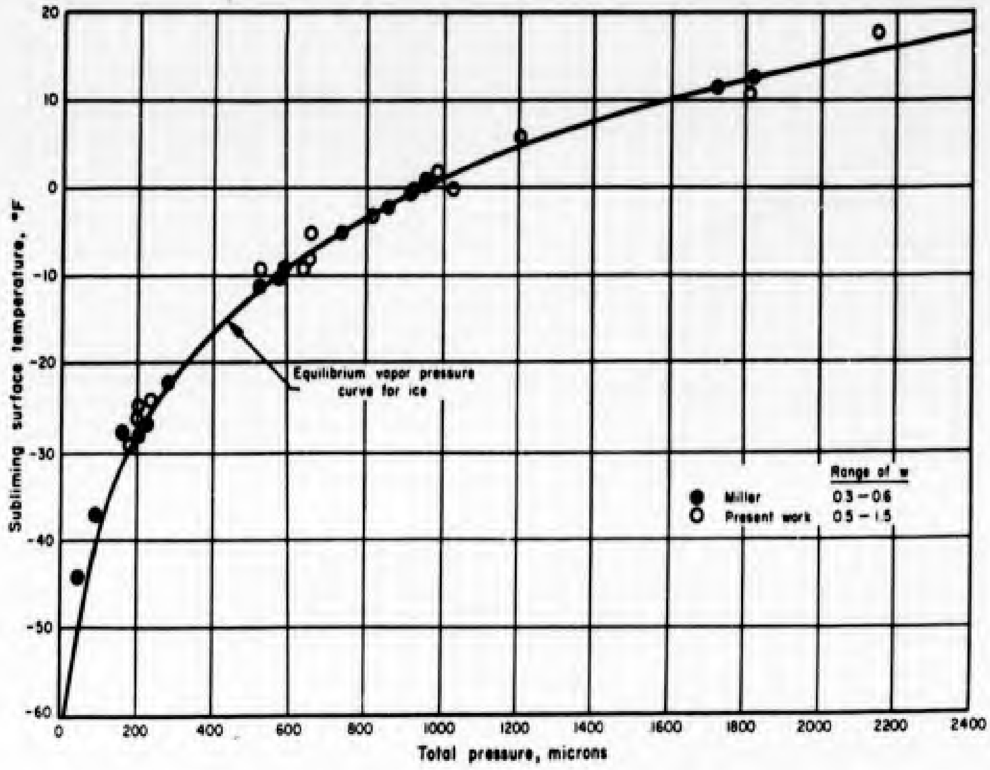


FIGURE 8. Subliming surface temperature vs. total pressure for pure ice.

similar lines for other runs were often irregularly curved. This nonlinearity was attributable to one or more of the following reasons:

1. Nonhomogeneity of the sample caused by solids redistribution on freezing.
2. Cracking of the ice cake near the thermocouples allowing drying to occur in the cracked cross-section.
3. Variable drying rate across the surface area of the sample.

Comparison of radiant with equivalent conduction heating of slabs was difficult because of the nonlinearity of many of the temperature gradient curves. It appeared that the amount of penetration of radiant energy into the sample was always very small. When high temperature radiation was used, caution was necessary to avoid toasting. Nonuniform drying further complicated the problem since portions of the sample were dry while others were still wet. To avoid any toasting whatsoever, the heat input had to be controlled to avoid overheating the dryest portion of the slab. Operating experience indicated that radiant heating is probably unsuitable for materials in which even slight "browning" may ruin the product (e.g., foods).

Occasionally, a run was made in which the frozen slab melted at the tray-ice interface. When this occurred, the sample broke loose from the tray, sometimes violently. The loosened sample then proceeded to dry from the bottom and sides, subsequently increasing the danger of toasting. It was this melting which limited the tray loading. For a given subliming surface temperature and drying rate, the

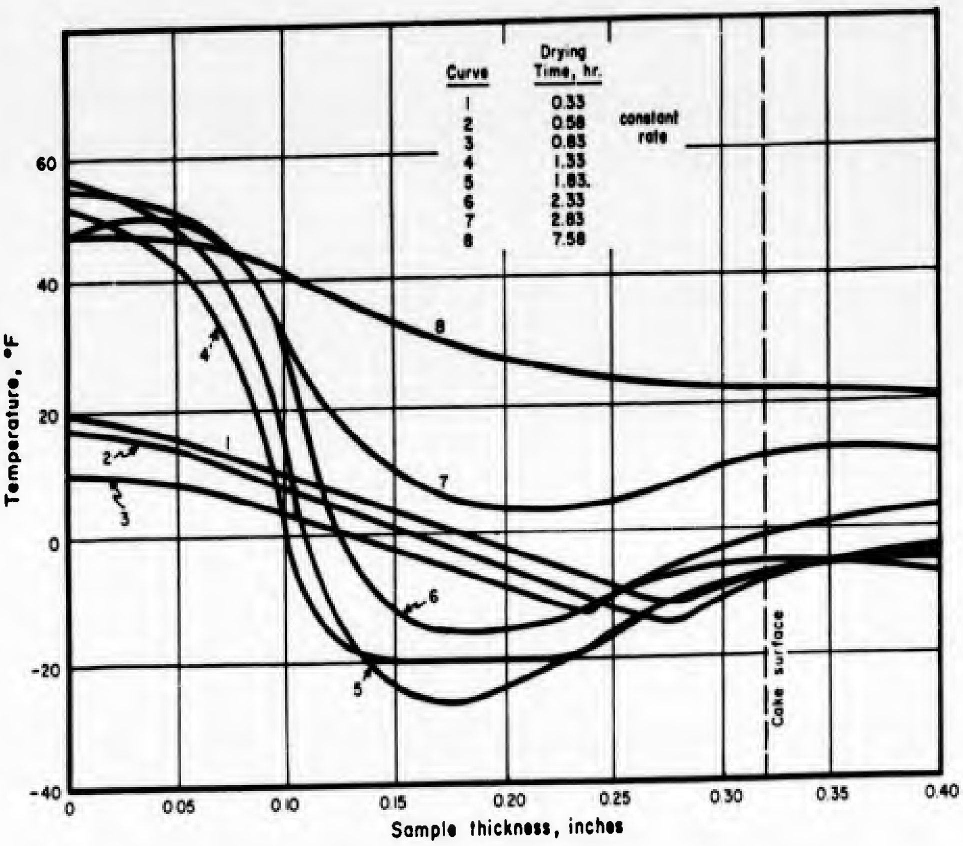


FIGURE 9. Temperature gradients in freeze-drying non-fat milk (run 74).

sample thickness should be such that the temperature at the tray-ice interface remains below the melting point.

Mass Transfer

The Effect of Pressure. The estimated subliming surface temperature near the start of the constant-rate period is plotted versus the total operating pressure for most of the solid-drying runs of this study in Fig. 11. The vapor pressure curve for ice is also shown. In contrast with pure ice sublimation, many of the data points are at higher temperatures than those corresponding to the vapor pressure curve (i.e., $P_s^* > \Pi$). This temperature elevation was indicative of the additional mass transfer resistance of the solid although a small portion of it was attributable to vapor pressure lowering by dissolved salts. Since the data represented in Fig. 11 were initial data, the resistance must have been caused by a surface film or crust. In general, the interface temperature increased with total pressure and, at a specified operating pressure, the ice vapor pressure curve provided the lower limit for the sublimation.

Cake Conductivity. In order for the cake conductivity, C, to have remained constant during a run, the subliming surface temperature, for constant drying conditions, should have increased as the dry cake layer thickness, L-x, increased. In Fig. 12, the estimated values of T_s^*

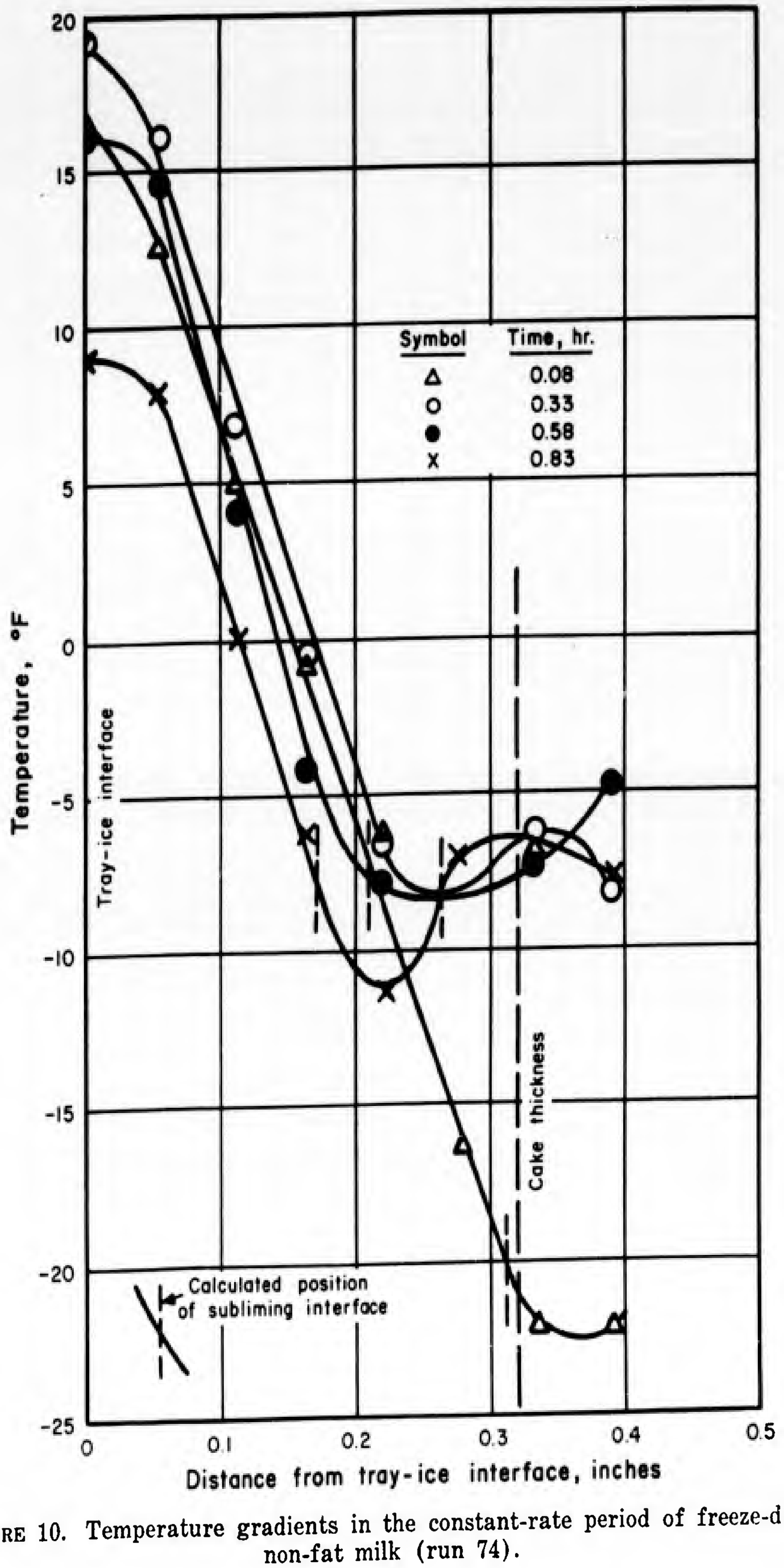


FIGURE 10. Temperature gradients in the constant-rate period of freeze-drying non-fat milk (run 74).

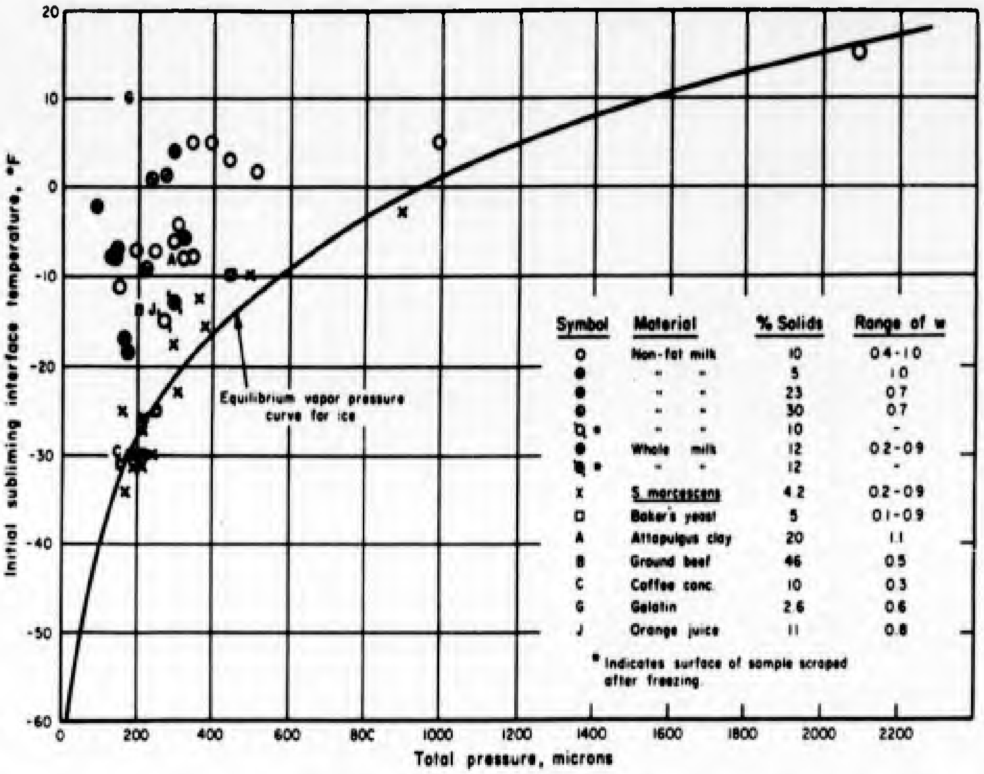


FIGURE 11. Initial subliming interface temperature vs. total pressure for various materials.

for several non-fat milk runs of different solid contents are plotted against the time of drying in the constant-rate period. A regular increase of T_s* with time did not occur, and the conductivity was therefore variable. The calculated conductivities for one of these runs (No. 74, 10.2% solids) at various drying times are given in Fig. 13 and Table 2. The variation of C with drying time also was explainable by nonhomogeneity of the dry cake, cracking or thawing of the sample during drying, and point-to-point differences in sublimation rate. For the other non-fat milk runs referred to in Fig. 12 and Table 2, conductivities were estimated at only one drying time. Calculations of conductivity for several other substances which were freeze-dried are listed in Table 3. Generally speaking, the conductivity of the dry surface crust increased with solid content for equivalent drying times.

TABLE 2
Estimation of Cake Conductivities
Non-fat Milk

Run	% Solids	Time hr	Constant Rate lb/(hr)(sq ft)	Pressure, microns	Cake Conductivity lb/(hr)(ft)(atm)
74a	10.2	0.08	0.694	200	12.6
74b	10.2	0.33	0.694	250	8.8
74c	10.2	0.58	0.694	245	12.9
74d	10.2	0.83	0.694	245	19.4
75	23.1	0.42	0.732	280	6.9
77	4.6	0.42	0.990	250	13.4
78	30.2	0.50	0.746	240	7.0

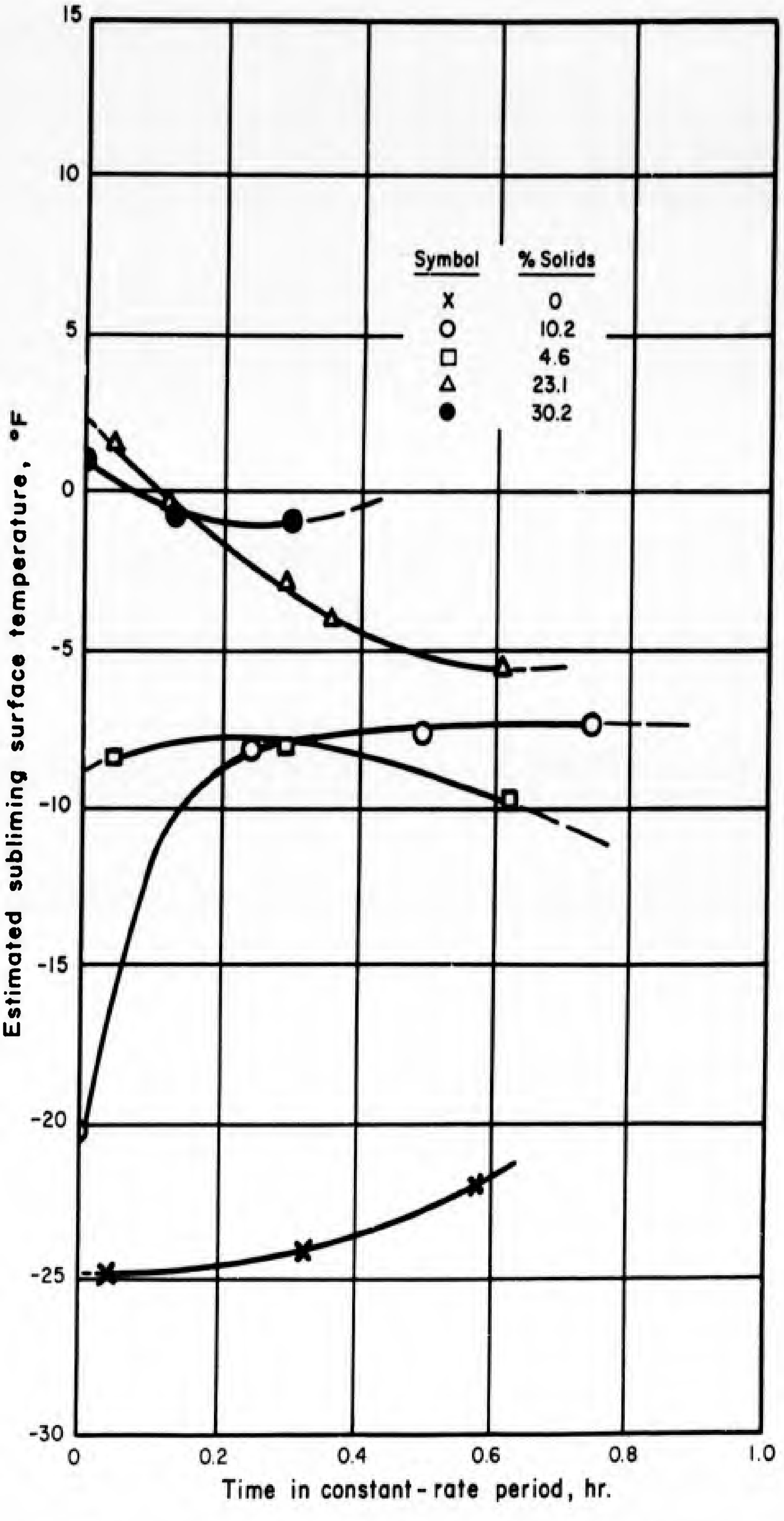


FIGURE 12. T_s^* vs. time in the constant-rate period (non-fat milk).

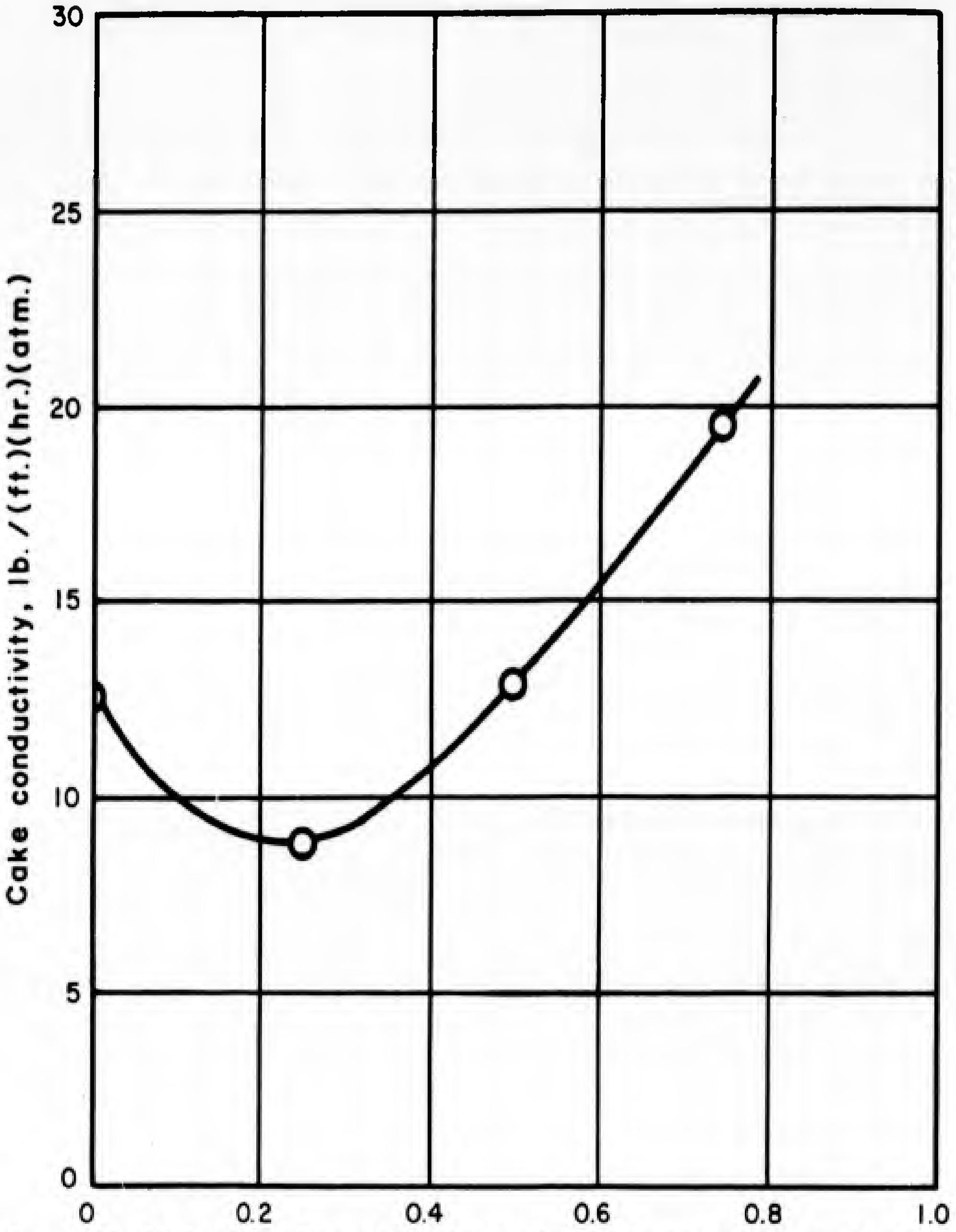


Figure 13. Estimated cake conductivity vs. drying time in constant-rate period (non-fat milk).

Several runs were also made to determine whether freezing had an effect on the initial conductivity of non-fat milk. Rate of freezing had only a slight effect. One sample whose frozen surface was scraped prior to drying did have significantly greater conductivity. The solid content of the scrapings was 15.1% as compared to 8.8% for the unfrozen feed.

The Drying Cycle

Dryer Performance. A typical performance record for the freeze-drying of a frozen slab (in this case, orange juice) is shown in Fig. 14. A decrease in drying rate always coincided with a reduction

TABLE 3

Cake Conductivity for Different Materials

Run	Material	% Solids	Constant Rate lb/(hr) (sq ft)	Pressure, microns	Cake Conductivity lb/(hr)(ft)(atm)
28	Coffee concentrate	10.0	0.324	155	15.8
54	Baker's yeast	4.9	0.938	450	26.1
70	Whole milk	11.8	0.912	250	18.1
82	*S. marcescens*	4.3	0.789	500	66.0
92	Orange juice	10.8	0.763	240	6.2
93	Gelatin	2.6	0.632	210	1.4

in heating rate. Reasons for this reduction were the rise of the sample temperature to a prescribed maximum or thawing of the frozen solid. Curves for various materials plotting drying rate versus moisture content are drawn in Fig. 15. The plots are seen to be typical drying curves, with a heat-up period, a constant-rate drying period, and a falling-rate period. The critical moisture content, at which the drying rate begins to drop, varied considerably for any material, even under similar drying conditions. Uneven drying and nonuniform sample thicknesses were major causes of the variation. Critical moisture was not found to be dependent on sample thickness or drying rate. Typical values for the materials freeze-dried in this study are listed in Table 4. Lower critical moistures would probably be obtained by scaling-up the freeze-dryer tray, since uneven sublimation at the samples edges caused by conduction heating from the tray sides, would then be minimized.

TABLE 4

Typical Critical Moisture Contents Obtained in Tray Freeze-Drying of Frozen Slabs

Material	% Solids	Slab Thickness, in.	Critical Moisture Content, lb H_2O/lb solid
Bacterial Suspension *(S. marcescens)*	4.2	0.320	5.4
Non-fat milk	10.2	0.320	3.3
Whole milk	11.8	0.350	3.2
Coffee	10.0	0.485	4.0
Attapulgus clay	19.6	0.550	2.2
Orange juice	10.8	0.360	1.7
Gelatin	2.6	0.450	13.2
Ground Beef	46.0	uneven	0.65
Baker's yeast	4.9	0.290	8.8

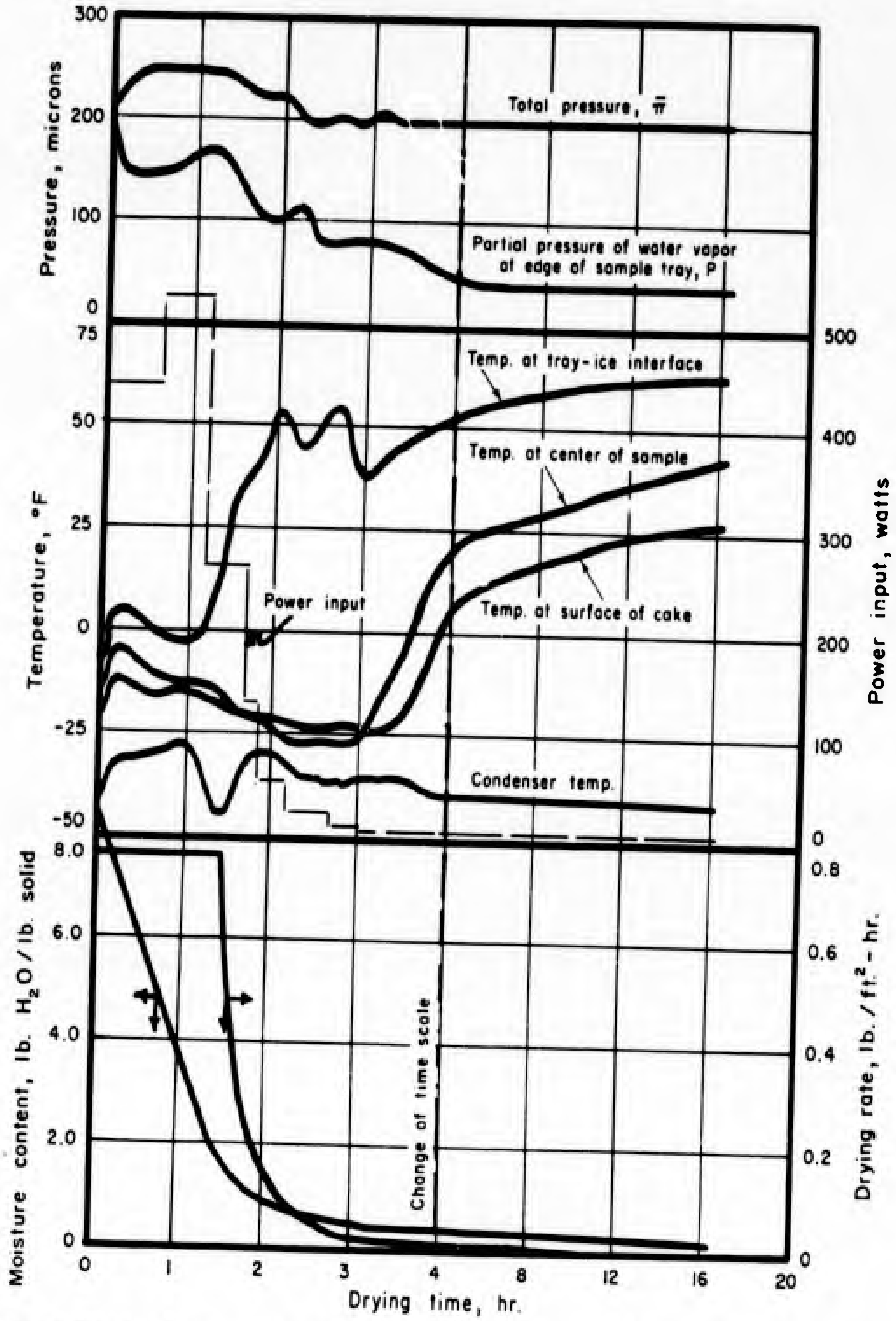


FIGURE 14. Performance record for the freeze-drying of an orange juice slab.

In general, over-all drying times were proportional to tray loading. It was usually more difficult to prevent uneven drying or overheating for the more heavily loaded batches. The major effect of sample thickness for given drying conditions was to increase the sample temperature at the tray-ice interface (see Equation 13).

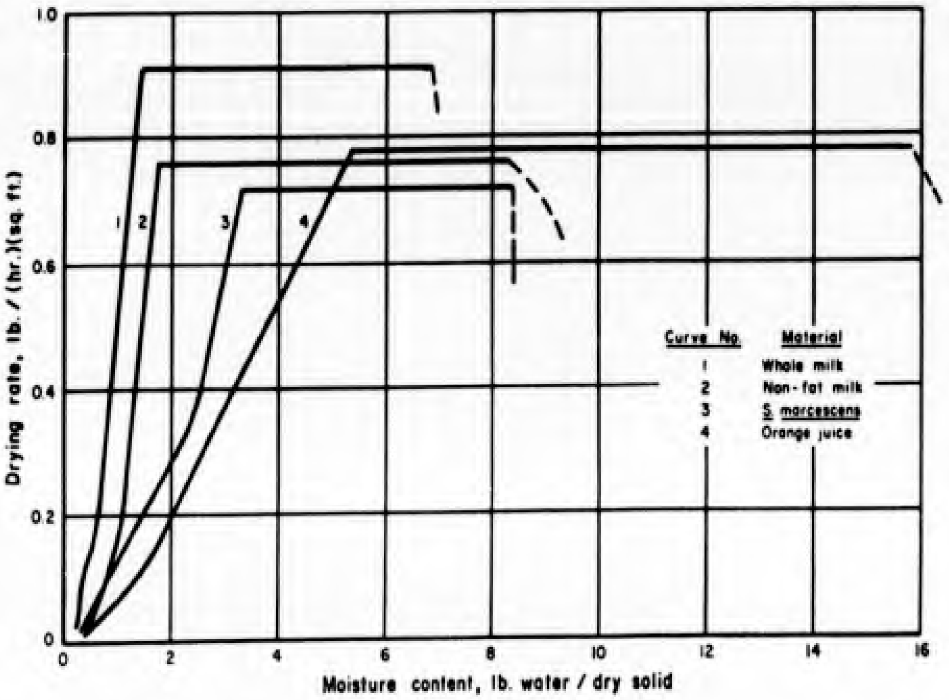


FIGURE 15. Rate vs. moisture content for freeze-drying several materials.

Freeze-Drying Tests in a Commercial Pilot Dryer

Performance data acquired from Proctor and Schwartz Co., Inc., for the freeze-drying of various materials in trays are summarized in Table 5. All of these tests were performed in the Proctor Freeze-Drying Research Unit, an apparatus which held two trays, each 1.6 sq ft in area. Radiant energy heating was used. The condenser consisted of a refrigerated coil placed at the rear of the drying chamber. Over-all drying rates were in the range 0.04 to 0.25 lb of water removed per sq ft/hr. No specific information in regard to product quality was available although it was claimed that the products were as good or better than similar dry materials from other sources.

Conclusions

The variables of energy input and sample loading govern the rate of freeze-drying. The variables of total pressure, condenser temperature, and structure and type of the freeze-dried solid primarily affect the drying temperature level.

In pure ice sublimation, the interphase mass transfer resistance is not important unless total pressure is less than 150 microns for a drying rate of 1.0 lb/sq ft/hr. It is doubtful that this resistance is ever significant in freeze-drying of solids.

Molecular diffusion was the primary mass transport mechanism observed in the apparatus of this study. It would be of interest to see

TABLE 5

Pilot Plant Performance Data*
Proctor Freeze-Drying Research Unit
(2 trays, each 1.6 sq ft)

	%	Press.,	Drying Temp., °F		Rate, lb/sq ft/hr		Drying
	Solids	μ	Init.	Final	Init.	Over-all	Time, hr
Leather	40	100	–26	124	—	0.035	3.8
Coffee extract	20	120	–10	103	0.43	0.16	4.7
Penicillin	1.0	50	–35	100	0.48	0.20	4.0
Grape juice	16	100	–15	118	—	0.04	21.5
Polyvinyl acetate emulsion	35	150	20	101	0.49	0.25	2.8
Dihydrostreptomycin	29	60	5	110	0.25	0.17	12.4
Cocoa	27	200	0	114	0.49	0.09	23.3
Sodium glycinate	10	140	–20	128	0.29	0.05	19.0

* Data for this table obtained from the records of the Proctor and Schwartz Co., Inc.

if drying rates could be accelerated by directing a high velocity air jet across the surface of the sample being dried.

Reasons for variability of the calculated cake-conductivity are: 1) nonhomogeneity of the dry solid, 2) cracking of the dry cake layer because of thermal stresses, and 3) point difference in the drying rate across the sample surface. Precautions should be taken to avoid freezing and drying conditions which induce nonuniform drying rates.

Good dryer performance depends on having good thermal contacting between the sample and container, minimum spacing between evaporator and condenser, and adequate pumping capacity. Drying tests usually must be performed to determine optimum sample loading. In this study no advantage for drying thin films was found.

The technique used for characterizing mass transfer through the partly-dry, freeze-dried layer could profitably be extended. Because of the complexity of dry-cake structure, it is suggested that model systems be used as a first approach.

Acknowledgment

The cooperation of the Proctor and Schwartz Co., Inc. in allowing the use and publication of company research data is gratefully acknowledged.

Notation

A = heat or mass transfer area, sq ft
B = thickness of tray bottom, in.
C = cake conductivity, lb/(ft)(hr)(atm)
d = flow channel (or ice crystal) diameter, ft
D = diffusion coefficient, sq ft/hr
h = film heat transfer coefficient, Btu/(hr)(sq ft)(°F)
J = transitional flow correction factor
k = thermal conductivity, Btu-ft/(hr)(sq ft)(°F)
K = geometric factor for noncircular flow channel
L = cake thickness, ft
M = molecular weight
p = partial pressure of air, microns
P = partial pressure of water, microns
q = rate of energy transfer, Btu/(sq ft)(hr)
Q = rate of air leakage, (cu ft)/hr
r = radius, ft
R = gas constant (no subscript)
R = resistance to heat or mass transfer (with subscript)

S = pumping rate, (cu ft)/hr

T = temperature, °R

w = rate of mass transfer per unit area, lb/(sq ft)(hr)

x = variable distance in ice or solid, ft

Z = distance from cake to condenser, ft

z = variable distance from cake surface in gas phase, ft

Greek Letters

β = function defined in Equation 4

Γ = function defined by Equation 5

λ = latent heat per unit mass, Btu/lb

π = total pressure, microns

σ = accommodation coefficient

Subscripts

a	refers to air
b	refers to the container wall, usually the tray bottom
c	refers to the condenser surface
i	means ice or refers to the gas at the cake interface
m	refers to the heating medium
o	refers to the gas at the subliming interface
s	means "sublimation" or refers to the subliming interface
v	refers to vapor
w	refers to water

Superscript

* represents equilibrium value

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METHODS OF APPLICATION OF MICROWAVE ENERGY IN INDUSTRIAL PROCESSES

B. O. M. GALL AND R. A. LA PLANTE

Introduction

Dielectric heating by radio frequencies has a unique property: the ability to transfer energy and create heat directly into the interior of nonconducting materials. Materials heated in this way are usually poor electrical conductors and also poor thermal conductors. Infrared heating of such materials by convection or conduction presents a well-known problem, namely, the necessity to establish a relatively large temperature gradient in the material in order to increase the speed of heating. This problem is overcome by the use of radio frequency dielectric heating. RF heating has become more attractive with the years because it has been possible to increase the power levels and efficiencies of RF generators.

For many years, power at frequencies up to 30 mc/sec. has been used for industrial dielectric heating applications. It has been found, however, that certain types of heating problems are still difficult to solve at these frequencies. Some of the better (more lossless) dielectrics cannot be heated easily. Thin films or sheet materials and materials with a high water content were also found difficult to heat. For reasons which will be shown in a moment, some of these problems have been solved by using higher frequency energy, say 200 mc/sec. These solutions were often difficult and expensive, with much effort being required in the equipment and applicator design. Let us consider the reasons for these heating problems and propose a solution which is applicable to a great number of them.

The general formula for radio frequency dielectric heating is:

$$P \alpha E^2 f \epsilon'' V$$

where

P is the power dissipated in the dielectric per unit volume.

E is the electric field strength in the dielectric.

f is the frequency.

ϵ'' is the dielectric loss factor.

V is the volume of the dielectric.

This simple expression shows that the power dissipated per unit volume is directly proportioned to the square of the field strength, and the first power of the frequency and the loss factor. To increase the heating rate, it is necessary to increase one or more of these three quantities.

The better dielectrics have a loss factor which is very small. The loss factor, being an intrinsic property of the material, cannot be changed. One of the simplest ways to increase the heating rate in these materials is to increase the electric field strength. Unfortunately, it is often found that the electric field strengths required at conventional radio frequencies (around 30 mc) used for this work are so high that problems of voltage breakdown in the dielectric or in the equipment used finally limit the heating rates severely. This is also true of thin films or sheet materials where arcing and subsequent puncturing of the material limits the usefulness of the technique. For materials of high water content, the vapors released during the heating cycle tend to break down and arc in the high electric fields. This limits the technique again. So the increase of electric field strength is by no means the general solution, although for many applications it is nevertheless adequate.

If one could increase the dielectric loss factor of the material, it would also increase the heating rate, other things being equal. Normally, this is not done because the loss factor is an intrinsic property of the dielectric. We should point out a possibility, however. Most dielectrics have a loss factor which is temperature-sensitive, increasing with temperature. Many materials have loss factors which increase strongly above certain critical temperatures. One could, therefore, actually change the dielectric loss factor by preheating the dielectric to the critical temperature in a conventional way and then complete the heating with the RF technique. It would appear, however, that the unique property of RF dielectric heating is minimized in this process.

The third way to increase the heating rate is, of course, to increase the frequency. In addition to the increase predicted by the heating formula, there is often (but not always) a secondary increase caused by the fact that the dielectric loss factor of some materials increases with frequency. In the discussions which follow, this secondary effect is by no means a prerequisite for the success of the techniques described. Other problems present themselves as the frequency is increased. Normally, the material to be heated is exposed to the electric field by placing it between the plates of a capacitor (Fig. 1). As the frequency is increased, one finds that the heating becomes more and more uneven, being most pronounced when the dimensions of the material to be heated are of the order of a wavelength. Standing waves are found on the applicator and multiple-feed techniques must be used to minimize them. Other factors which make the capacitive applicator ineffective as the wavelength approaches its dimensions are that it radiates an appreciable amount of energy and that its impedance as a capacitor is so low that it is difficult to drive it efficiently.

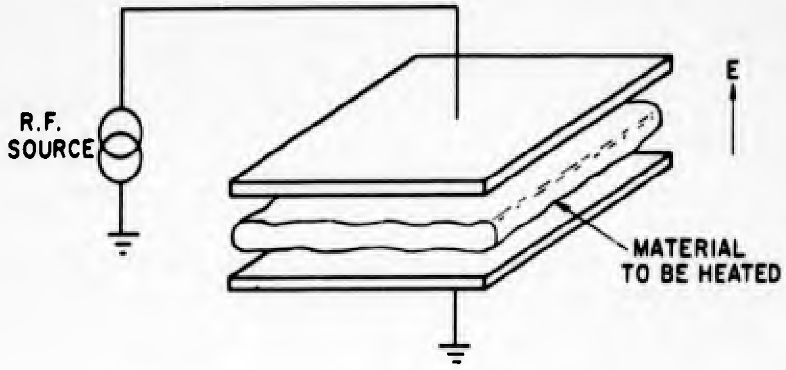

FIGURE 1. Capacitive heating applicator.

It has been found, however, that increasing the frequency to the point where the material to be heated is large compared with a wavelength is quite effective. Unique methods of application of the energy then can be used, and the problems encountered are of a different nature. An obvious limitation on the maximum frequency which can be used practically is the limited availability of electron tubes which will generate useful power at these high frequencies with a reasonable cost and efficiency. Another limitation is the penetration depth.

The dielectric loss factor ϵ'' comes from the definition of the complex dielectric constant

$$\epsilon = \epsilon' - j\epsilon''$$

Energy is stored according to the value of ϵ' and energy is dissipated according to ϵ'' when an RF electric field is established in the dielectric. Since the electric field must be applied from outside the dielectric, the amplitude of the electric field diminishes as it penetrates the material. As energy is dissipated in elements of the material nearest the surface, the total energy available to elements of volume deeper in the material is decreased. This appears as a decrease in the electric field. The field therefore undergoes a decay in penetrating the material. The penetration depth is defined as the distance at which the electric field intensity has been reduced to $\frac{i}{e}$ (0.37) of its value at the surface of the material.

The penetration depth is inversely proportional to the dielectric loss factor and the frequency. At a given frequency, if one material has a higher loss factor than another, it will have a smaller penetration depth than the other. As one increases the frequency, the penetration depth of a material also decreases (unless its loss factor happens also to decrease with frequency). The advantage of penetration by microwave heating can be lost if the frequency is made too high.

At the 1947 Conference at Atlantic City, several frequency bands were allocated for industrial use. We have selected the microwave band with a wavelength of about 10 cm (2450 mc/sec.) for the following reasons:

1. The frequency is high enough to permit suitable power densities for industrial use without objectionable electric field strengths.
2. There is still a reasonable penetration depth in most materials.
3. It has proved possible to construct generator tubes of a suitable power and a reasonable price, life, and efficiency.

The generator tubes we have used were magnetrons designed for continuous power output of 2 kw and 5 kw at 2450 mc/sec. The efficiency of the tubes is between 50 and 60% and we estimate the operating tube cost (for the 2 kw tube) at about 3.5 cents per RF kilowatt-hour of operation.

Five methods of application of the microwave energy have been investigated and will be described below. It should be borne in mind that energy at this frequency is usually conducted via coaxial transmission lines or by waveguides which are metal pipes of various cross-sections. A typical rectangular waveguide at this frequency is about 2 by 4 inches in cross-section.

Method I. The Rectangular Waveguide

The first and simplest means for heating material with microwaves is to mount an inclined tube through the broad wall of the waveguide (Fig. 2). The tube is usually mounted with only a small inclination to the waveguide axis to minimize electrical reflections and to provide a reasonable distance inside the waveguide over which the heating in the tube can take place. The material to be heated is then transported through the tube at a suitable rate for the heating power available and for the temperature rise required.

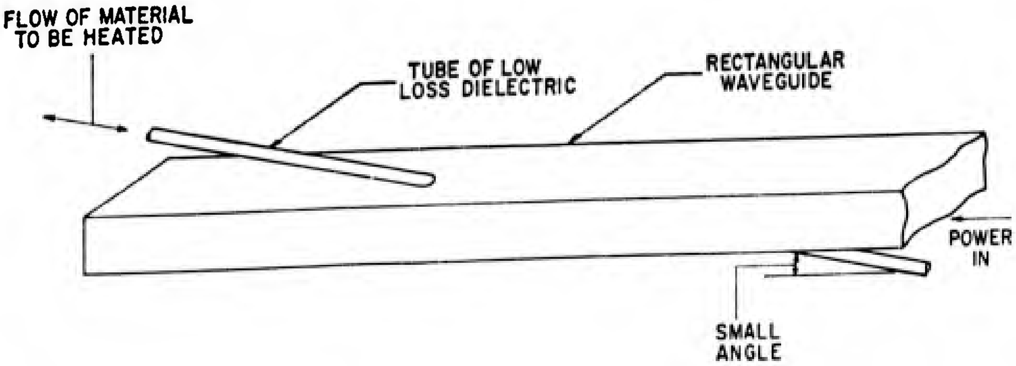

FIGURE 2. Rectangular waveguide applicator.

This configuration has been used extensively to make high-power terminations or dummy loads, as well as to make high power attenuators. For this purpose, water or other lossy liquid flows through the tube. The power from microwave generators has been measured for many years in this way by measuring the flow rate of the liquid and the temperature rise caused by the microwaves. These quantities and the specific heat of the material can be used to calculate the power absorbed by the liquid.

Many variations of this arrangement are possible, but each operates on the same principle.

Method II. The Circular Waveguide

A second type of waveguide, circular in cross-section, can be used in a way similar to Method I. A low loss dielectric tube is placed along the axis of the circular waveguide and microwave energy is absorbed according to the lossiness of the material being transported inside the tube. One principal difference between Methods I and II is the orientation of the electric field patterns in the respective waveguides. The pattern in the rectangular waveguide, shown simply in Fig. 3, is stable and quite predictable for any combination of waveguide dimensions and frequency which permit the existence of what is called the TE_{01} mode. This mode has the field distribution as shown. At 2450 mc/sec. it would be necessary to use rather large-sized waveguide before other field patterns or modes could exist. This, and the fact that the electric field is known to be in a direction across the narrow dimension of the waveguide, have given rise to the widespread use of rectangular waveguide.

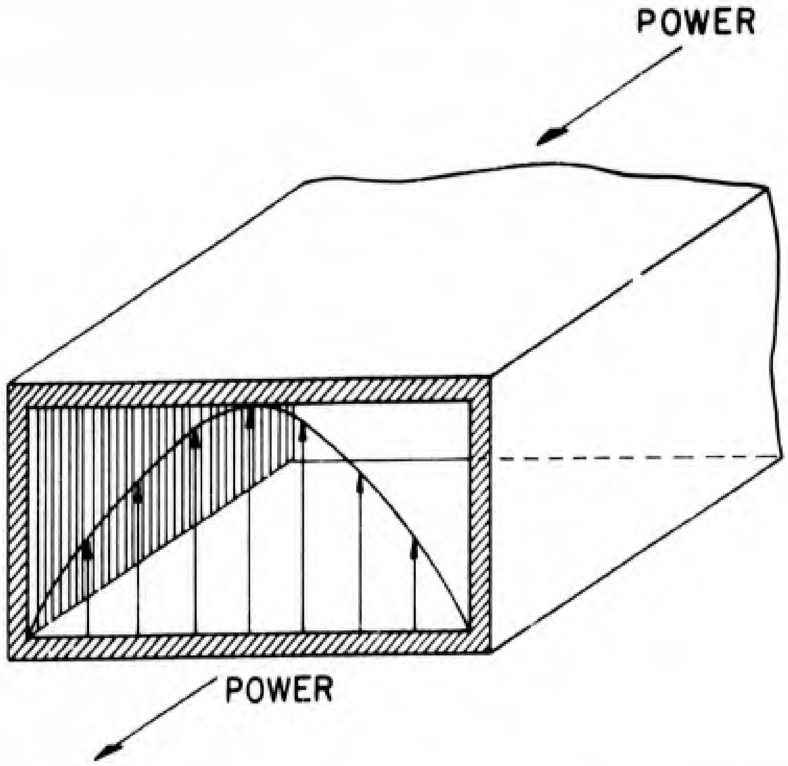

FIGURE 3. Electric field pattern in rectangular waveguide, TE_{01} mode.

In the circular waveguide applicator, however, we take advantage of a special mode of field pattern, called the TM_{01} mode. In this mode, the electric field pattern is concentrated along the axis of the waveguide, where the material to be heated is situated. The field pattern is shown simply in Fig. 4. By transporting the material to be heated along the center of the waveguide, as in Fig. 5, it will be exposed to the highest electric fields and will intercept the greatest amount of energy.

Again, many variations of this principle are possible.

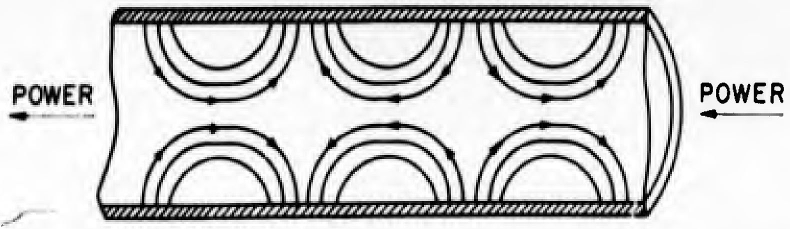

FIGURE 4. Electric field pattern in circular waveguide, TM_{01} mode.

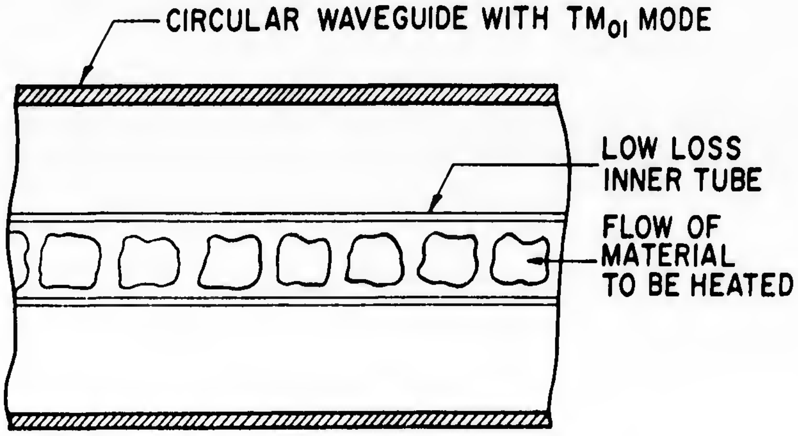

FIGURE 5. The circular waveguide.

Method III. The Folded Waveguide

If a narrow slot is cut in the broad side of a rectangular waveguide midway between the sides and extending along the length of the waveguide, very little change is observed in the RF properties of the waveguide. A second slot can similarly be cut in the opposite broad side with no sizable effect on the waveguide, electrically. These slots would then permit the introduction into the waveguide of thin dielectric films or sheet material to expose them to the electric field within (Fig. 6). If power were passed through the waveguide and the material were lossy, it would absorb power and be heated. Negligible power escapes from the slots. This is a principle which has been in use for many years like Method I for the control or dissipation of power in waveguides, in attenuators, or dummy loads.

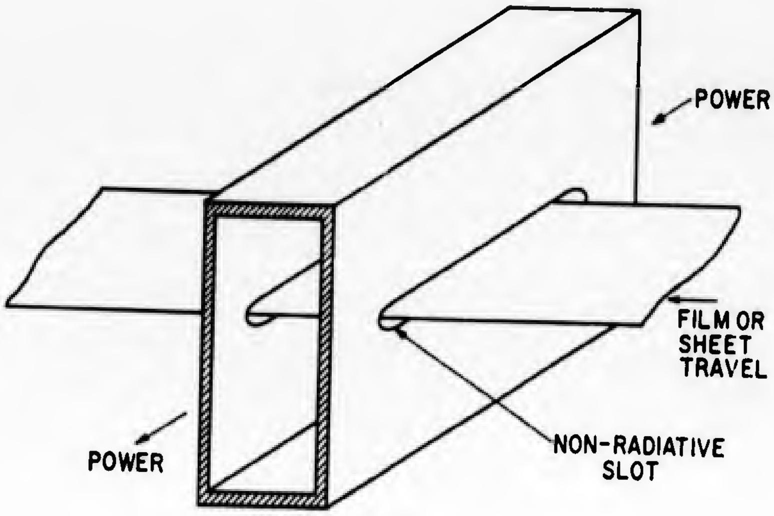

Figure 6. Slotted waveguide.

For many types of materials, however, the passage through the waveguide does not result in sufficient heating to be practical. All the power not absorbed by the film or sheet would ordinarily be absorbed by a water column or dummy load. This can lead to very poor heating efficiencies where most of the RF is wasted.

To overcome this problem, the waveguide has been folded back on itself many times forming a zig zag or meander. In this way, the power not absorbed during the first passage by the film or sheet is turned around for a second passage, a third, and so on (Fig. 7). During its passage through each succeeding waveguide, the sheet absorbs an additional amount of energy until a large percentage of the total available power has been transferred to the sheet. A closer look shows that the increase of the number of folds gives diminishing returns. Since the sheet absorbs some power during its passage through the first fold, less power is available for the second fold, for the third fold, and so on. Economy dictates the number of folds which is best for each individual problem.

Method IV. The Closed Cavity

Microwave energy can be introduced into closed metal cavities providing the cavity has suitable dimensions. The cavity can have various shapes, but for simplicity we shall consider the cube or rectangular parallelepiped. The dimensions of a cavity for microwave use must be of the same order or larger than the wavelength. RF energy introduced into the cavity is stored throughout the cavity space and produces a pattern of standing waves. The nature of the pattern will be discussed shortly. Lossy dielectric material introduced into the cavity will absorb energy and the temperature of the material will increase throughout its volume.

With a lossy or absorbing material in the cavity, it is possible to couple the microwave generator to the cavity with a minimum of

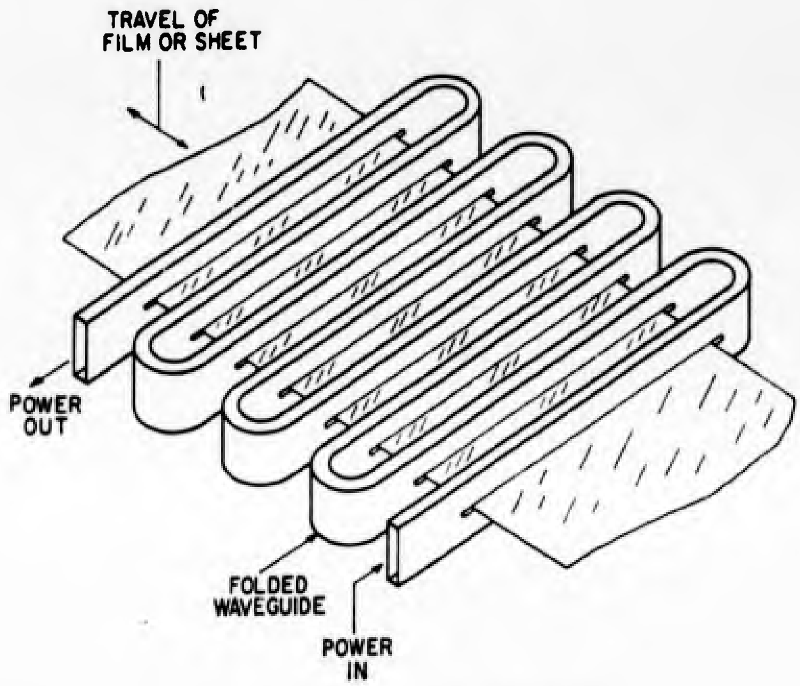

FIGURE 7. The folded waveguide.

undesirable electrical reflections from the cavity. With no loss in the cavity, it is impossible to prevent the reflections, and without special safeguards the generator may be damaged. Therefore, the closed cavity can be used to heat dielectric materials, but care must be taken that some energy will be dissipated within the cavity or the generator may be damaged. A simple illustration of the closed cavity is shown in Fig. 8.

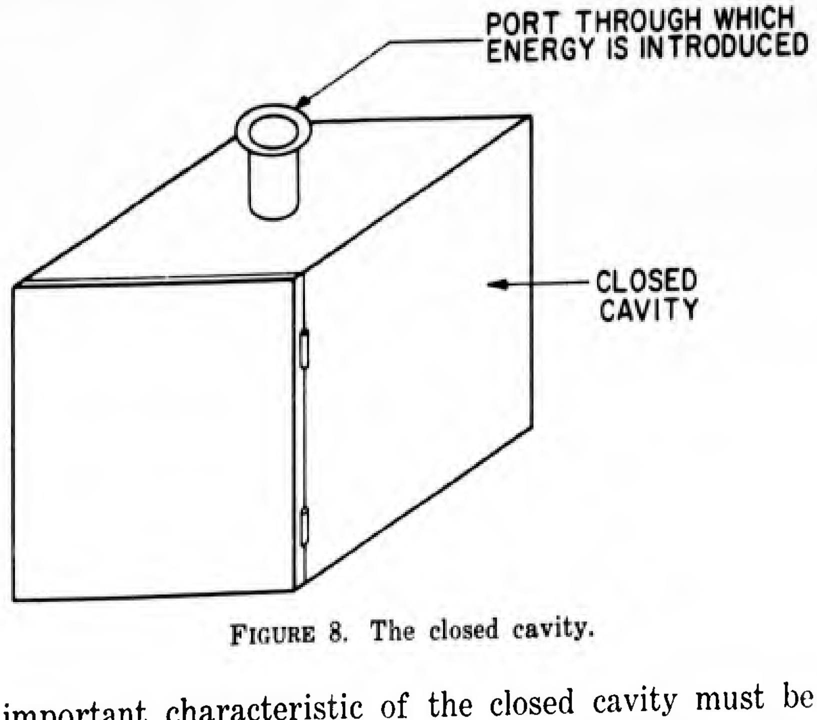

FIGURE 8. The closed cavity.

An important characteristic of the closed cavity must be noted. The intensity of the electric field is not constant over the volume of the cavity. It approaches zero at the metal walls and at a plurality of points within the cavity volume, depending upon dimensions, frequency, and so on. Therefore, there are inherently points of concentration and cancellation of the field pattern giving rise to an unequal distribution of heat. The thermal conduction of the material being heated will tend to equalize the hot and cold spots, but the degree of equalization will depend upon the thermal time constant of the material, which is a function of the thermal properties and mass of the material and the distance between hot and cold spots. A smaller power input to the cavity and consequent slower heating time will give rise to fewer difficulties with hot and cold spots than a larger power input and faster heating times.

A principal advantage of the RF heating process, however, is its penetration, its ability to achieve rapid heating by the transfer of energy immediately throughout a volume, rather than to a surface alone as with radiant heat. The problem of unequal heating, therefore, nullifies this advantage to a large extent. An additional technique, fortunately, can be used to achieve a considerable degree of even heating.

By making the cavity-generator combination suitable for what is called multi-mode operation, the field patterns within the cavity can be shifted in a position periodically so that hot and cold spots move around. The closed cavity, like the waveguides described, can support certain field patterns or modes depending upon the dimensions of the cavity, the frequency, and certain other things. If the cavity has the right dimensions, a small change in the generator frequency can cause a changeover to a different mode or field pattern. A change in shape of the cavity, or boundary effects, can also be used to switch modes. These changes are usually used in combination to shift modes.

A commonly used boundary-effect change is produced by a device in the cavity called a field stirrer. The stirrer is usually a metal fan-like device which is rotated at slow speeds by a small motor (Fig. 9). Most often the stirrer is situated in the cavity near the port through which the microwave energy enters. As the stirrer rotates, the blades pass close by the port influencing the generator frequency directly as well as changing the mode pattern in the cavity by changing the boundary effects.

At 2450 mc/sec., it is possible to use cavities with dimensions of the order of one to two feet on a side and with generators of 1, 2, and 5 kw of microwave power available interesting effects can be obtained. Industrial processes, however, quite often require higher energy input than is afforded by 2 kw or even 5 kw.

Two generators can be coupled to the same cavity but there are considerable difficulties with avoiding mutual interference, and possible damage to the generators (Fig. 10). Just as power enters through a port, it can leave through the same port and thus one generator can deliver power to the other with possible damaging effect. Certain arrangements are possible to minimize this interaction. It has been

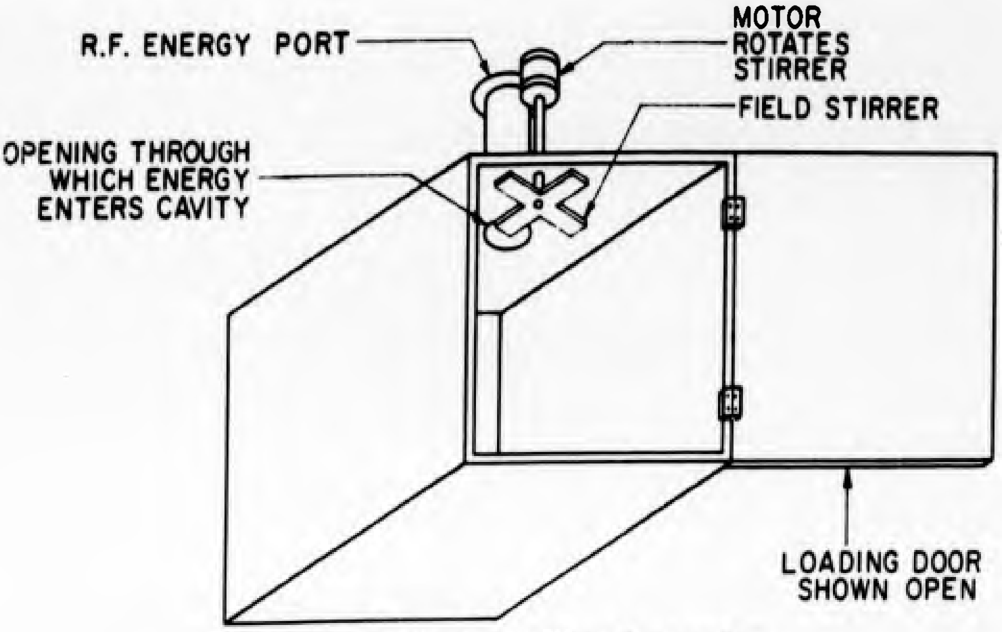

FIGURE 9. Cavity for multi-mode operation.

impractical to try to couple more than two generators in this way to the same cavity. Therefore, the power levels attainable are limited by the unit power of the generators. For industrial processes, it is also desirable that the microwave energy be applicable to work pieces on a conveyor. Of course, with any method, it is essential to avoid the radiation of microwave energy to prevent hazards to personnel and to maintain good power efficiency.

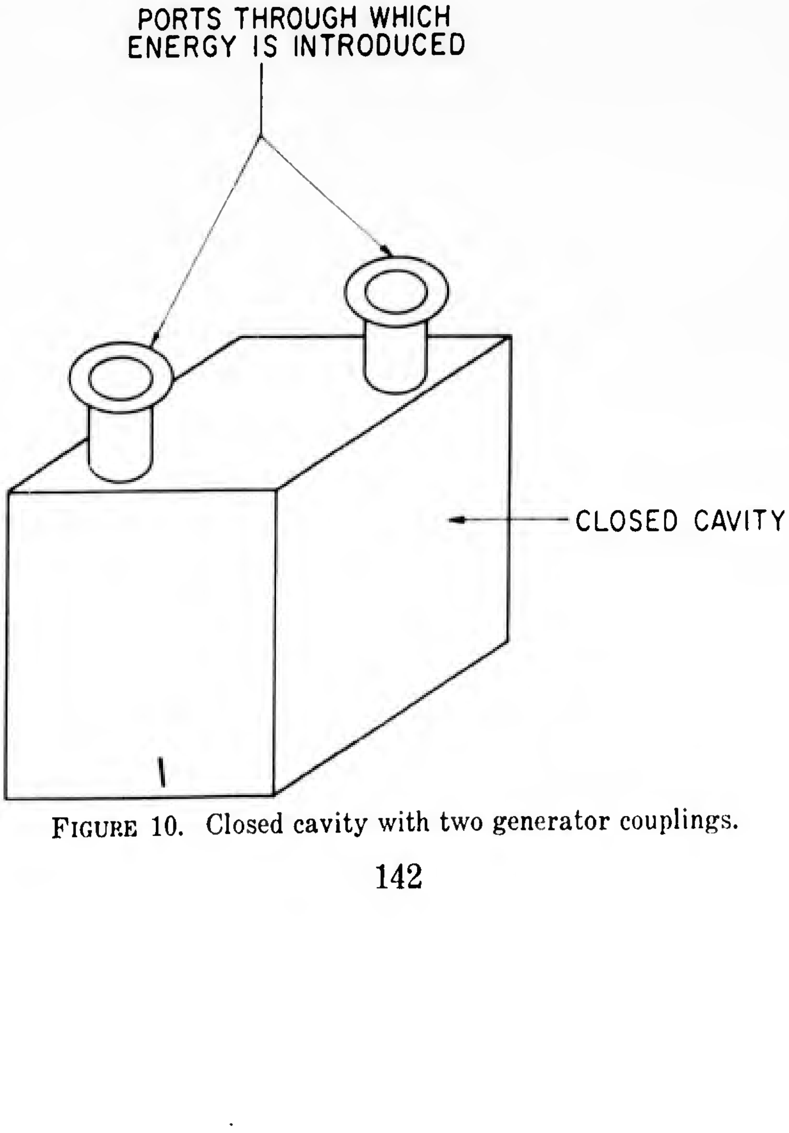

FIGURE 10. Closed cavity with two generator couplings.

The use of a cavity with a conveyor system has been investigated with the idea that the addition of many cavities along the conveyor would solve the power limitation problem. A cavity was made with slots in opposing walls through which a conveyor belt passed (Fig. 11). Material could be placed on the belt, move into the heating area of the cavity, and pass out the other side. For practical purposes, the slots must have a certain height and width or very little useful work could be done. Within the cavity, however, the electric fields are not polarized uniformly (as in rectangular waveguide, for example) and a substantial portion of the cavity power is radiated from the slots. These slots in experiments have measured about 4 or 5 by 12 inches.

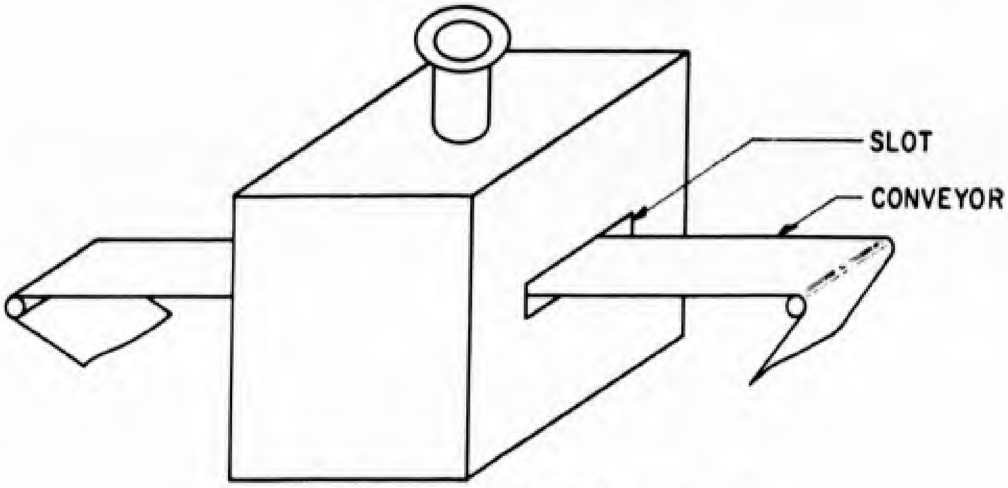

FIGURE 11. Cavity with slots for conveyor.

It is possible in principle to build RF chokes around the slots, the chokes being adjusted to the generator frequency. In Fig. 12 is seen the type of construction resulting. In order to reduce the radiation to a tolerable level, it was necessary to use quite a number of chokes (five on top and bottom of the conveyor). The resulting design was so frequency-sensitive that changing the generator (which might cause a small frequency change) required the readjustment of all of the chokes. The introduction of the material to be heated also disturbed the adjustment making the design much too cumbersome to use. In all, there were twenty chokes which would require frequent readjustment. Other methods were sought, therefore, to solve the conveyor problem.

Method V. Zone Heating

One approach to the reduction of the radiation from the ports through which the work-pieces must enter and leave the heating area on the conveyor was to polarize the fields properly at the ports. The following solution[1] was found. A radiating element was made from a thin slice of a parabolic cylinder (Fig. 13) which was closed on top

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[1] Patents applied for.

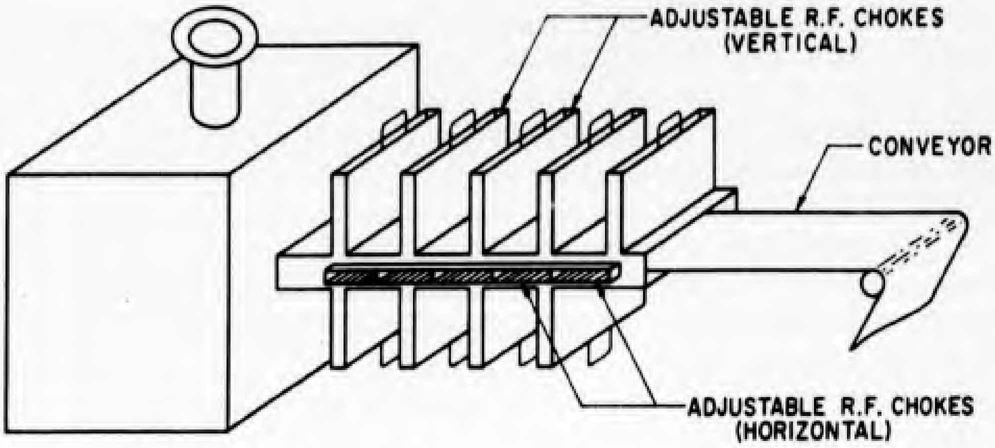

FIGURE 12. Cavity with conveyor slot and RF chokes.

and bottom. Power was introduced by a stub antenna at the focus of the parabola and was focused outward to the mouth of the radiator. In this structure, the electric field is oriented in a known way, or polarized, and can better be controlled in the completed machine.

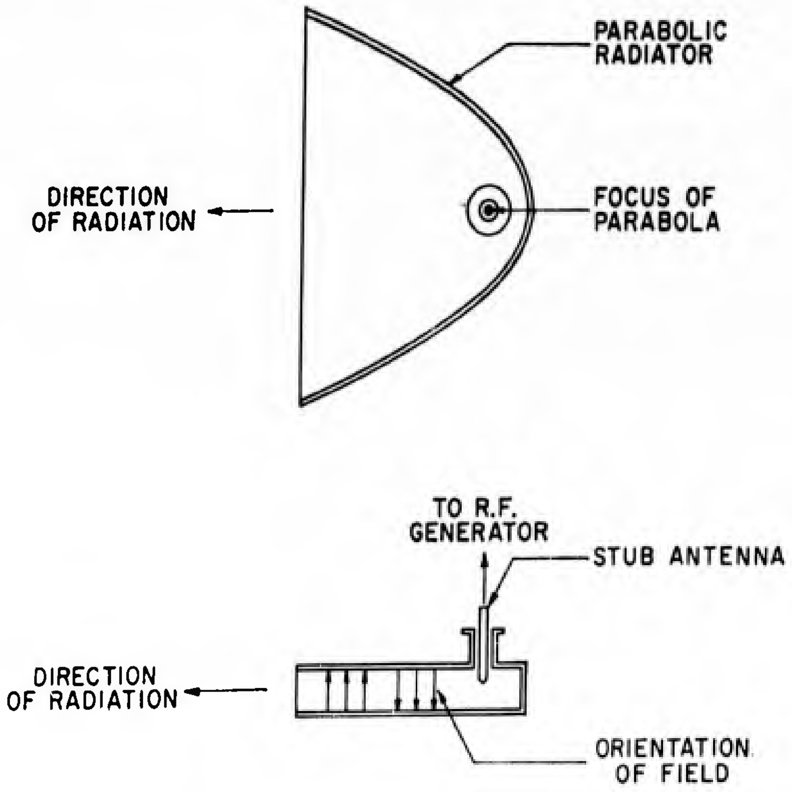

FIGURE 13. Parabolic radiator.

The parabola at its mouth is slightly wider than the conveyor belt with which it is to be used. It is mounted, in the first machine mode, with its mouth across the width of the belt and its plane at right angles to the belt. A number of these can be mounted in a relatively short length along the belt (Fig. 14). When so mounted, they are joined by metal work which then completely encloses the belt except at the entrance and exit ports, forming a rectangular tunnel through which the work-pieces travel. The particular polarization of the electric field in the parabolas restricts the propagation of the energy out of the ports.

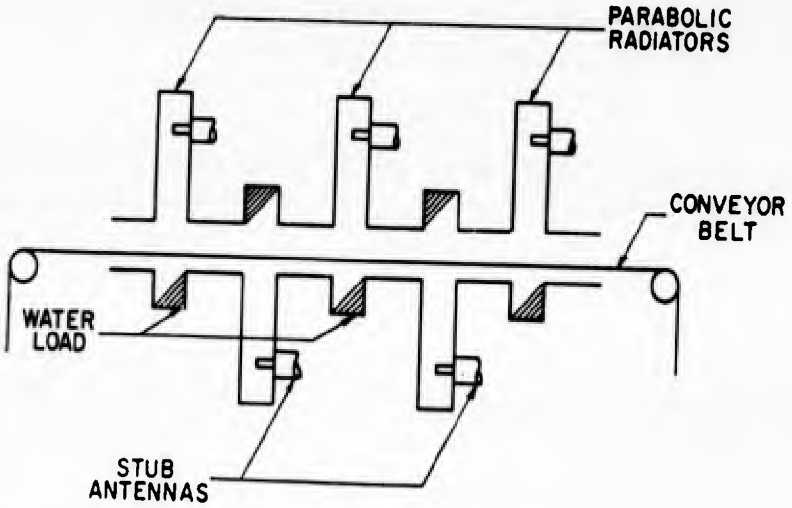

FIGURE 14. Zone heating.

The direct radiation given by the structure of Fig. 13 would not produce even heating across the width of the belt. This is corrected by the addition of secondary radiators in the parabola which yield a highly uniform heating pattern. In the experimental machine which was made, the conveyor was about 30 inches wide and the clearance above the belt was about 2 inches. The parabolas provided heating zones across the belt which were also about 2 inches wide. These particular dimensions were chosen to suit a specific application to reconstitute refrigerated or deep-frozen meals. Many variations are possible.

Unlike the closed cavity, the zone-heating machine can be operated with no load in it without damaging the generators. Beneath (or above) each parabola is a water load which absorbs all of the radiation not already absorbed by work on the belt. In the slotted cavity, several hundreds of watts were lost by radiation from the ports (out of 2 kw). In the experimental zone-heating machine, with a total power of 10 kw only a few watts were radiated. Where necessary, this too can be greatly reduced by single chokes, much simpler than for the slotted cavity.

The 10 kw machine, as an example, then gives the possibility of heating 100 to 150 lb of food per hour from deep-freeze temperatures to 75°C, or double the quantity of refrigerated food.

The advantages of the zone-heating method are numerous. First, a very uniform energy distribution across the width of the belt can be achieved. Second, the work passes in and out of heating zones as it progresses through the machine. This allows thermal equalization, between heating zones, of any non-uniformities in heating which may have occurred. Third, the total power of the machine is not limited by the generator size, since any number of zones can be used, each with as much, for example, as 5 kw. Fourth, there is very little stray radiation. Fifth, an empty conveyor belt will not damage the machine. And sixth, as the electrical properties of the material being heated change with temperature, the individual parabolas can be adjusted to maintain maximum efficiency in the process.

Summary

We have shown simply some of the principles underlying microwave dielectric heating and discussed several problems and their solutions. The limitations on frequency and power were also discussed. Finally, five methods were shown for the application of microwave energy with brief explanations of the inherent and design difficulties which can be expected. We conclude that sufficient possibilities exist in the technique of applying microwave energy to make the technique of great potential use.

SESSION No. 4

IMPROVED INSTRUMENTATION FOR FREEZE-DRYING OPERATIONS—EXPLORING NEW APPROACHES

CLINTON O. CHICHESTER, *Moderator*

LIMITATIONS AND OPPORTUNITIES FOR HIGH FREQUENCY ENERGY IN THE FREEZE-DRYING PROCESS

ROBERT V. DECAREAU

Freeze-drying is a process characterized by a long drying cycle and low production rates. The processing cycle is long because the method of heat input becomes progressively less efficient. The transfer of heat across the increasingly thicker dry layer is necessarily slow because the dry layer represents a rather effective insulator. High frequency heating overcomes this limitation. This form of energy for all practical purposes does not see the dry portion, but passes unimpeded through it to the ice portion where it is absorbed and converted to the heat necessary to effect sublimation of the ice.

Many have said that the application of high frequency energy to the freeze-drying process is impractical because it is not economical. Others have suggested that, since heat transfer is the limiting factor in the process, the use of high frequency energy should be investigated as it offers a means of freeze-drying at a maximum rate. Although the literature is not replete with studies of this nature, a good deal of useful information has been developed and some work is in progress. It is hoped that the discussion to follow will shed additional light on the subject.

Let us concern ourselves, first of all, with some of the factors which must be considered when using high frequency energy, such as choice of frequency and design problems, and defer a discussion of the economics of the process until later.

Table I lists some of the sources of high frequency energy which are available. The frequencies shown in this table were allocated by the Federal Communications Commission for industrial, scientific, and medical uses. These are commonly referred to as ISM frequencies. Such equipment may be operated with unlimited radiation on these assigned frequencies and within the specified tolerances. No license is required as long as the design and operation of the equipment complies with the technical limitations set forth by the Commission for such equipment and as long as the equipment has been certificated (1). Operating within these tolerances presents some difficulties, particularly at the higher frequencies, because the characteristics of the product change during heating. This change presents a fluctuating load to the energy generator which in turn results in a frequency shift. Because of this situation the Commission established an allowable radiation limit of 10 microvolts per meter at a distance of one mile from the equipment. This tolerance may not be too difficult to meet with present relatively low powered sources being used in the microwave frequency range, but most likely will offer some reason for concern when higher powered sources become available. The reason for the Commission's concern is the possible interference with communications which are in the same range of frequencies.

TABLE 1

Sources of High Frequency Energy

Source	Frequency in Megacycles Per second	Power in Kilowatts
Dielectric Heaters	13.56 ± 6.78 Kc 27.12 ± 160.0 Kc 40.68 ± 20.00 Kc	as high as 500
Magnetrons and Klystrons	915 ± 25 Mc 2450 ± 50 Mc 5850 ± 75 Mc 18000 ± 150 Mc	0.8 to 5.0

Of more importance, perhaps, is the control of radiation in the vicinity of the equipment where radiation may be far more intense and is a potential hazard to workers there. According to one writer (2), as of December 1959 there was no "medically substantiated case of human injury from microwave energy." The possibility, however, cannot be dismissed, especially when much higher powered sources become available for industrial heating purposes. Considerable governmental research is being carried out on this subject. Much of the concern has been with the danger to personnel operating near very high powered radar devices such as those on the DEW line, on radar picket ships, and on Texas Towers.

Before proceeding further, a few definitions are in order. The terms dielectric heating and microwave heating are often used interchangeably, and although the effect is essentially the same, there are differences between the two. Perhaps a more suitable way to differentiate between them is to refer to dielectric heating as capacitive heating. The capacitor in this case consists of the electrodes and the material to be heated. This assemblage may be in the form of a sandwich, or if the product is cylindrical the electrodes may be shaped accordingly. Electronic generators for capacitive heating convert electrical energy to radio-frequency energy with the assistance of an oscillatory circuit and vacuum tubes. Their power may attain 500 kilowatts while the frequency may vary between 100 kilocycles and 1000 megacycles. A generator of this type consists of one or more vacuum tubes (such as triodes) which oscillate at the desired frequency, an adaptor unit which transfers the energy to the workpiece, a transformer to heat the filaments of the vacuum tube, a rectifier which provides several thousand volts of direct current plate voltage for the tube, and a control unit to assure the correct voltage supply to the transformer and rectifier.

Microwave heating, on the other hand, generally refers to the frequency range from 300 megacycles to 30,000 megacycles. Thus it actually extends down into the range of capacitive heaters. The equipment in this range employs special tubes such as magnetrons and klystrons, but does not feed the energy to a capacitor. Instead, the energy is fed to a resonant cavity in either one of two ways: by direct insertion of the tube into the cavity as in the case with some microwave cooking equipment currently on the market or by wave guide transmission in which case the magnetron may be located at some distance from the cavity. The latter technique has a certain amount of flexibility which is lacking in the former. In an oversimplification, the direct feed system beams energy into the cavity in the way a light bulb illuminates a room, while the latter is more like a plumbing arrangement in which the energy flows in the way water flows through pipes into a tank. The argument for microwave heating over capacitive heating may be elucidated by considering the equation for power generation:

$$P = 2\pi f \times E^2 e'' \times 0.0885 \times 10^{-12}$$

where

P is the power in watts per cubic centimeter converted to heat in the workpiece.

f is the frequency of the alternating electrical field in cycles per second.

E is the rms field strength in volts per centimeter.

e'' is the loss factor of the dielectric (workpiece).

To increase the heating rate, either the field strength or the frequency may be increased. Since there are limits to which the field strength may be increased, limits imposed by the breakdown strength of the residual gases, the alternative is to increase the frequency, thus the reason for considering the microwave frequencies. The loss factor also tends to increase with frequency, a fact which helps to increase the heating rate.

Characteristics of Radio-Frequency Energy

Energy in the radio-frequency range obeys the laws of optics in that it is reflected, transmitted, and absorbed. Thus metals reflect such energy; glass, ceramics, paper, and plastics transmit it; and food materials absorb it and it is converted into heat. In relatively simple terms food materials are heated by radio-frequency energy because foods have for the most part a high moisture content, and water molecules have a large electric moment. Therefore, in the presence of an electrical field the water molecules tend to align themselves with the field. Since the field is reversing its polarity millions of time per second, the water molecules in attempting to align themselves move past each other and cause considerable frictional heat to develop. In the case of frozen food materials, the restricted movement of the water molecules allows the energy to penetrate a greater distance. Fig. 1 shows the effect of frequency on the penetration of high frequency energy into ice, suet, and water. The ordinate is the depth in centimeters at which half of the incident energy has been absorbed. From the curve it can be seen that energy at 2450 megacycles per second will penetrate about 800 centimeters into ice before half of the energy has been absorbed. The penetration at 915 megacycles is about twice as great.

Information concerning the dielectric properties of food materials in the frozen and unfrozen state has been published (3). These data indicate that the penetration into frozen foods is considerably less than into ice. Recent studies (4) tend to confirm these data (Table 2).

TABLE 2

Penetration of Microwave Energy into Frozen Foods at 1000 Megacycles per second

Food Material	Temperature (°C)	Loss Factor	Penetration (cm)
Beef, raw (3)	–15	0.75	5.8
Peas, whole, boiled (3)	–15	0.5	7.9
Pork, raw, ground (3)	–15	8.16	0.66
Potato, boiled (3)	–15	0.9	6.1
Spinach, boiled (3)	–15	6.5	1.44
Squash, baked (3)	–15	0.15	3.7
Ice (5)	–12	0.00288	1980.0
Peach sections (4)	–18	0.45	—

Equipment Design

There are several approaches which we may consider in designing a microwave freeze-dryer:

1. All microwave batch unit.
2. All microwave continuous unit.
3. Dual energy batch unit.
4. Dual energy continuous unit.

One embodiment of the first unit is illustrated in Fig. 2. It consists of a standard freeze-drying unit with a removable tray rack, the trays and rack constructed of a non-radio-frequency absorbing material. The energy is introduced through a system of wave-guide feeds so that in the event of the failure of one of the microwave generators, a replacement could be made without shutting down the equipment.

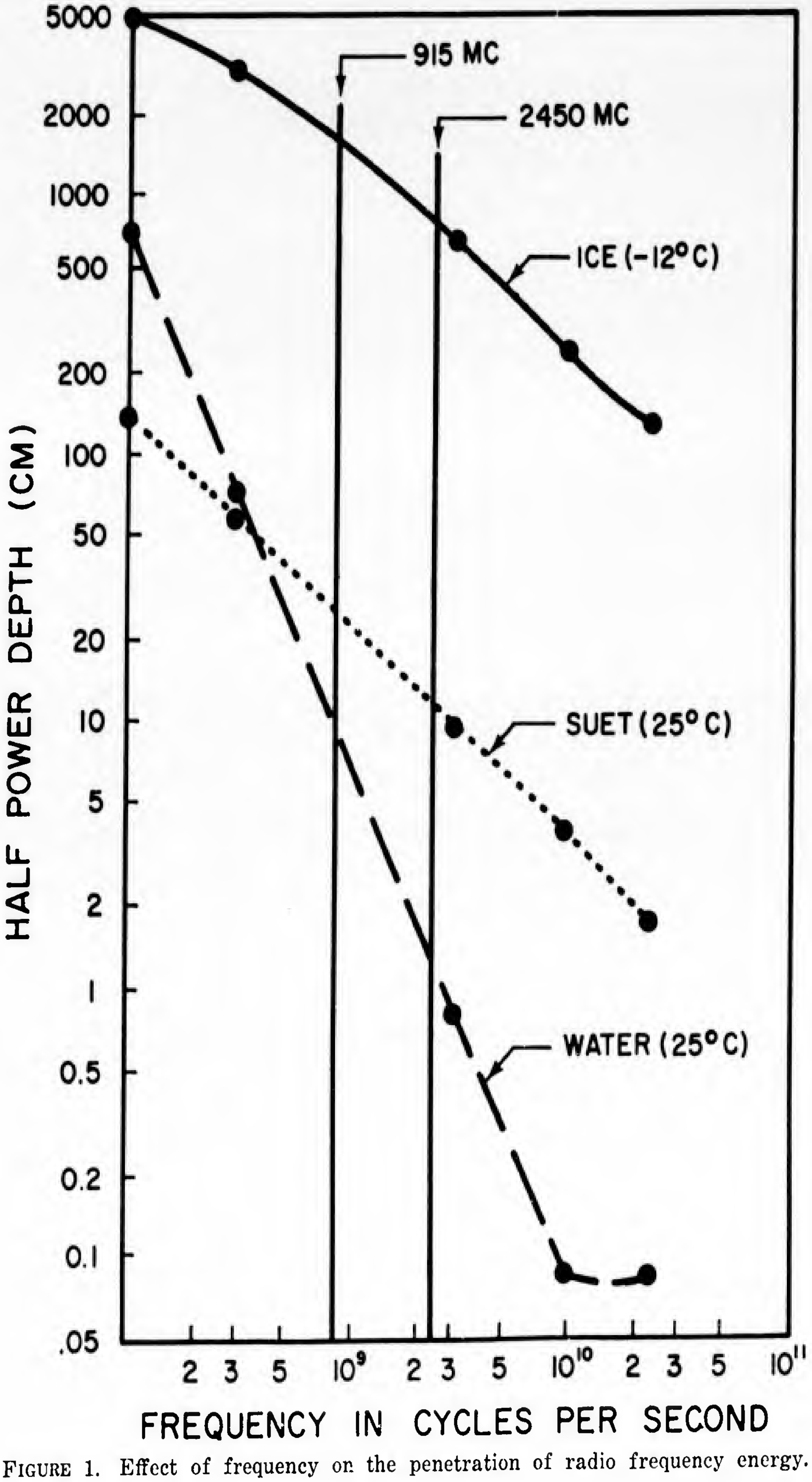

FIGURE 1. Effect of frequency on the penetration of radio frequency energy.

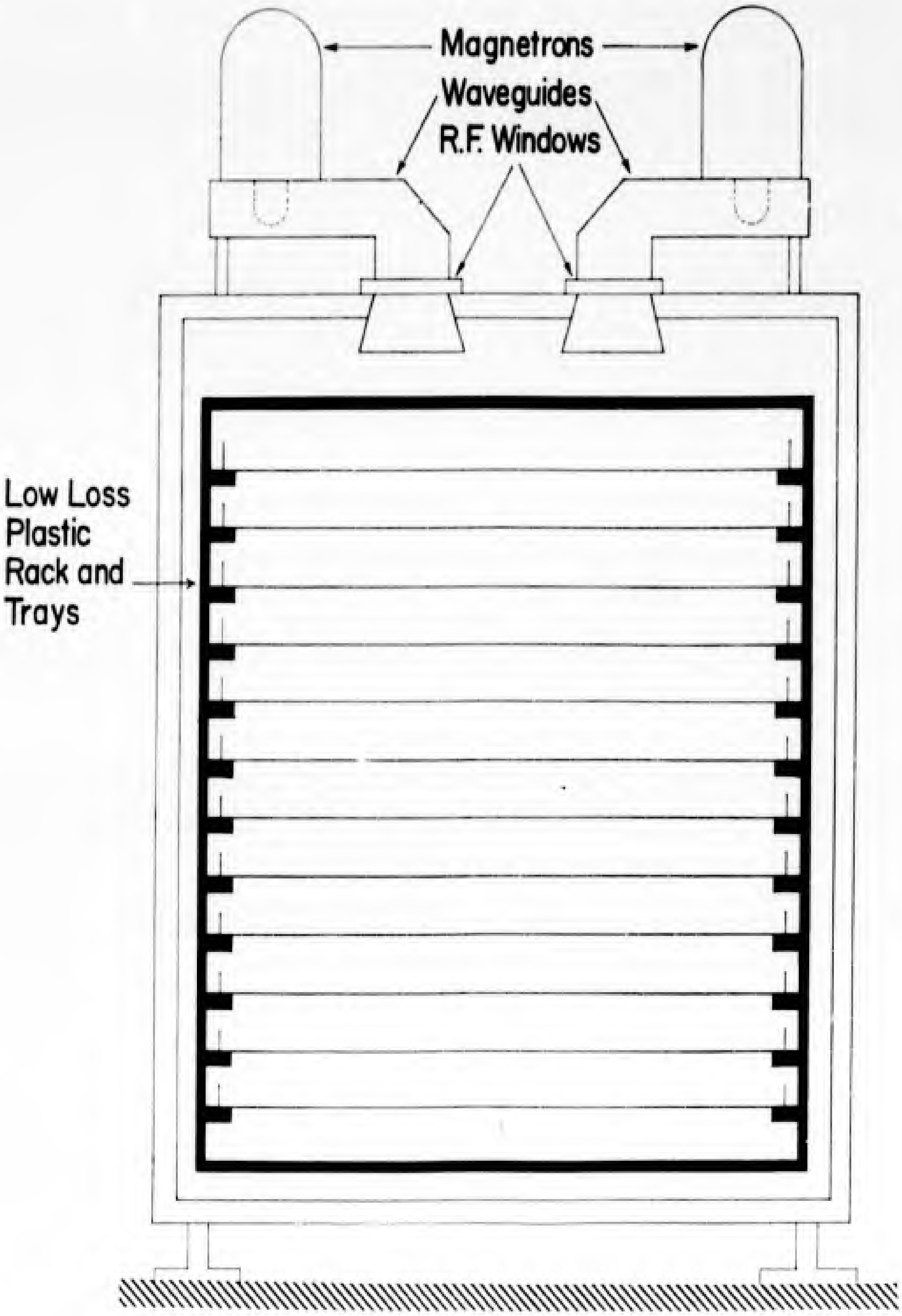

FIGURE 2. Microwave freeze-dryer.

The continuous unit could be designed in several ways, possibly using a belt to carry the product or it could be of the truck and tunnel type of design. This would depend to some extent on the processing volumes involved. Fig. 2 can also be an end view of a truck and tunnel freeze-dryer, equipped with suitable air locks at either end.

From experience gained with an experimental microwave freeze-dryer, it was realized that the construction of a production-sized unit presented several formidable problems, the major problem being the lack of a suitable microwave energy source. At that time only one and two kilowatt sources were available. To take advantage of the short process times possible using microwave energy, it would have been necessary to supply sufficient energy to sublime over 100 pounds of ice per hour. Since the complete conversion of one kilowatt of radio-frequency energy into heat of sublimation would result in only 2.8 pounds of ice sublimed per hour, about 25 to 50 such radio-frequency generators would have been required. Such a grouping would result in a rather complex device. Recently, however, a five-kilowatt magnetron has appeared on the market. This device simplifies considerably the problem of an all radio-frequency freeze-dryer. It is quite conceivable to direct the energy of eight to ten such magnetrons into a single standard freeze-drying cabinet which has a normal loading capacity of about 1000 pounds of product. The ability to control the input of eight to ten such packets of energy also provides flexibility. The heat input to be increased or decreased immediately, i.e., without the delay caused by the inertia of the heat source. The third design approach, the dual energy unit, would take advantage of the relatively high economic efficiency of conventional dryers during the constant rate drying period and employ radio-frequency energy during the otherwise falling rate period. In this design it would not be possible to introduce all of the radio-frequency energy through the top of the cabinet because it would be reflected by the top metal platen. Instead, advantage would be taken of the fact that the spacing between the platens is adequate to support the propagation of the energy waves, and the energy would therefore be introduced from the cabinet sides as illustrated in Fig. 3. Fortunately it is also possible to split the output of a single generator and move it along several pathways; thus, the energy of a single magnetron could be used to heat two or three shelves of product. Since most of the moisture has been removed from the product at this stage, much less energy is needed to finish the process. We would expect to derive certain economies from this approach.

Other factors which need to be considered pertain particularly to the radio-frequency portion of the equipment.

1. Radio-frequency shielding is necessary to protect gaskets and "O" rings from the heating effect of the energy. Proper internal shielding will eliminate the need for building an external shield around the entire equipment, a move which could seriously hamper the operation of the equipment.
2. Radio-frequency chokes at viewing ports and across the vapor conductor are essential. Ordinary copper window screen is adequate for this purpose and will not impair vision into the chamber, nor will it permit the energy to pass through the vapor conductor to resublimate ice from the condenser plates.

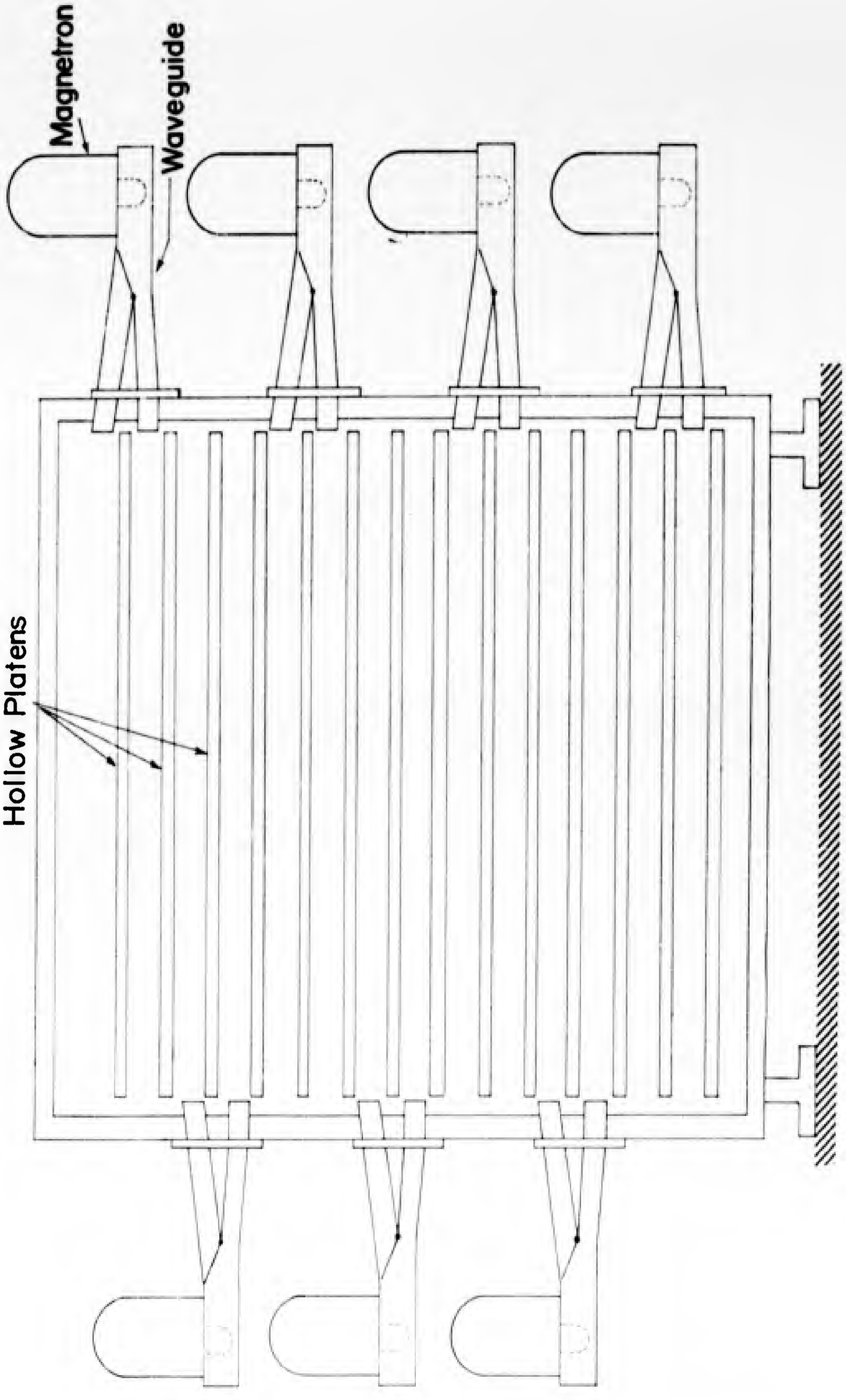

FIGURE 3. Dual energy freeze-dryer.

3. Door interlocks are required so that the chamber door may not be opened if the energy is still radiating into the chamber.
4. Radio-frequency cutouts actuated by the pressure controller

will protect the product in the event of pump or refrigeration failure.

5. Energy input control by product temperature would tend to maximize the sublimation rate and reduce the input toward the end of the cycle so that product overheating does not occur. Since the use of thermocouples is not possible in the presence of radio-frequency energy, some other technique such as the Leybold system of barometric temperature measurement will be required (6).
6. Uniform energy distribution techniques are presently employed in high-frequency cooking devices. A mechanism known as a mode stirrer (Fig. 4), which to all appearances is a fan, is positioned in the chamber to intercept the energy waves and distribute the energy by reflection. Its location in a lightly loaded chamber is unimportant since most of the energy strikes the stirrer because the load intercepts only a small fraction of the energy flux. In a heavily loaded chamber such a large fraction of the energy flux is intercepted by the load that the scattering effect must be imparted as the energy leaves the generator.

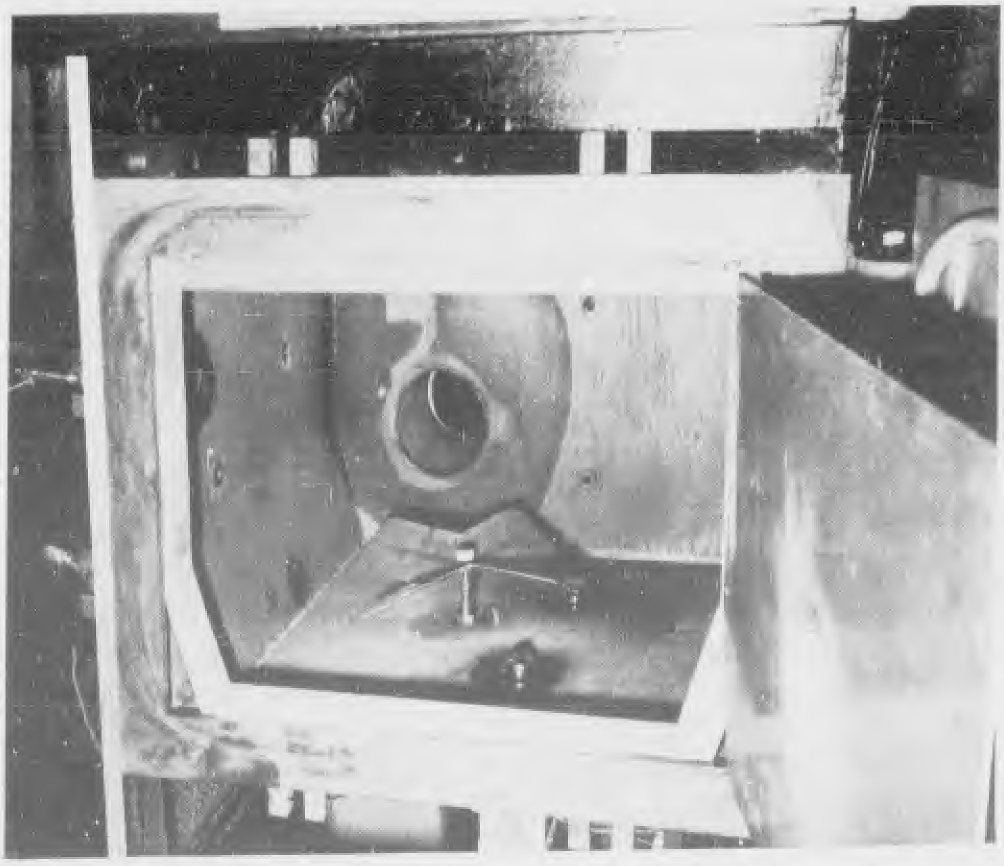

FIGURE 4. Illustration of a mode stirrer in a microwave cavity

7. **Power tube protection techniques may be necessary. Thermal cutouts would turn the energy generator off if it should overheat as a result of its absorbing an excess of reflected energy. Such techniques as isolation can be used to intercept reflected energy. Some preliminary work with radio-frequency freeze-drying indicates that a much longer tube life can be expected in such a process since it operates for longer periods than in its normal role in restaurant cooking applications where short cycles of less than one minute is the usual experience. Thus, thermal cutouts and isolation techniques would tend to further improve tube life. One firm guarantees the life of their magnetron for six months of continuous use and states the conversion efficiency to be 60%.**

Fig. 5 illustrates an experimental freeze-dryer employing microwave energy at a frequency of 2450 megacycles per second. The inner door is held firmly against a flange at the front of the chamber by rubber pads on the main door. The inner door thus serves to confine the energy within the drying chamber and, in this way, not only prevents energy radiation, but also protects the gasket on the chamber door. Also illustrated are low dielectric loss plastic shelves which permit the energy to pass through to the lower shelves. Although not completely obvious from this figure, maximum use can be made of the chamber space; whereas, in conventional shelf dryers considerable vertical space is taken up by the hollow metal shelves. The absence of manifolds and other plumbing connections in this design allows the entire load to be supported freely on a weighing device so that the progress of drying can be easily followed. Such a device is present in the equipment shown. It consists of an aluminum plate resting on three points, one of which is a transducer. The change in the force exerted on the transducer, as the product dries, is reflected in a voltage reading on the voltmeter to the right of the chamber. This reading is then interpreted in terms of the change in weight of the product. The voltage so generated could also be used to control the radio-frequency energy input so that a particular rate of drying could be maintained.

The rather small values for the penetration of radio-frequency energy into various frozen foods, which were shown earlier, seem to be at variance with results obtained in the experimental microwave freeze-dryer. These results demonstrated clearly that each shelf load, with the exception of the top shelf which was receiving radiant heat from the Pyrex caps over the magnetrons, dried at a relatively uniform rate. This action suggests that equal increments of energy were being absorbed by each shelf load and that the energy therefore penetrated through the entire stack, a product depth of at least 12 centimeters. Unfortunately, the equipment was too small to determine how tall a stack of shelves could be uniformly dried. Table 3 shows

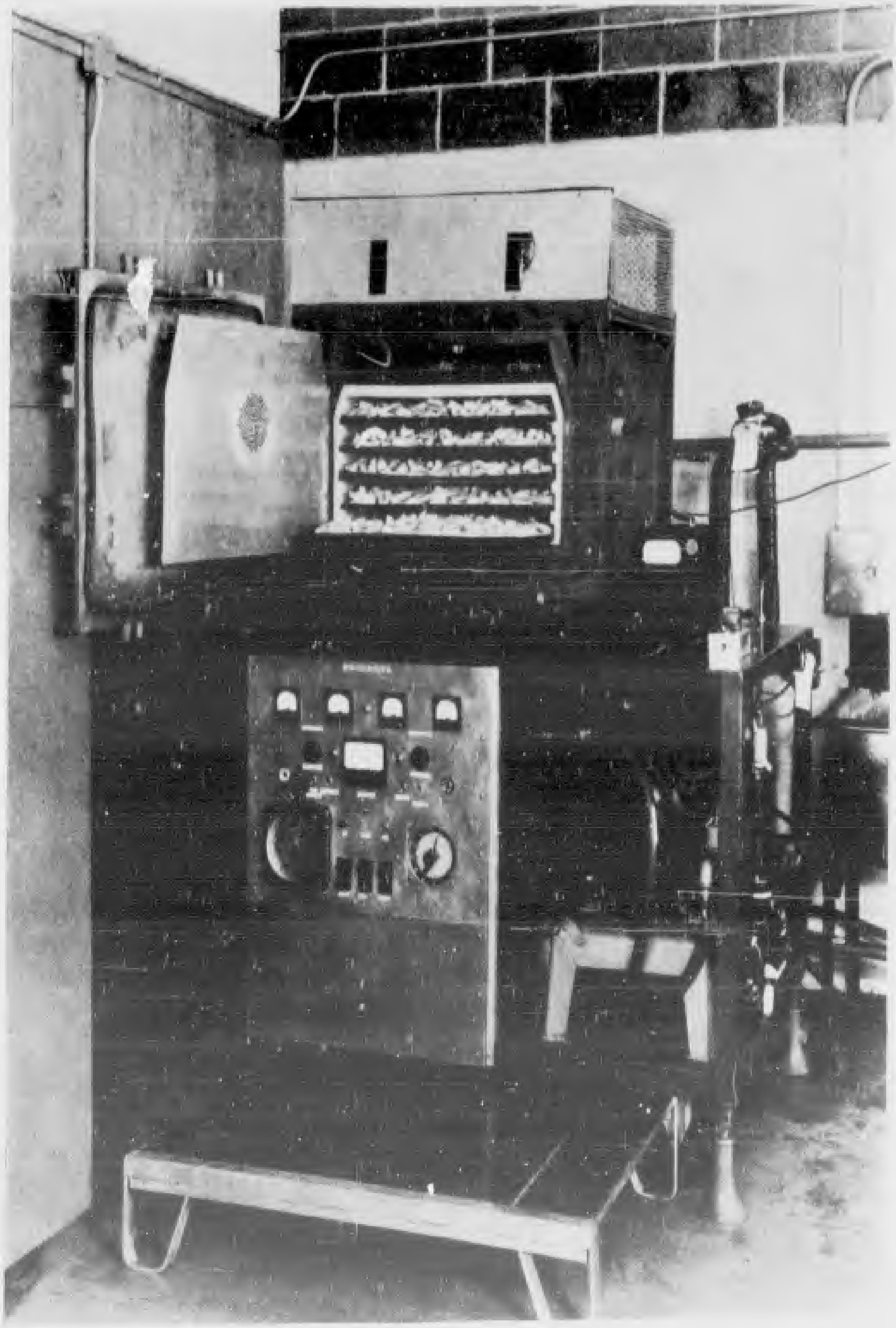

FIGURE 5. Experimental microwave freeze-dryer.

the weight losses per hour from each shelf. The top shelf was moved to the bottom rack position after each weighing and the other shelves moved up one position. The initial shelf loading was about 6.5 pounds. The results also show a rather uniform rate of drying until the sixth hour when the rate falls off, indicating that the system is becoming mismatched. The temperature of the Pyrex globes, as measured by means of pyrometer, during the weighing periods also showed a rise in temperature at this time demonstrating that more energy was being absorbed while less was being used by the product.

TABLE 3

Weight Changes During Microwave Freeze-Drying of Shrimp

Elapsed Time (hours)	Weight Loss in Pounds				Total Loss in Pounds
	Top Tray	Other Trays			
1	1.25	0.72	0.90	0.69	3.56
2	1.38	0.83	0.70	0.69	3.60
3	1.23	0.72	0.77	0.87	3.59
4	1.22	0.77	0.89	0.80	3.68
5	1.13	0.88	0.82	0.75	3.58
6	1.08	0.69	0.66	0.79	3.22
7	0.94	0.63	0.57	0.59	2.73

Process Economics

It is important now to consider what effect the use of radio-frequency energy will have on the process cost and whether the increased production rate resulting from the shorter drying cycle can justify the cost of this form of energy. The all microwave process is compared in this analysis with the dual energy process and the conventional process. The bases for comparison are presented in Table 4.

TABLE 4

Cost Comparison Basis

Product	Frozen Raw Beef Steak
Moisture Content	75 per cent
Thickness	One inch
Operating Pressure	1 mm
Vacuum Chambers	Typical of those used for commercial scale production
Dimensions (feet)	5 × 5 × 6 approx.
Number of Shelves	17 in conventional and dual energy units 34 in microwave unit
Shelf dimensions	1 × 44 × 60
Spacing between shelves	2.75 inches
Shelf material	
Conventional	Hollow steel
Dual energy unit	Hollow steel
Microwave	Low loss material
Chamber Loading	
Shelf area use	90 per cent
Meat (wet basis)	
Conventional	1377 pounds
Moisture	1032 pounds
Dual energy unit	Same as conventional
Microwave	2754 pounds
Moisture	2064 pounds

TABLE 4—Continued

Processing Rate	11,016 pounds per day
Operation	24 hours per day, 300 days per year
Drying Cycles	
Conventional	
Drying	23 hours
Load-Unload	1 hour
Dual energy unit	
Drying	11.0 hours
Load-Unload	1 hour
Microwave	5.5 hours
Load-Unload	30 minutes
Number of Chambers	
Conventional	8
Dual energy units	4
Microwave	1
Maximum Water Vapor Rate	
Conventional	300 pounds/hour
Dual energy units	270 pounds/hour
Microwave	515 pounds/hour
Vacuum System	
Vapor pumping	Four-stage steam ejector system
Steam supply	100 psig steam
Steam required	
Conventional	3700 pounds/hour
Dual energy units	3500 pounds/hour
Microwave	5300 pounds/hour
Water required	
Conventional	625 gallons per minute
Dual energy units	600 gallons per minute
Microwave	1080 gallons per minute
Roughing pump	10 hp mechanical vacuum pump
Capacity	300 CFM
Vapor lines and valves	Typical of commercial freeze-drying system
Microwave Equipment	
Tube type	Magnetron 5 KW rating
Tube life	3000 hours at rated output
Electrical efficiency	50%
Tube cost	$140/KW
Power supply cost	
Installed microwave capacity	$200/KW
Dual energy units	37 KW/Chamber
Microwave	134 KW
Utilities	
Steam	$0.80 per 1000 pounds
Water	$0.02 per 1000 gallons
Electricity	$0.015 per KWH
Labor and Supervision	
Operating Labor	
Conventional	2 man-hours per cycle
Dual energy units	1 man-hour per cycle
Microwave	0.5 man-hour per cycle
Supervision	20 per cent of operating labor

TABLE 4—Continued

Quality Control	$2.50 per chamber cycle
Overhead	150 per cent of operating labor
Maintenance	
Conventional	4 per cent of installed equipment cost per year
Microwave	6 per cent of installed equipment cost per year
Taxes and Insurance	2 per cent of installed equipment cost per year
Depreciation	10 per cent of installed equipment cost per year

One inch thick beef steak was chosen as the product in this comparison for two reasons: first of all, some information was available for both microwave and conventional freeze-drying of this product, and secondly, the drying of thick materials presents a challenge to the conventional process while emphasizing a major advantage of the microwave process. The processing rate was based on the daily throughput possible with a single microwave unit of a size compatible with available microwave generating sources. Equipment requirements for the other processes were then scaled up to this production level. Actually a much shorter microwave process is possible, but because of the need for extremely high powered microwave sources and for the tremendous vapor handling capacity, it was felt that the comparison would be unrealistic at this time.

The dual energy process is based on reducing the moisture content to 10 per cent by conventional means, assuming that this can be accomplished in 10 hours, then applying radio-frequency energy in the same chamber to remove the remaining moisture in one hour. Such a unit could handle two loadings per day. A tube life of 3000 hours and a conversion efficiency of 50% are assumed. A 23-hour cycle is taken for the conventional process for the convenience of this comparison even though a slightly shorter cycle is conceivable.

The drying chamber dimensions are those of one manufacturer of such equipment. Normally the chamber contains 17 usable shelves. A framework with low loss plastic trays is substituted for the one inch thick hollow metal shelves when the equipment is used for the microwave process. Dimensionally it is possible to place 34 of these trays in the chamber thus in effect doubling the loading capacity. Therefore, one microwave unit with a six-hour processing cycle can process as much as eight conventional units in 24 hours. Loading and unloading is also considerably simplified since an entire rack can be rolled out of the freezer and into the chamber without the necessity of transferring the trays from the rack to the shelves of the dryer, an operation which consumes a substantially greater amount of time. Actually in all of these comparisons the time allotted for loading and unloading is more than ample since the use of steam ejectors in this instance eliminates the delays encountered in defrosting condensers.

The loading schedule for the conventional process requires the presence of operating labor to load and unload a chamber every three hours. The one hour allotted allows for shutting down, unloading, loading, starting up the equipment, and then seeing that the equipment is operating properly. The total labor requirement is 16 man-hours per day. For flexibility in the event of steam ejector failure, two sets of steam ejectors are included, each to handle four cabinets.

The schedule for the dual energy units, one unit to be loaded every six hours, requires a total of eight man-hours per day. It is felt that simplicity of the microwave unit requires only two man-hours per day.

All units are equipped with suitable alarm systems to alert operating personnel in the event of equipment failure. It is assumed that operating personnel are otherwise employed in the vicinity of the drying equipment.

TABLE 5

Comparison of Freeze-Drying Costs of Beef Steak Processed by Various Methods

Processing Rate: 11,016 pounds per day, 300 days per year

	Conventional	Dual Energy	Microwave
	(cents per pound of water removed)		
Utilities			
Steam:			
Vacuum	0.860	0.815	1.215
Heating	0.624	0.312	—
Water:			
Vacuum	0.214	0.209	0.375
Electric Power:			
R. F. Circuit	—	0.107	1.07
Roughing pump	0.0065	0.0065	0.0032
Hot water pump	0.104	0.104	—
Magnetron Usage	—	0.166	1.65
Labor			
Operating Labor	0.34	0.17	0.0425
Supervision	0.068	0.034	0.0085
Quality Control	0.242	0.242	0.121
Plant Overhead	0.51	0.255	0.064
Maintenance			
4% of investment	0.389	0.198	0.096
6% of investment	—	0.0717	0.065
Taxes and Insurance			
2% of investment	0.195	0.123	0.062
Depreciation			
10% of investment	0.975	0.616	0.309
5% of investment	0.4875	0.308	0.1545
	5.0150	3.7372	5.2357
Total Capital Investment	$241,591	$152,368	$76,655
Initial Tube Cost	—	$ 20,720	$18,760

The results of this economic study are shown in Table 5. The dual energy process is shown to be the least expensive process on the basis of operating costs. The microwave process, in spite of the low initial investment, is slightly more expensive than the conventional process. These operating costs take into account the fact that the magnetrons would need to be replaced about every six months in the microwave process, but only once in about five years in the dual energy process.

In some instances the microwave process might still be the process of choice, particularly if space were at a premium. A specific example might be the freeze-drying of fish aboard fishing vessels at sea. The dual energy process, while not demonstrating as much economy of space, would be expected to have some of the advantages of the microwave process, such as a low finish temperature. This feature would presumably have some relationship to product quality.

It can be said in summary that microwave energy should be seriously considered as a heat source for freeze-drying. Magnetron generators are available which can provide the power needed to handle current production rates. As a result of improved tube life and greater operating efficiency, the cost of microwave energy is not prohibitive. And finally, the dual energy process offers considerable promise as an economical freeze-drying method.

Acknowledgments

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The author wishes to express his appreciation to the following companies for their assistance in the economic analysis of freeze-drying:

F. J. Stokes Corporation
Croll-Reynolds Company
Raytheon Company
Amperex Electronic Corporation

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METHODS OF DETERMINING FREEZE-DRYING PROCESS END POINTS

B. KAN

Introduction

In batch freeze-drying processes as we know them today, it is generally found that in any given load of food being dried the various individual pieces that make up the load will dry at different rates. This differential drying may be due to pieces being of different thickness and size, to non-uniformity of heating in the equipment, to differences in the rate of freezing, or to variations in structure that may be expected in products such as meats. Uniformity of drying in a load is a desirable goal. On the other hand, since uniformity does not exist, it is necessary to have an end point indicator which can reliably inform us when all individual pieces in the load are dry. It is not necessary to dwell here upon the undesirability of stopping a freeze-drying cycle while ice-cores still remain in portions of the load. At present it is difficult to distinguish between the dry and the partially dry material. It is therefore common practice to apply a safety factor—to dry for a period beyond that considered "probably sufficient." In plant operations where cycles are running somewhere under 24 hours, it is usually convenient to allow a safety factor of a couple of hours. In a case where drying takes 21 hours, the addition of two hours of safety factor would not affect the productivity of the unit if it were being run with a single shift. Were drying times much lower or if multiple units were being run in staggered cycles, this safety factor becomes increasingly expensive and a reliable end point indicator correspondingly more desirable.

Mass Transfer

The first procedures that come to mind are those that directly measure the mass transfer of water, by weighing the condensed ice or measuring product weight loss. Aside from the mechanical problems involved in making these measurements and the need to know the initial weight of water in the product, it is felt that these methods are basically impractical because in the time period of greatest interest, namely, the end of the cycle, the drying rate is at its lowest and small differences in weight correspond to large segments of the drying cycle.

Thermocouple Measurements

At present it is a common practice to measure the internal temperature of selected pieces within the load using imbedded thermocouples and to use this measurement as an end point indicator. Towards the end of a drying cycle, the evaporative cooling because of sublimation of the ice is reduced and the internal temperature of the product rises. The process end point is taken to be that time when product temperature reaches some predetermined point.

Thermocouple measurements under these conditions are subject to error for a number of reasons. The manner in which the couple is imbedded will influence the reading. The exact location of the couple in the piece of food will also influence the reading. It is not only likely that heat transfer down the thermocouple will affect the temperature in the immediate vicinity of the junction, but it may even increase drying rate of the test piece. Aside from inaccuracies, the method suffers from a sampling problem. There is no assurance that the test pieces are representative of the slowest drying pieces in the load.

Vapor Pressure Rise

One procedure we have found to be especially valuable in laboratory work has been the measurement we term Vapor Pressure Rise (VPR). This method involves closing a valve between the drying chamber and the vapor handling system and measuring the rise in chamber pressure on an Alphatron® vacuum gauge. In our work we have standardized on measuring the VPR over a period of ten seconds. We have done enough work to realize there is much we do not understand about the observed data, but we do feel confident that when the VPR falls to a low value after a period of drying it indicates that it is time to stop—either the product is dry or it has case hardened or it is otherwise impermeable. We encountered the last phenomenon when we tried to freeze-dry a cranberry gel.

The actual values of VPR obtained during any given run will depend upon the nature and amount of product being dried, the volume of the chamber, in-leakage, and intensity of heating. Fig. 1 depicts the freeze-drying equipment used in our experiments. In one of our typical runs with one pound of beef sticks (½″ x ½″ x 1″) drying on a stainless steel mesh and heated by radiation from a cylindrical wall, the 10-second VPR readings might start at 50 microns, rise to a broad peak at 500 microns after an hour of drying, and steadily decrease thereafter to a value under 10 microns. In general we have found that a 10-second VPR of 10 microns in our equipment is a reliable indication that a product is dry.

The sensitivity of the VPR method is so high that it is possible to follow the drying of a single beef stick in a chamber whose volume is about sixty liters. In this experiment the VPR values ran 80, 55, 15, and 3 microns over a period of two hours and twenty minutes. These results are not surprising if one uses the gas laws to calculate that the 80 micron VPR represents the evolution of 5 milligrams of water vapor in 10 seconds.

It is obvious that leakage of air into the drying chamber will result in errors in the VPR value. If there is chronic leakage it is undesirable in itself and should be eliminated. An acceptable leakage rate for a 7′ x 7′ x 4′ chamber would be 5-10 microns/hours. Smaller chambers would have higher values. The most disturbing leakage would be sporadic leakage, but even in this case the system will "fail-safe"

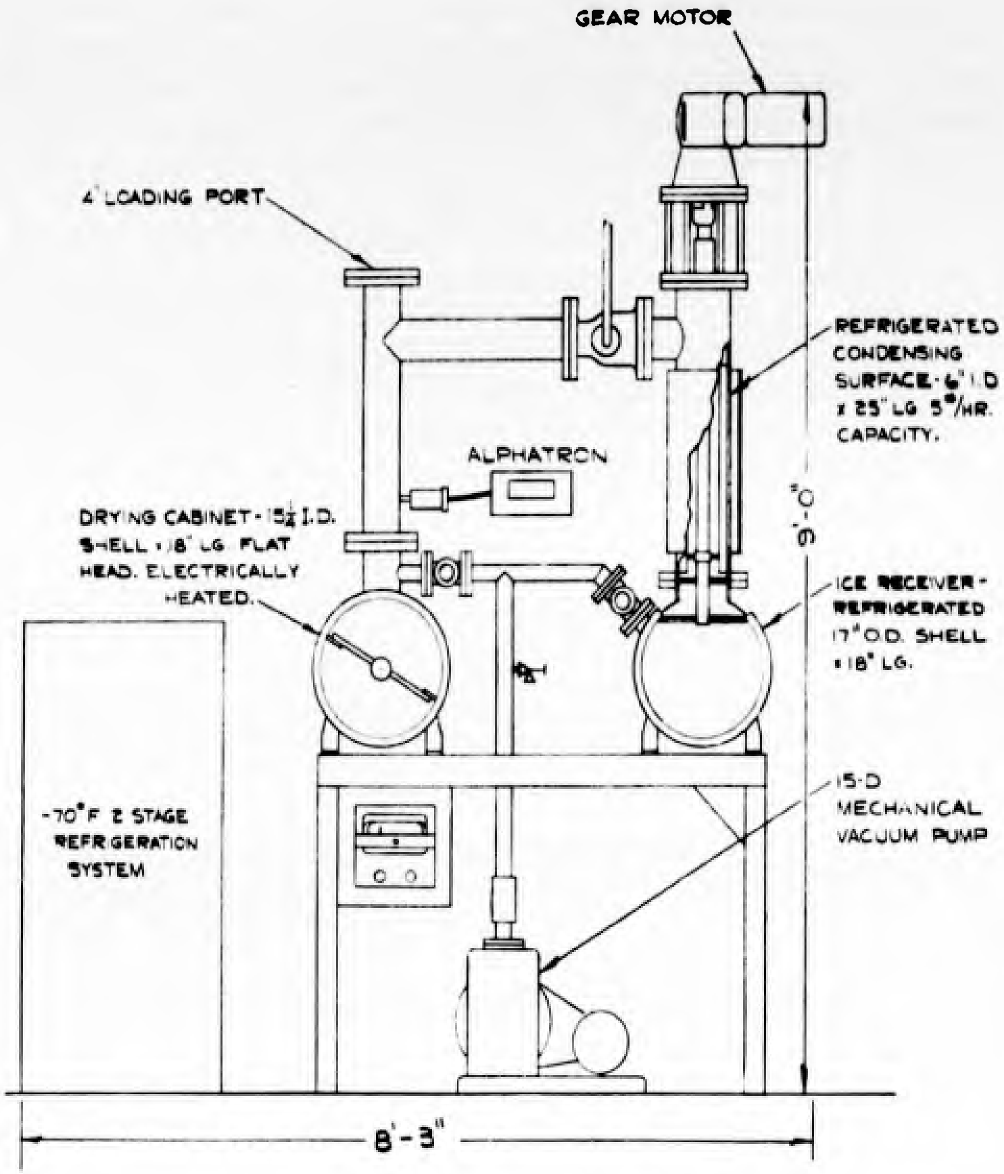


FIGURE 1. Outline of freeze-drying pilot plant. (NRC Equipment Corporation)

inasmuch as the main effect of the leakage will be to indicate an extra long drying cycle. A simple test for leakage would consist of isolating the mechanical pump from the chamber and condenser. A significant rise in pressure under these conditions would indicate leakage since the condenser would still be "pumping" all the evolved water vapor.

Vapor Sample Condensing

Another system of end point determination is designated as Vapor Sample Condensing or VSC. The procedure is best explained by referring to Fig. 2. The first schematic shows the Alphatron vacuum gauge measuring the chamber pressure. The second schematic shows the intermediate step: the trap and Alphatron are isolated from the chamber, taking what we term a vapor sample. In the last schematic a liquid nitrogen bath cools the trap causing a drop in the pressure of the isolated system. This drop in pressure is caused by contraction of noncondensable gases and by condensation of water or other condensables. The pressure drop caused by condensation is far more sharp than that caused by contraction so that presence of a large fraction of water vapor in the chamber atmosphere is readily detectable. Table 1 shows the results from a typical beef drying experiment in which the chamber pressure was controlled at about a millimeter by air bleed. One can observe that throughout the major part of the run the "cooled sample pressure" dropped into the ten micron range. The last

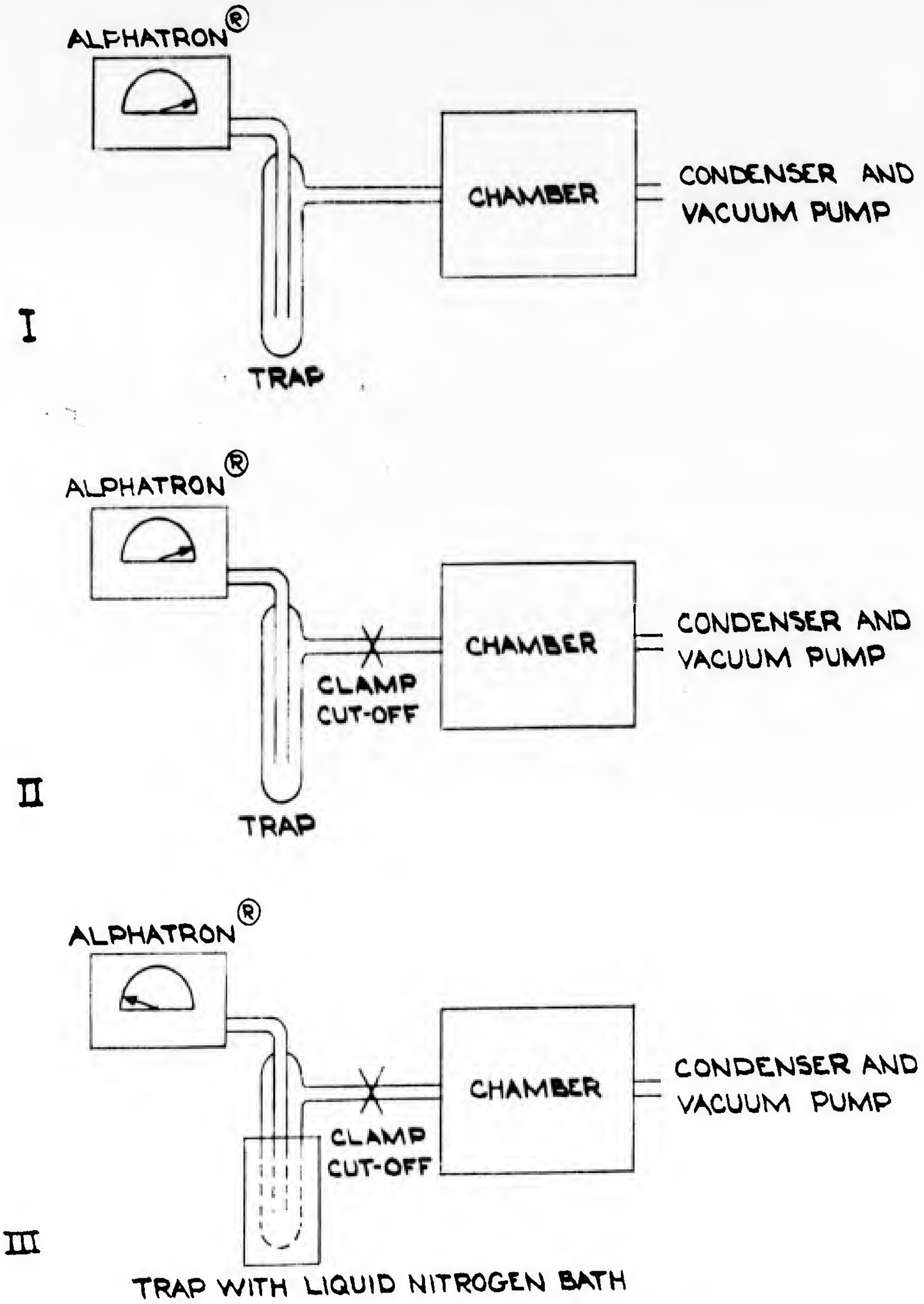


FIGURE 2. Vapor sample condensing system.

two readings, however, were progressively and significantly higher, which can be taken to indicate a decreasing partial pressure of water vapor because of decreased vapor evolution from the charge.

The VSC system was developed in runs where total pressure was controlled by air bleed. The system was found not to function when total pressure was raised by throttling the vapor line, for in this case there is no way to raise the partial pressure of non-condensables in the chamber and the VSC value does not change at the end of the drying cycle.

In commercial application this system would have advantages over the previous system in that its reading is less sensitive to chamber leakage and does not require the closing of valves. An air bleed, while undesirable, may not be too serious a drawback particularly if the VSC system is applied only towards the end of the drying cycle.

TABLE 1

Vapor Sample Condensing System

1000 microns operating pressure

Time (minutes)	Operating Pressure (microns)	Cooled Sample Pressure (microns)	10-Second VPR (microns)
30	900	10	200
60	900	10	500
90	1000	12	400
120	1200	12	600
150	1200	20	500
180	1100	10	500
210	1000	10	200
240	950	11	100
300	1000	95	25
330	1000	240	0

100 to 14 microns operating pressure

Time (minutes)	Operating Pressure (microns)	Cooled Sample Pressure (microns)	10-Second VPR (microns)
30	100	8	300
60	64	5	300
90	46	3.8	360
120	36	3.2	340
150	28	3.6	360
180	22	3.6	260
210	18	2.8	100
240	15	6	20
270	14	4.4	6
300	14	5.1	4

Farvitron as End Point Detector

The Farvitron is manufactured by E. Leybold's Nachfolger (Fig. 3). It is a radio-frequency mass spectrometer with rapid response. It measures masses from 2 to 200 and has an oscillographic display read-out. Its operating range is from 10^{-4} to 10^{-8} mm of mercury, which is below that of the freeze-dryer. It was therefore necessary to assemble the equipment shown in Fig. 4. The oil diffusion pump holds the pressure in the test dome to the 10^{-5} to 10^{-6} mm of mercury range even when the chamber is connected to the system. The Farvi-

FIGURE 3. Leybold-Farvitron.

tron head is thereby exposed to gas molecules flowing from the drying chamber, but at a low enough pressure so that it can be used. This set-up may be thought of as a "pressure transformer." The results are shown in Figs. 5 and 5A and Table 2. Initially a high peak at mass 28 was observed. This was attributed to nitrogen. As the dehydration began, the peak at mass 18 increased, presumably indicating water vapor. Because of the nature of the instrument, the peak at mass 28

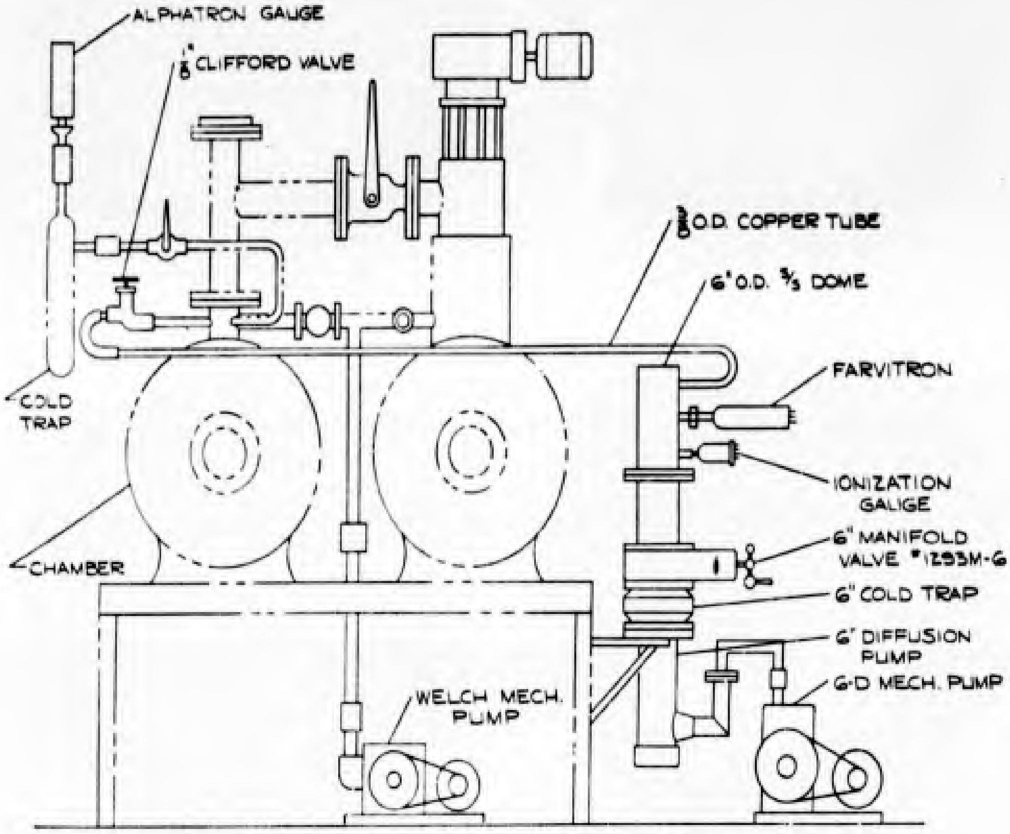


FIGURE 4. Equipment set-up for Farvitron tests.

TABLE 2

Frame	Time hours	Chamber Pressure (microns)	Dome Pressure ($\times 10^6$)	VPR (microns)
1	0	—	2.4	—
2	1	64	5.6	220
3	2	36	3.8	220
4	3	28	3.2	240
5	4	20	2.5	200
6	5	14	2.2	55
7	5½	10	2.0	12
8	6	12	2.0	4
9	6½	26	2.6	80*
10	6¾	52	3.8	4

* Chamber wall raised to 400°F.

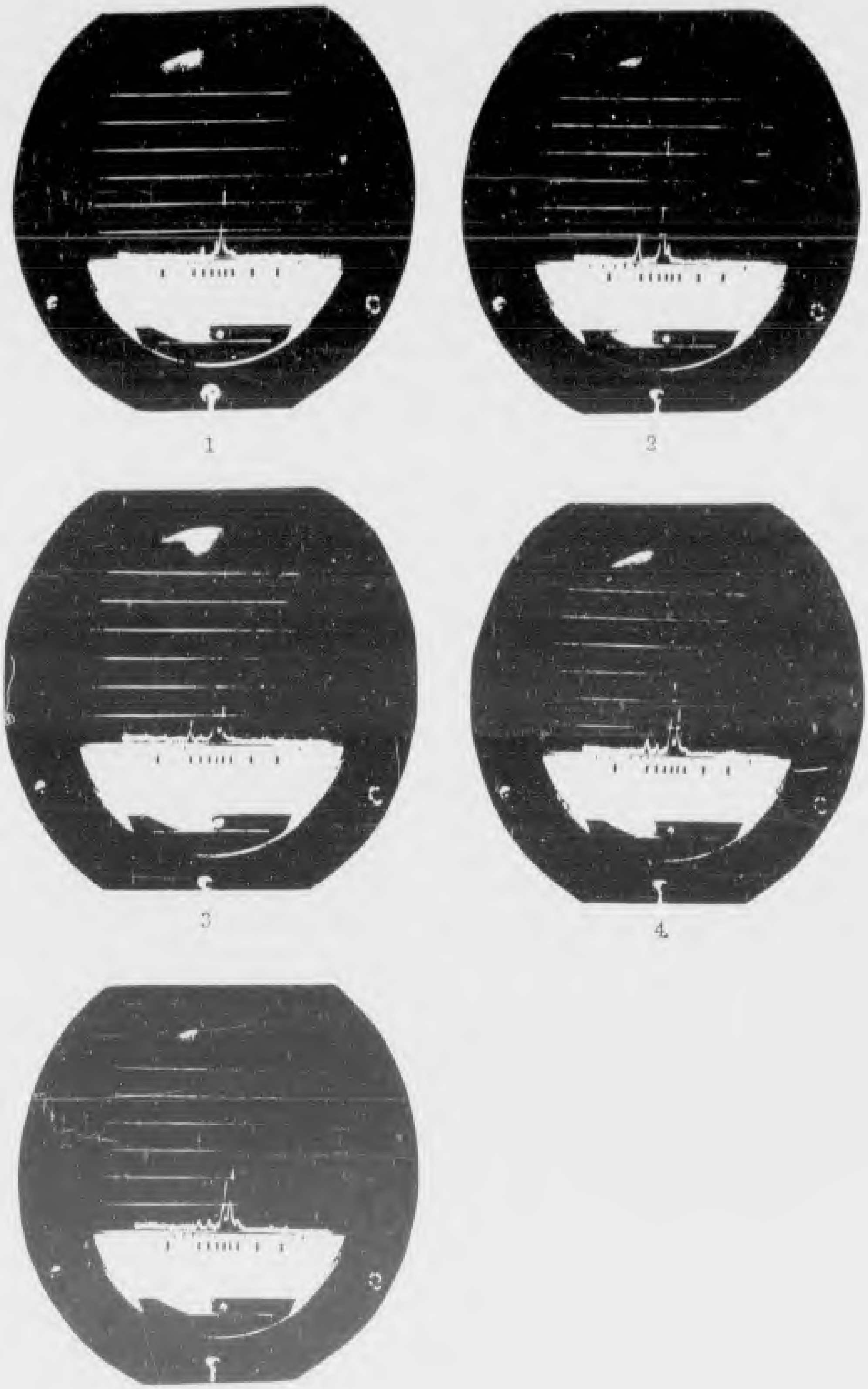
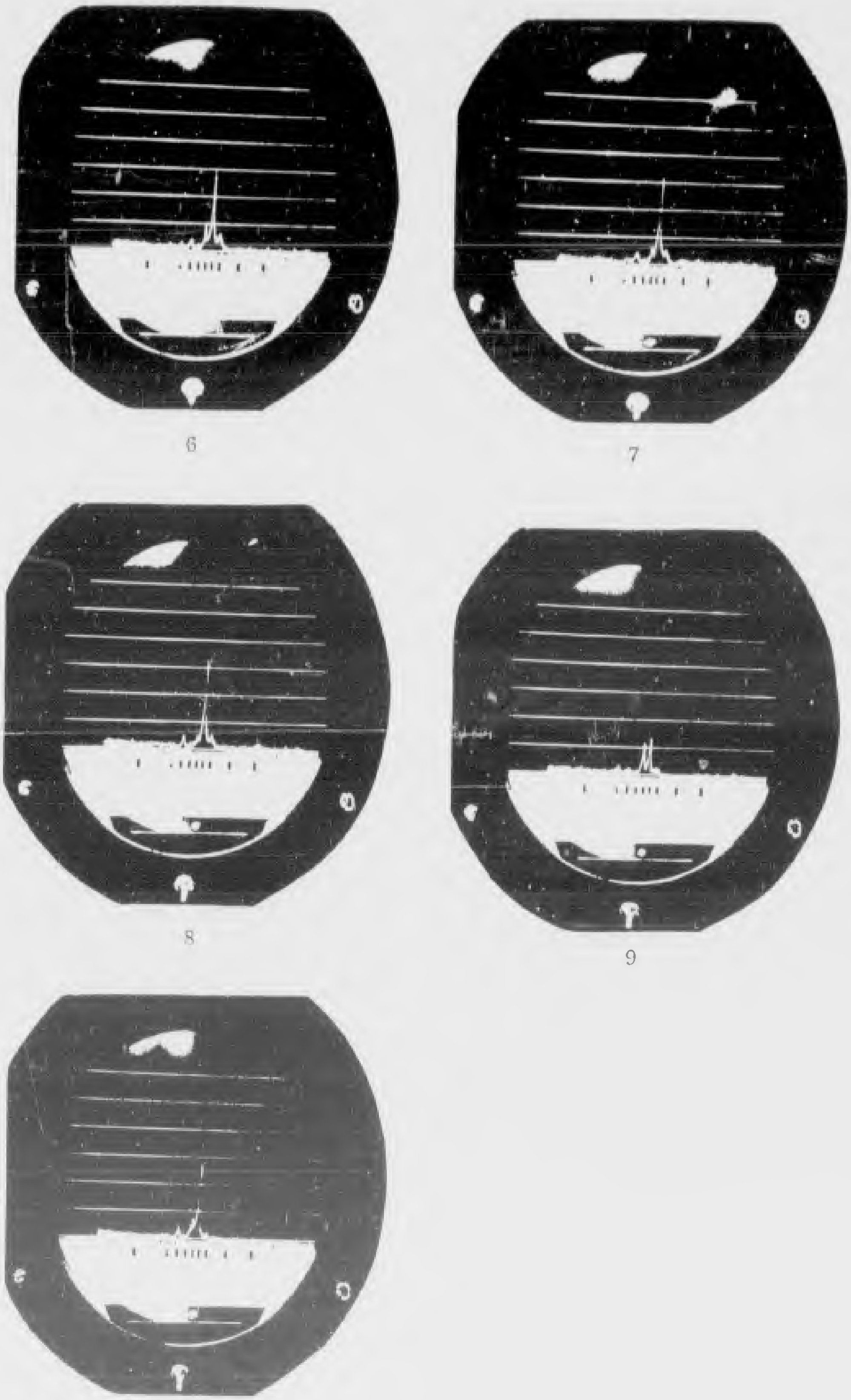

1
2
3
4
5

FIGURE 5. Farvitron read-out.

was depressed. As the process proceeded to its end, the mass 18 peak once again decreased and the mass 28 peak correspondingly increased back to its original value. At the end of the run, when the VPR read-



6 7 8 9 10

FIGURE 5a. Farvitron read-out.

ing indicated adequate dryness, the wall temperature was raised to 400°F in an attempt to obtain other peaks due to possible breakdown products. No such peaks appeared; however, the water peak again rose together with the VPR. After 15 minutes at 400°F, the water peak again decreased and the VPR also dropped to 4 microns. Presumably more tightly bound water was driven out by the drastic heating at 400°F. Two other runs were made with similar results. Thus, it is apparent that the Farvitron can be used to follow the progress of a drying cycle although it cannot be used to detect breakdown products of the beef. If the appropriate end point relationship between H_2O and N_2 peaks is established, the Farvitron can be used as an end point indicator. Frame 7 in Fig. 5A may be taken as the end point in the example presented.

The Farvitron method of end point determination is related to the VSC system in that a relationship between water vapor partial pressure and nitrogen partial pressure is used to indicate the end of the drying cycle. The difference between the methods is that whereas the VSC requires in-leakage sufficient to bring the chamber pressure up to about one millimeter, the Farvitron registers a nitrogen peak that is due to in-leakage at a much lower pressure. The Farvitron method therefore does not require the opening of a deliberate leak in the system, but depends on the sort of leakage that is actually hard to prevent.

Infrared Detection of Product Surface Temperature

In earlier work on freeze-drying of beef sticks, it appeared that when operating at total pressures less than one millimeter of mercury it was possible to scorch the meat without causing melting of the ice. High vapor pressure rises indicative of thawing were not observed, and the product was in all cases found to have dried without the typical hardening that occurs when beef is dried from the thawed state. It was concluded that product surface temperature was the limiting factor and that it should be sensed and controlled. In addition it was felt that a method of measuring product temperature that was more accurate than thermocouples would be of value as an end point detection method when used instead of thermocouples. There were some hopes that a scanning system could be developed so that temperatures in various places inside a dryer could be read. Attainment of this goal seems somewhat remote at present.

The Barnes Engineering Company, Stamford, Connecticut, manufactures infrared sensing devices that should be suitable for reading product surface temperatures. Their model R-4D1 Industrial Radiometer (Fig. 6) was obtained so that the feasibility of this measurement could be established. Briefly, this equipment consists of two units, a sensing head and an amplifier-power supply (Fig. 7). The sensing head contains an optical system of mirrors that focuses target radiation on the radiation detector—a thermistor bolometer. In front of the detector there is a chopper that opens and closes the sensing aperture at a 150 cycle per second rate. The chopper causes the detector alternately to "see" the incoming radiation and a reference

FIGURE 6. R-4D1 Industrial Radiometer.

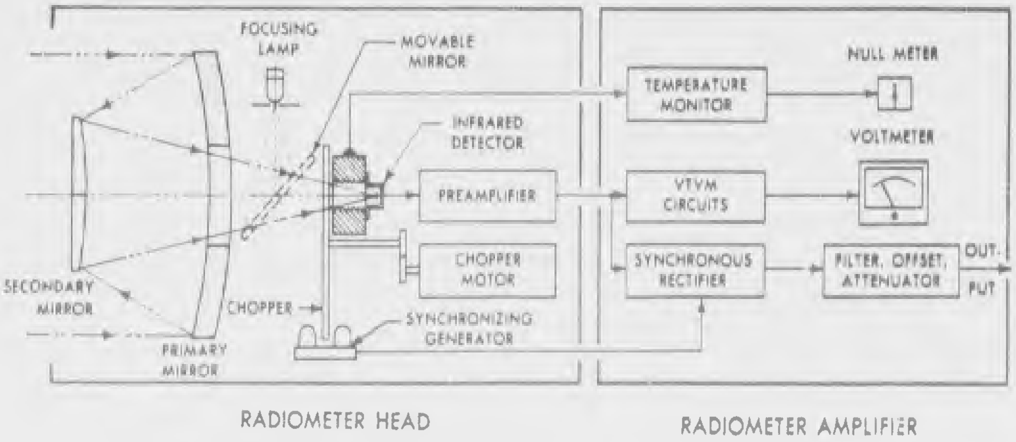


FIGURE 7. Generalized radiometer block diagram.

source near the detector. The result is an alternating signal whose peak to peak voltage is proportional to the difference between target and reference source radiation. The amplifier contains a vacuum tube voltmeter, a synchronous rectifier, and a temperature monitoring circuit for determining the temperature of the reference black body.

The radiometer head is pointed at the target and is focused either by projecting a focusing light spot or adjusting the distance scale and sighting the target through the periscopic sight. In the experiments reported here, readings were taken on the vacuum tube voltmeter which indicates the output of the radiometer amplifier.

The first experiments were an attempt to follow the surface temperature of beef as it was being freeze-dried. The radiometer was focused through a germanium sight port onto a piece of beef inside the drying chamber. The beef also had a thermocouple imbedded in its surface. It was found that a qualitative relationship between radiometer reading and thermocouple reading could be established. In other words, the radiometer did indicate the initial temperature, the decrease in temperature as the chamber was evacuated, and the subsequent steady temperature rise. Two difficulties were encountered: 1) the germanium window attenuates the signal, and this attenuation was apparently different at the beginning and end of the run; and 2) the equipment has a noise zone around ambient temperature wherein no meaningful readings can be obtained, and the noise zone is effectively enlarged by the attenuation due to the germanium window.

In the second group of experiments, various freeze-dried foods were fitted with thermocouples on their surfaces and placed in the chamber under atmospheric pressure. The germanium window was removed and the surface temperature of the food was read directly through the aperture. The temperature of the food was raised by the heated chamber walls. The readings obtained are shown in Figs. 8, 9, and 10. It is apparent that the emissivity of the various foods tested must be quite similar and all of them seem to be high which is favorable. It is not possible to determine which values are absolutely correct since the radiometer is considered the unknown, and the thermocouples themselves are subject to error.

These experiments have led to several conclusions as follows:

1. Infrared sensing can be used to measure the surface temperature of freeze-drying food, but is probably most useful when applied to continuous belt drying where product movement makes thermocouples hard to apply. Should the absolute accuracy of the radiometer be established, it would also have advantages over thermocouples since the latter are subject to various errors.
2. The infrared sensing element should be inside the vacuum chamber to avoid the attenuation caused by windows.
3. The equipment to do this job must be especially designed to operate in a vacuum. The equipment used in these experiments was not suitable for use in a vacuum and also included features not required for freeze-drying applications.

Summary

In summary, we have presented four methods that may be applicable to the determination of freeze-dehydration process end points. They were not presented as procedures that have been proved in the field, but as excursions in directions that we feel may prove to be fruitful.

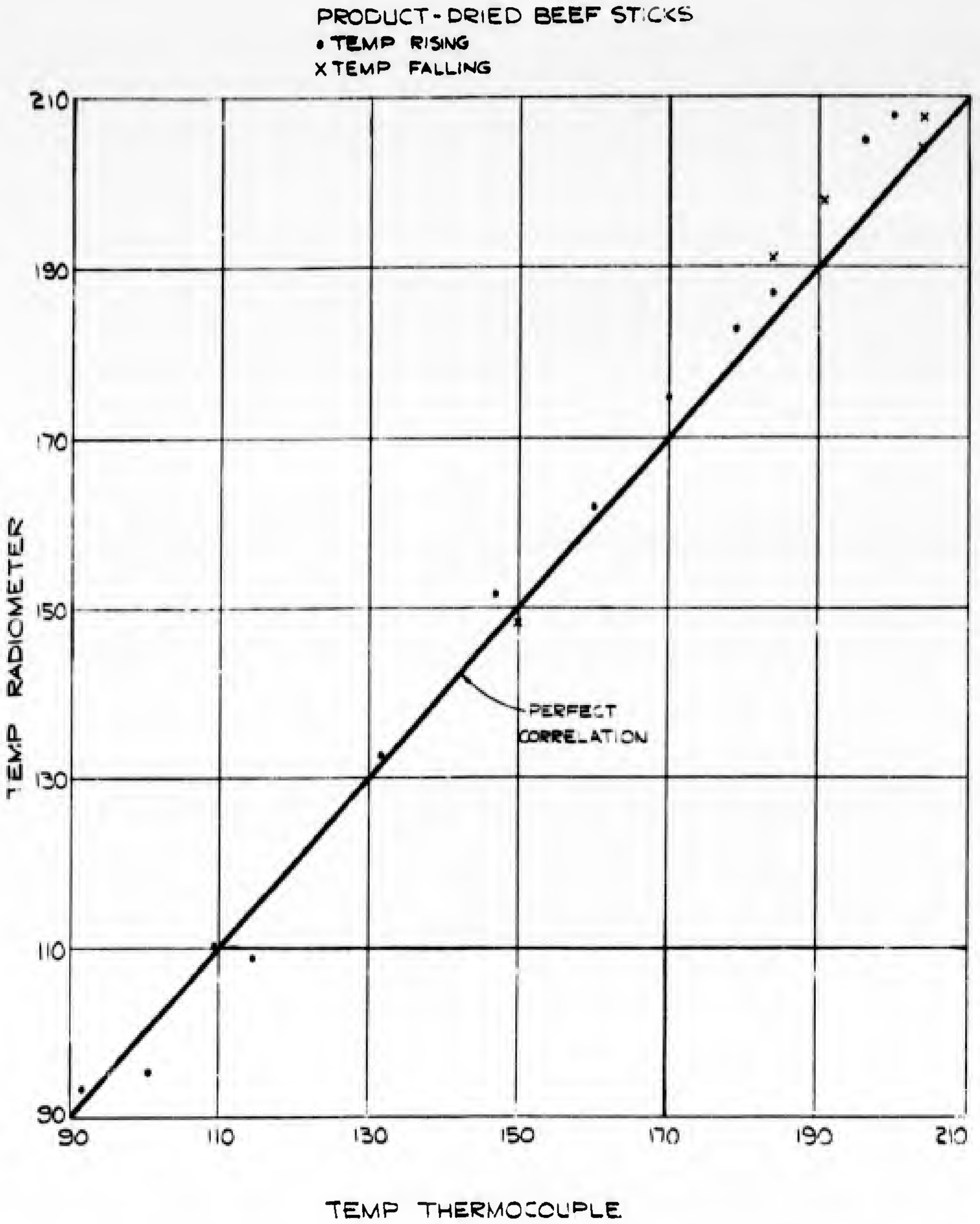


FIGURE 8. Correlation: radiometer vs. thermocouple temperature readings.

The first of these methods, we feel, is ready for use in laboratory investigations. The VPR measurement can be a real boon to people who must process food samples of many sorts that can be expected to dry at different rates.

In any event, we feel a first step has been taken towards the worthwhile goal of improved control and instrumentation of freeze-dehydration processes that, in the long run, will lead to more favorable economics and products of consistently superior quality.

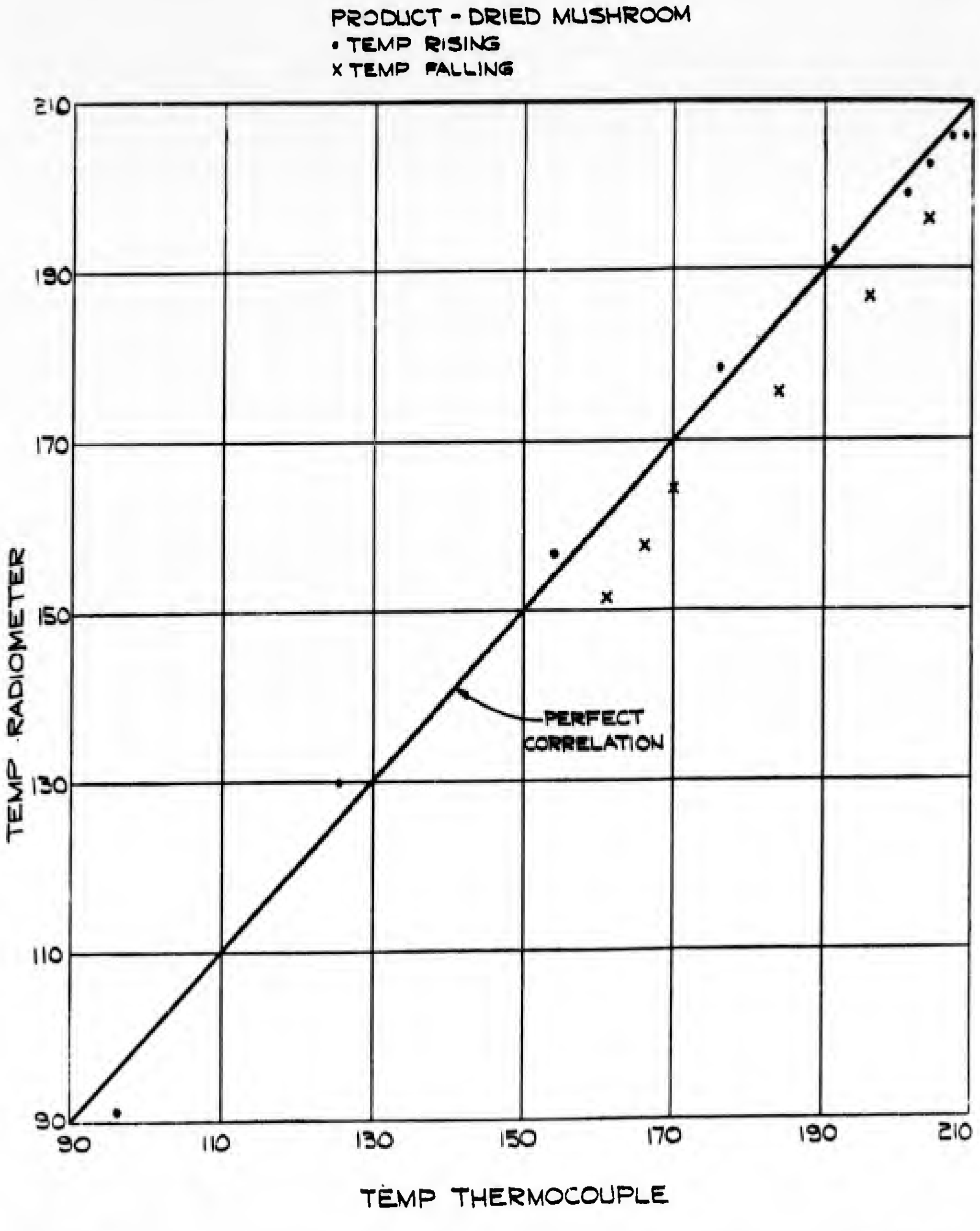


FIGURE 9. Correlation: radiometer vs. thermocouple temperature readings.

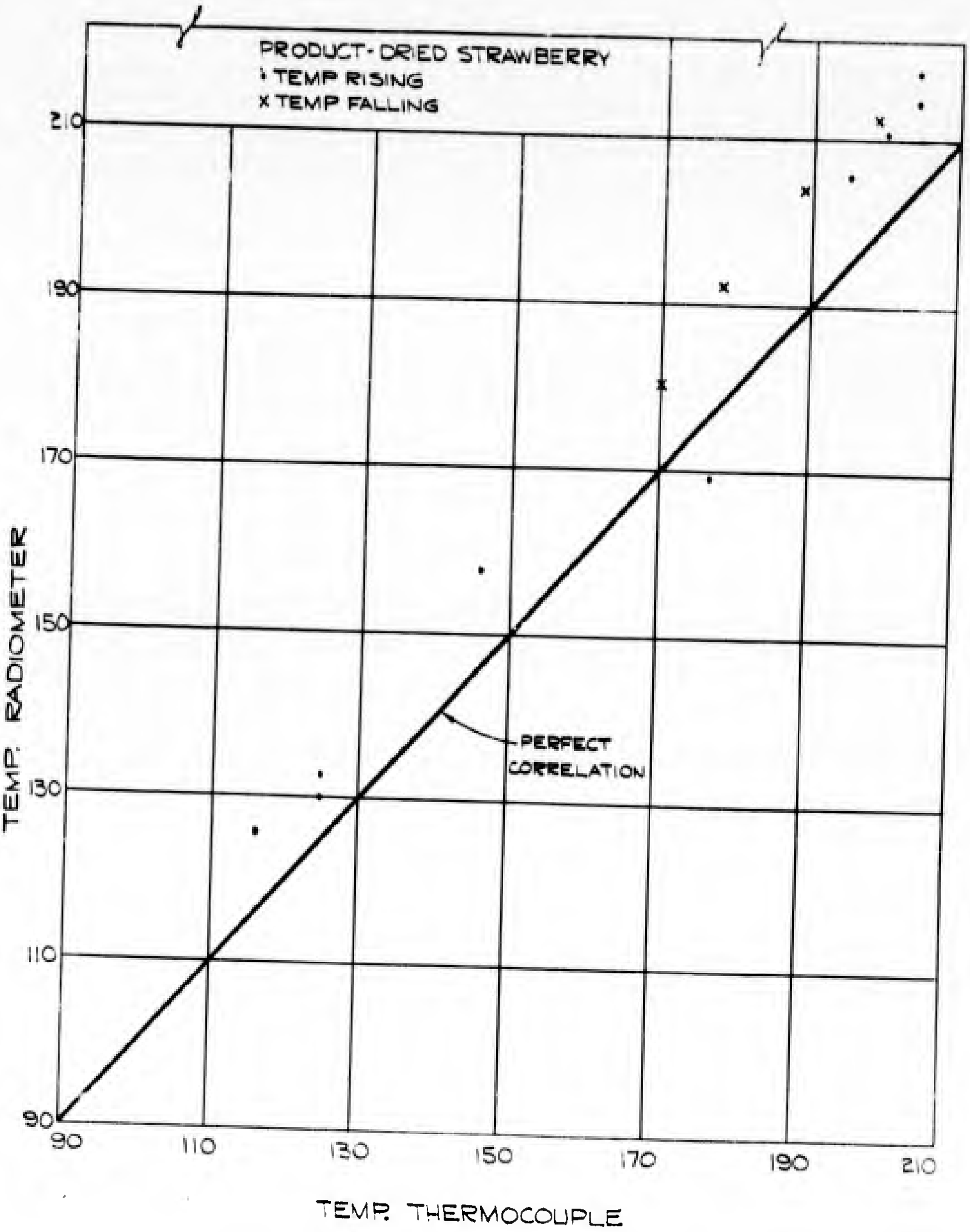


FIGURE 10. Correlation: radiometer vs. thermocouple temperature readings.

TEMPERATURE-MEASUREMENT AND CONTROL OF FREEZE-DRYING PROCESSES

G. W. OETJEN, H. EHLERS, U. HACKENBERG,

J. MOLL, AND K. H. NEUMANN

Considering the various factors which influence the quality of a freeze-dried product and the economy of the process, one has to deal with groups of information which seem to be incomparable and difficult to separate into significant and less significant ones. Data of shape, size, and behavior of the food material itself are intensively mixed with technological information such as heat and mass transfer, temperatures, and total or partial pressure. In an attempt to define and measure only one of these factors, i.e., the temperature of the ice surface during the freeze-drying operation, a simple and reliable technique has been developed which is called "barometric" temperature measurement. By this method the temperature of the ice surface is measured by its corresponding equilibrium water vapor pressure. If the shut-off valve in Fig. 1 is rapidly closed, the pressure in the chamber rises within a few seconds to its equilibrium value, called "evp." At the operation pressure of a freeze-dryer, the amount of ice to be evaporated to obtain this evp is very small, i.e., one gram of ice per m^3 chamber volume as an order of magnitude. (Assuming that such a chamber is filled with 200 kg of material even in a nearly dry state, 10% moisture content, less than 1 per mill of the residual ice would be necessary for this measurement). Furthermore, if the measured ice temperature is used to control the heat input of a quickly reacting heating system, the temperature of the ice can be kept constant within ±1° or 2°C. A vacuum steam system, operable at any desired temperature between cooling water and 100°C, used as a pressure system up to 140°C has been chosen and has proved to be adequate for this purpose. The temperature measurement in the installation schematically shown in Fig. 1 is given in Fig. 2, demonstrating the very constant level of temperature. Fig. 3 shows the photograph of this control-system built into an industrial unit.

Besides the predetermined and constant temperature level, it was also observed that the rate of evaporation of ice per unit of time and the surface area were considerably increased by this method. The results were reported at the freeze-drying meeting in September last year in Chicago. At that time many questions were raised about these phenomena. By publishing the results of measurements carried out in recent years, we hope to contribute to a theoretical understanding of the complete process.

Fig. 4 shows the schematic drawing of a laboratory unit for measuring heat transfer. The measuring system is placed in a vacuum chamber which is then pumped down to 10^{-5} mm Hg. Then the gas or vapor to be studied is admitted at the desired pressure; a small

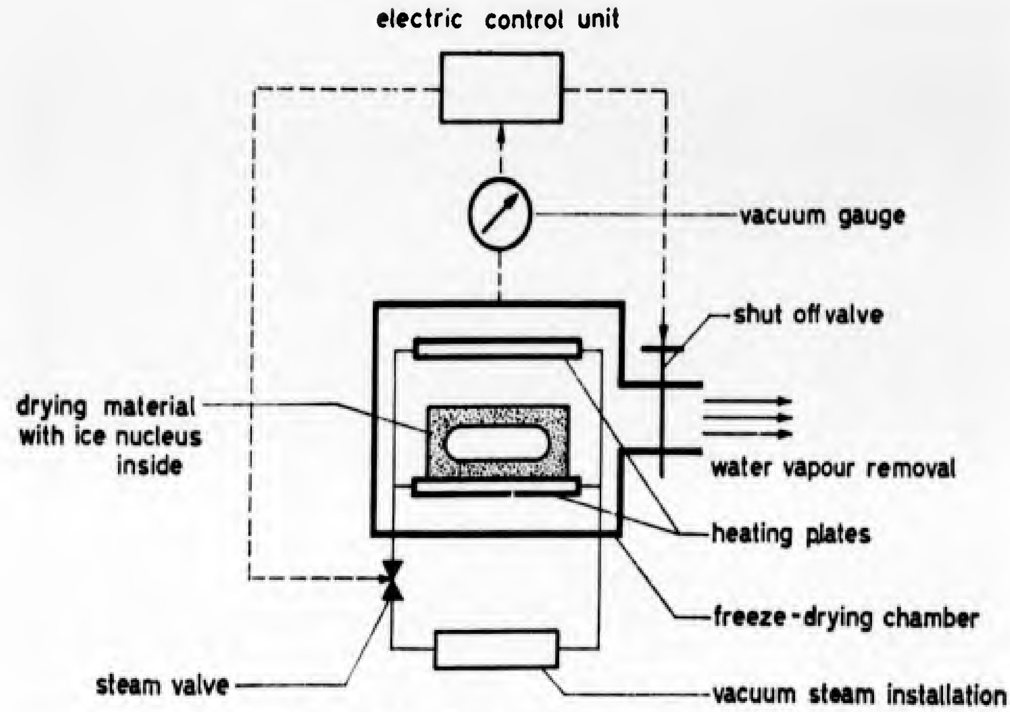


FIGURE 1. Principle of automatic temperature control.

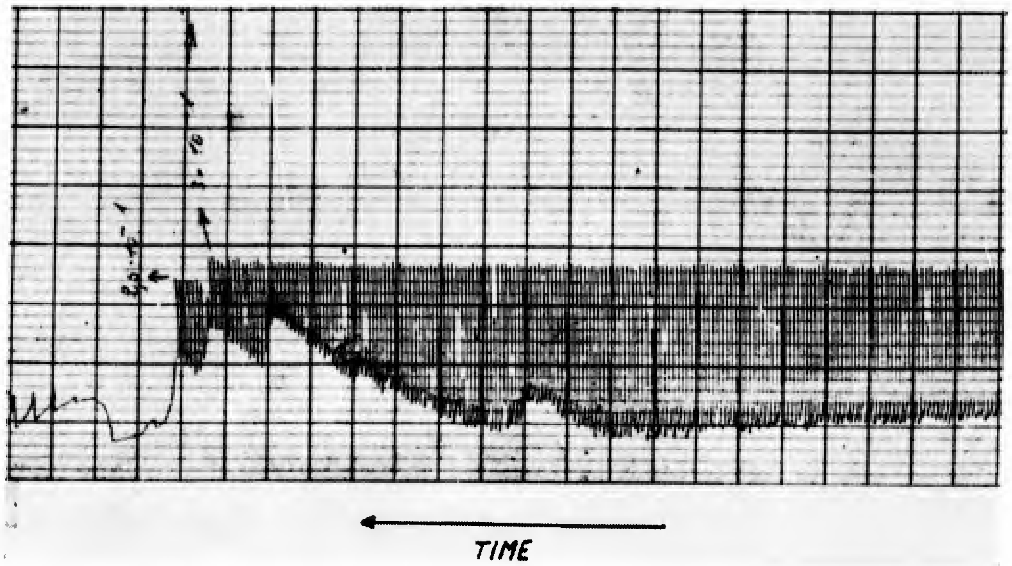


FIGURE 2. Pressure record of automatically controlled freeze-drying process.

flow of the gas is maintained to insure a continual negligible air pressure (below 3-4 x 10^{-4} mm Hg). The lower plate and the ring plate around the measuring plate are kept at two different constant temperatures, i.e., 20° and 30°C respectively. The measuring plate is electrically heated and automatically controlled to keep its temperature close to that of the surrounding plate. The necessary energy to maintain the desired temperature of this inner plate is recorded. At equilibrium conditions, this energy is the heat transferred from plate 1 to plate 2. Photographs of the installation are shown in Figs. 5 and

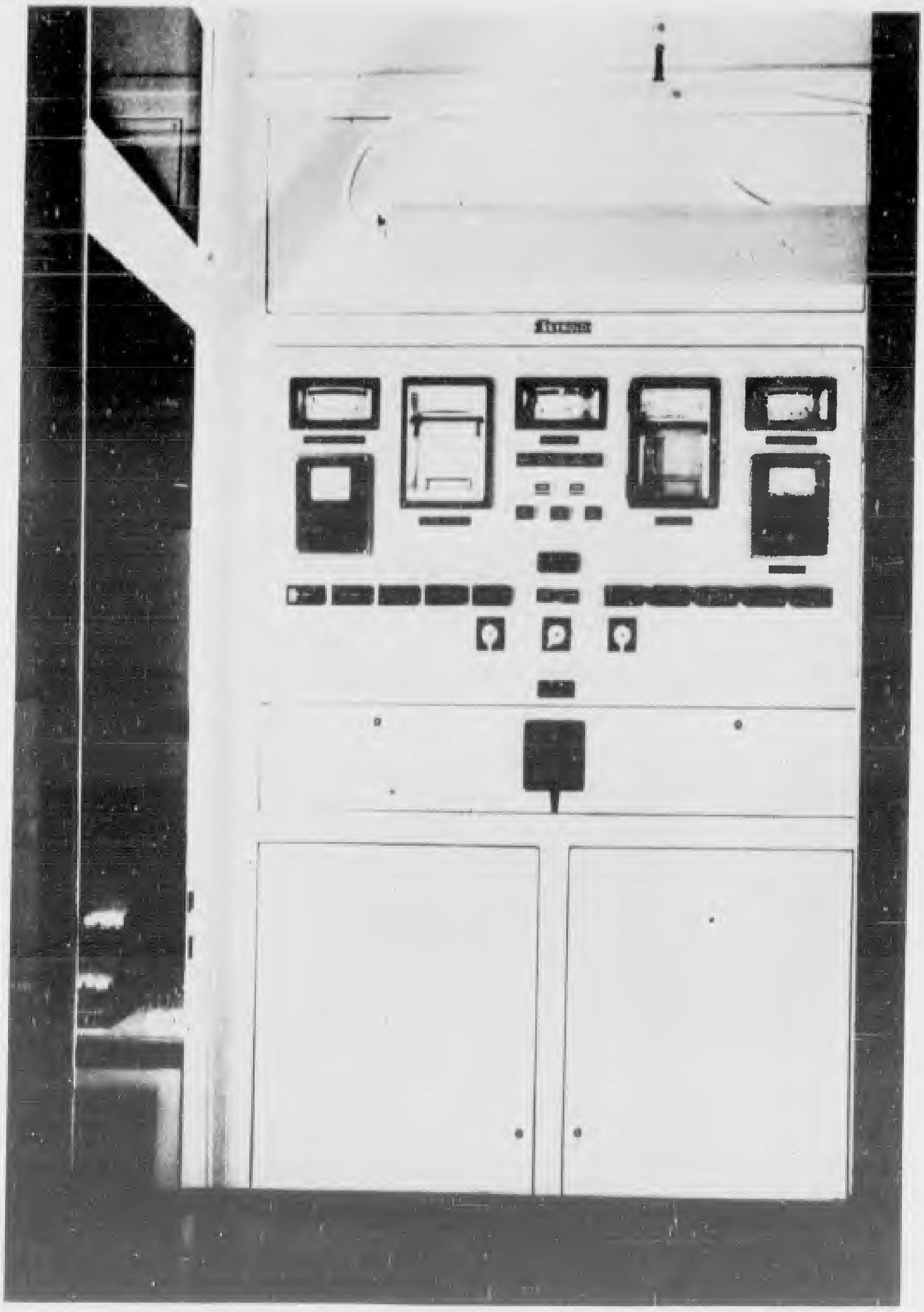


FIGURE 3. Control panel of automatic freeze-drying plant.

6. Fig. 7 shows the heat transfer of water vapor measured in this apparatus to prove that the known physical behavior of the mentioned gases is reproduced in this installation. Fig. 8 shows the results of heat transfer measurements when one layer of fish or cauliflower is placed between the plates. In both cases the distance between the upper plate and the material was reduced from 11 mm to zero. Then heat transfer was also measured with a certain pressure between the

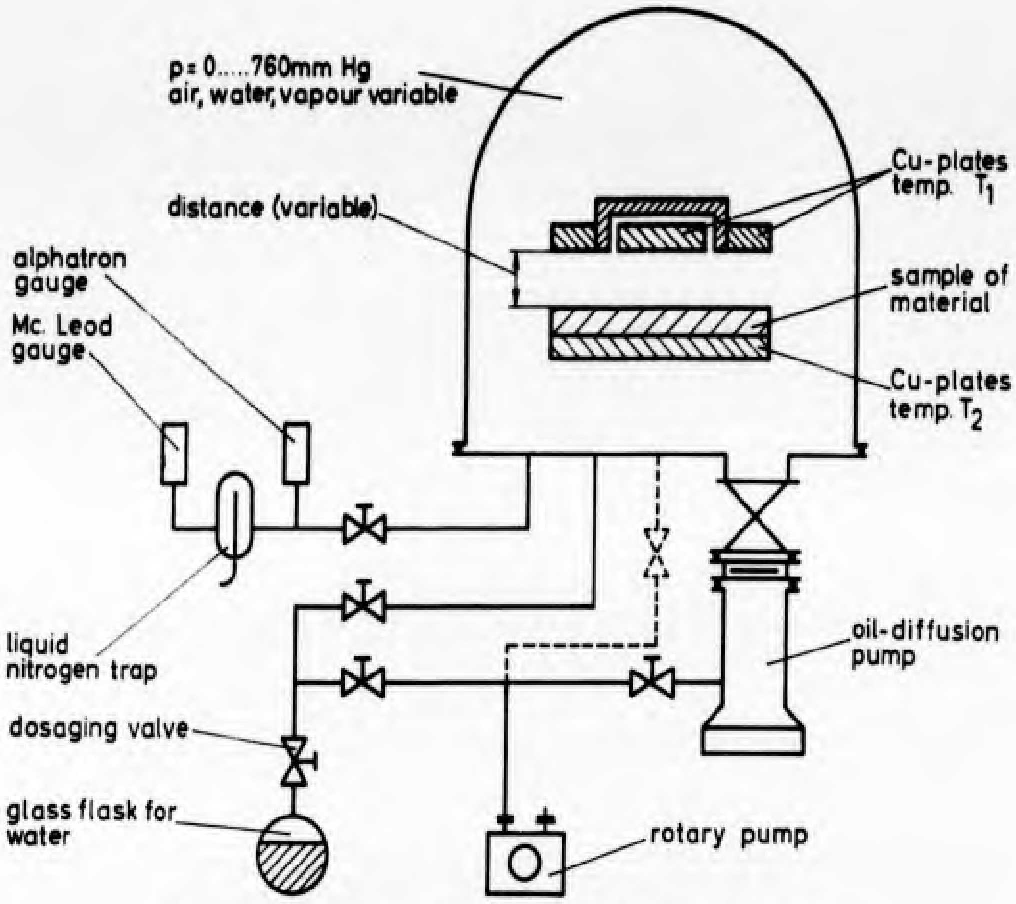


FIGURE 4. Apparatus for measuring heat conductivity.

plates. The results are very straightforward: Heat transfer increases as the space decreases between the material and the plate, but no further increase can be measured when additional pressure is placed on the material by pressing the plate (without a deformation and therefore a smaller thickness of the material itself). The actual measurements with fish were carried out with steaks of 19 mm thickness. The cauliflower was cut in small pieces of approximately 15 mm length. A layer of 11 mm thickness was formed for the measurements with cauliflower. The results of Fig. 8 demonstrate that the amount of energy transferred under corresponding conditions is approximately 1.65 units higher for fish than for cauliflower. Furthermore, the curves show how much the heat transfer is increased by raising the operation water vapor pressure and what can be gained if the pressure is voluntarily increased from time to time (during the barometric temperature measurement). The curves for the total heat transferred can be fairly well approximated by the equation:

$$Q_{\text{total}} = \frac{Q_s + \frac{D_w}{d_w} \times F \times \Delta T}{1 + \frac{D_w}{d_w} + \frac{Q_s}{F \times \Delta T} \times \frac{d_f}{D_f}}$$

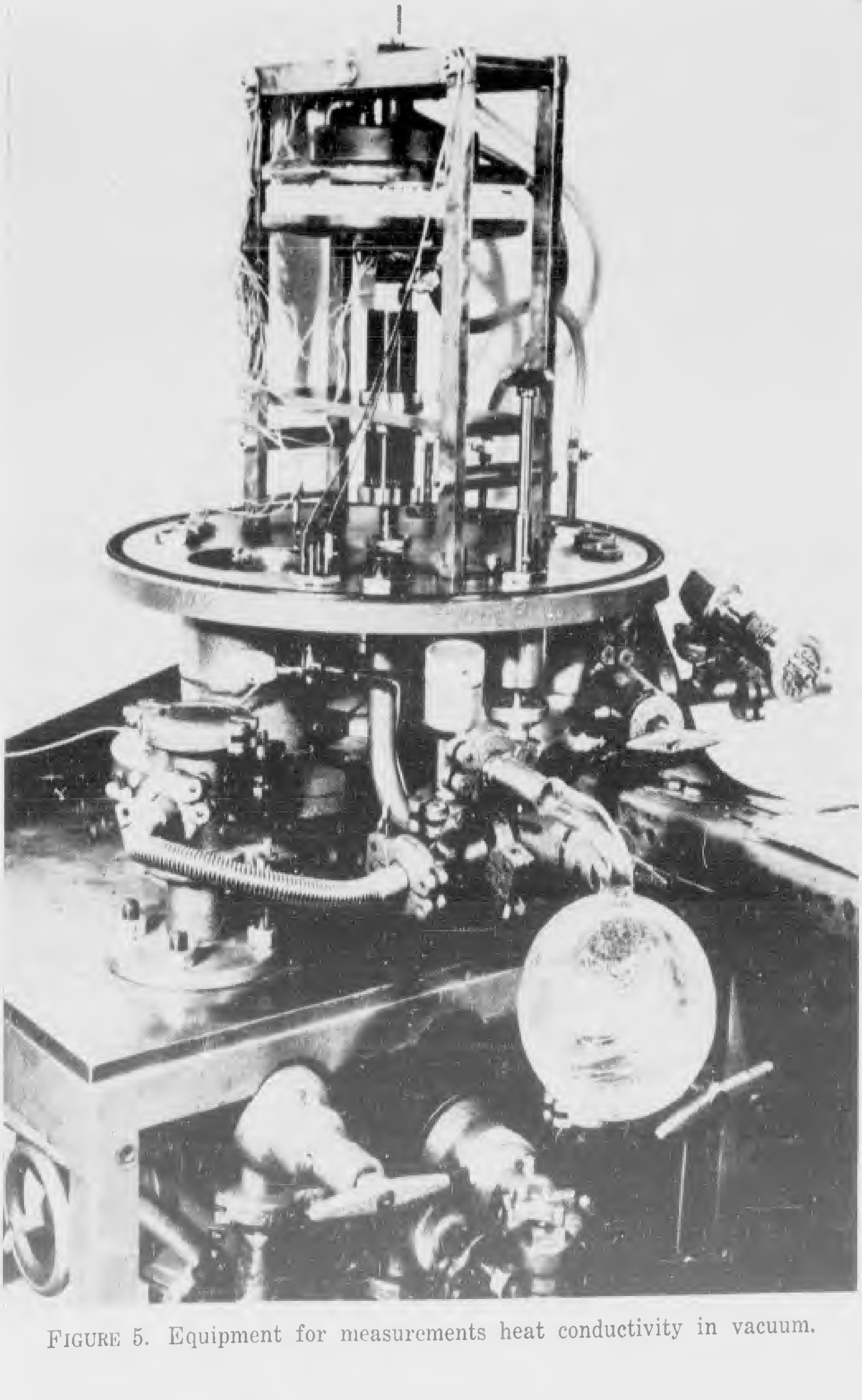


FIGURE 5. Equipment for measurements heat conductivity in vacuum.

Q_s = energy, transferred by radiation.

D_w = heat transfer coefficient of water vapor.

d_w = distance between heating plate and fish.

F = area of fish.

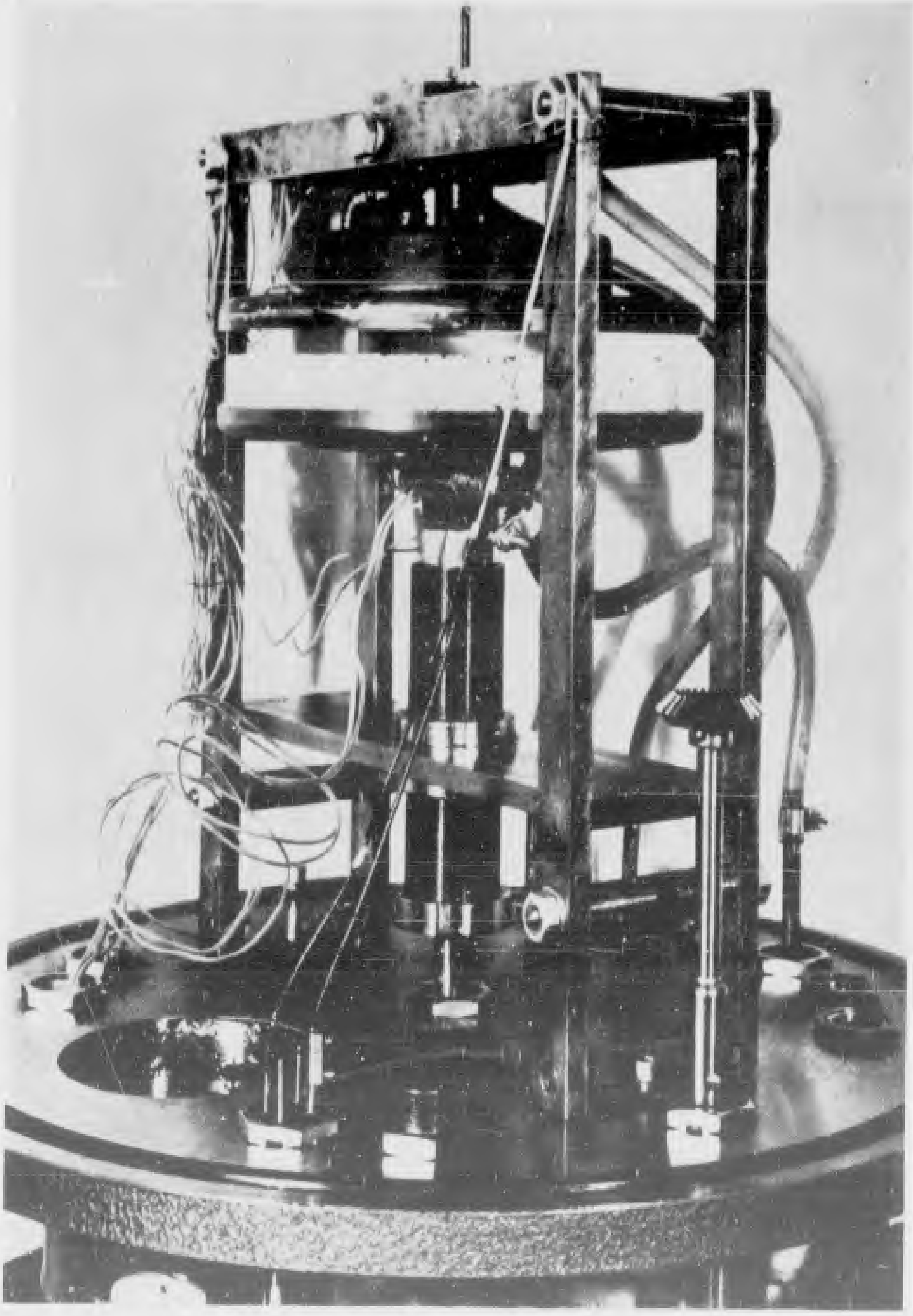


FIGURE 6. Double plate measuring device for heat conductivity.

d_f = thickness of fish.

D_f = heat transfer coefficient of water vapor through fish.

ΔT = temperature difference between heating plate and ice.

(At pressures below 3-4 x 10^{-1} mm Hg some corrections are necessary to obtain full agreement with the experiments). Another

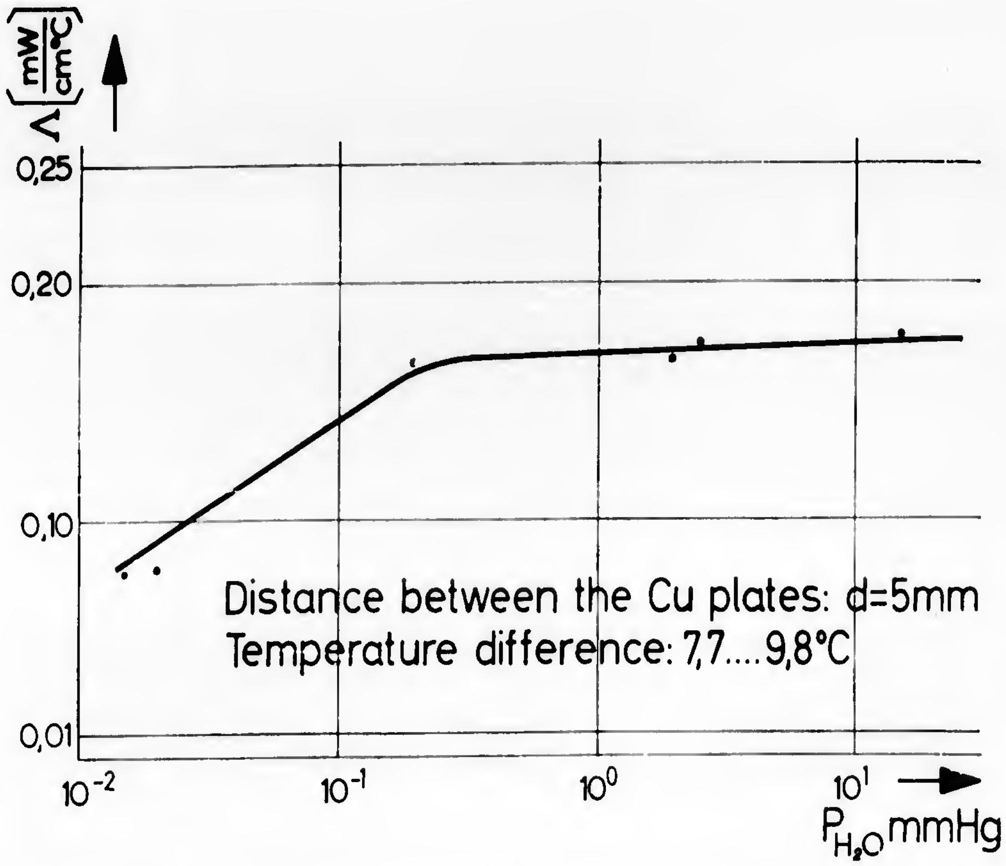


FIGURE 7. Coefficient of heat conductivity for water vapor at different pressures.

important equation follows, which gives the maximum temperature of the fish as long as no direct contact exists between heating plates and fish:

$$T_{\text{fish/vapor}} = T_{\text{ice}} + \frac{Q_{\text{total}} \times d_f}{F \times D_f}$$

at $\Delta T = 110°C$ and $d_f = 1$ cm

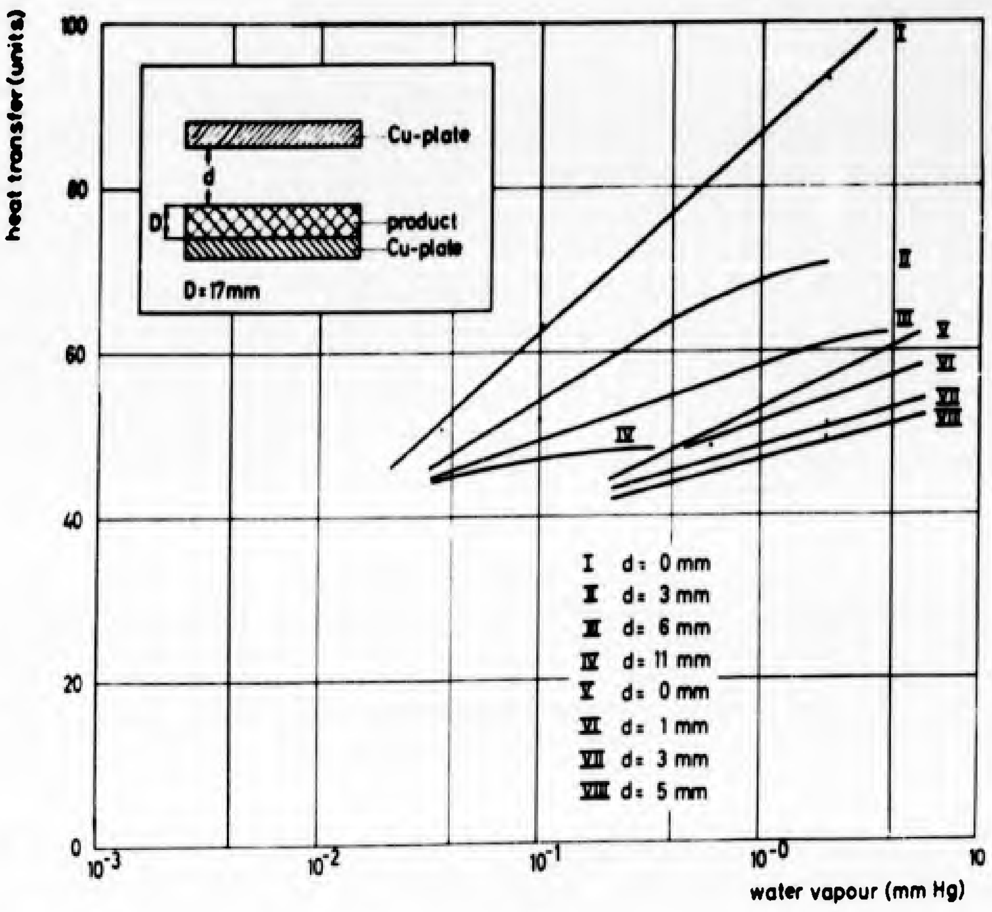
$$T_{\text{fish/vapor}} \approx T_{\text{ice}} + 50°C$$

With these results it is possible to calculate the necessary drying time for fish steaks of, for example, 15 mm thickness. With a heating plate temperature of 100°C and accounting for the variability in thickness of steaks, the drying time for the following operation pressures during the process would be:

2 mm Hg	5 to 7 hours
0.4 mm Hg	6 to 8 hours
0.1 mm Hg	7 to 9 hours

It should be noted that these two-hour differences in time result theoretically from only 1 mm variation of the distance between plate and material. For all practical purpose these calculated figures are as near to operational data as one can get.

If the same calculation



I ... IV: fish
V ... VIII: cauliflower

FIGURE 8. Heat transfer of different freeze-dried products.

$$t = \frac{L \times \rho}{2D \times \Delta T} \times d_F^2$$

L = sublimation heat

ρ = density

D = heat transfer coefficient (fish or cauliflower)

is used for the drying of cauliflower (20 mm layer, 70°C heating plates, and the density of the material 0.6 g/cm^3). The resulting drying time is 50% longer than that for fish steaks. With the lower density of the cauliflower (which results in a lower load per surface area), the possible throughput per day and unit of surface area goes down by a factor of 2.5.

So far only the heat transfer to the ice surface has been used to determine the minimum possible drying time for certain ice and heating plate temperatures. The other limiting factor of the process is the mass transfer from the ice surface through the already dried material to the chamber and the steam ejector or condenser system. A second laboratory unit has been built to study mass transfer problems.

Fig. 9 shows the schematic drawing of this installation which consists of a complete freeze-drying unit with vacuum chamber, heating plates, condenser, vacuum pumps, temperature control, and so forth. The product to be dried is placed in a "standard" tray (as mentioned below) and this tray is connected with a balance.

Figs. 10, 11, and 12 show this equipment in a sequence of 3 photos. The built-in automatic electrical balance permits the weight of the product to be determined at any specific time or periodically every few minutes throughout the whole process. Fig. 13 shows a diagram of such measurements.

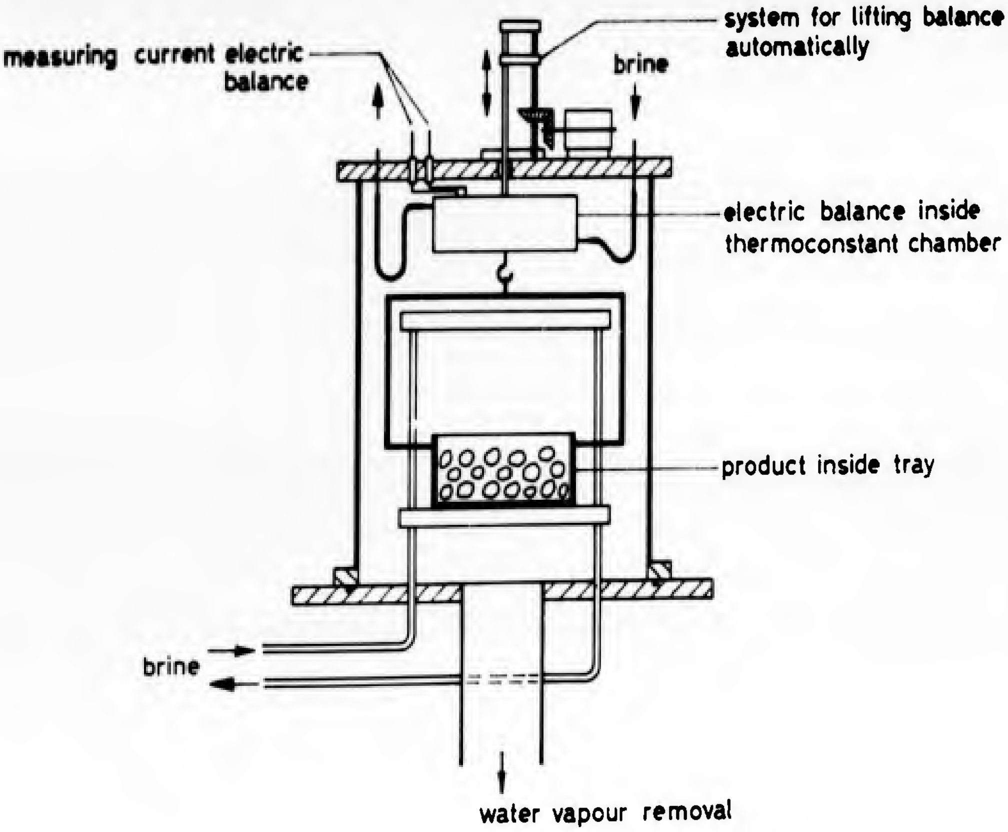


FIGURE 9. Drying chamber with electric balance.

Dr. Carman has shown how the flow of gases through porous materials can be calculated. Harper and Chichester have reported heat transfer coefficients and pressure drops in beef, peach, and apple which are in the same order of magnitude as our measurements. If these equations are combined with the above mentioned equation for heat transfer, the pressure drop resulting from the flow of water vapor evaporated by the respective flow of heat is given by the following equation:

$$\Delta p = k \frac{\eta \times D \times \Delta T}{B \times L}$$

k = a constant.

η = viscosity of the gas.

B = permeability.

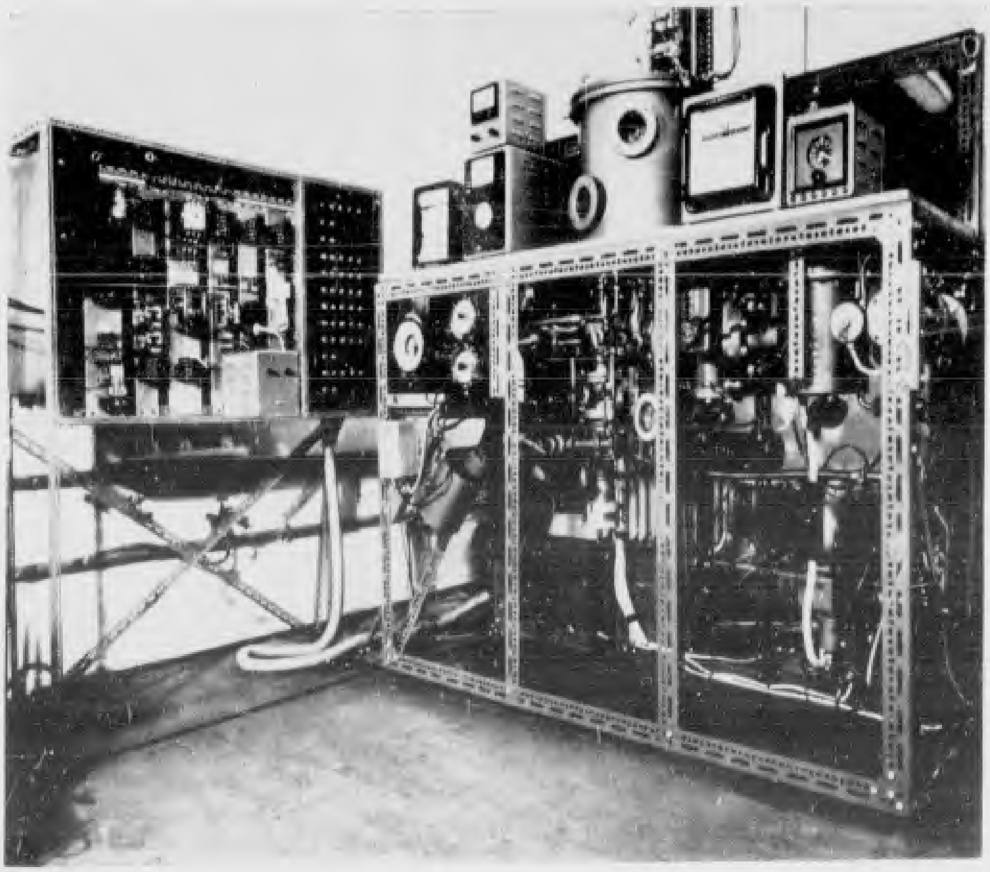


FIGURE 10. Laboratory freeze-dryer with temperature control and automatic balance.

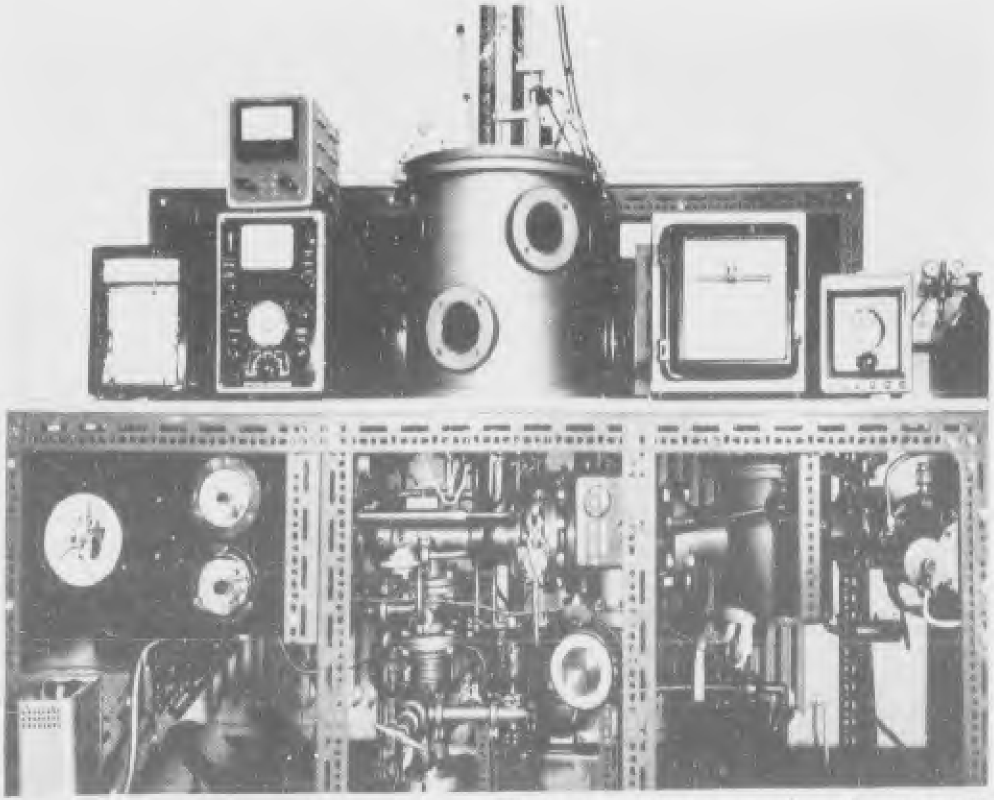


FIGURE 11. Laboratory freeze-dryer with temperature control and automatic balance.

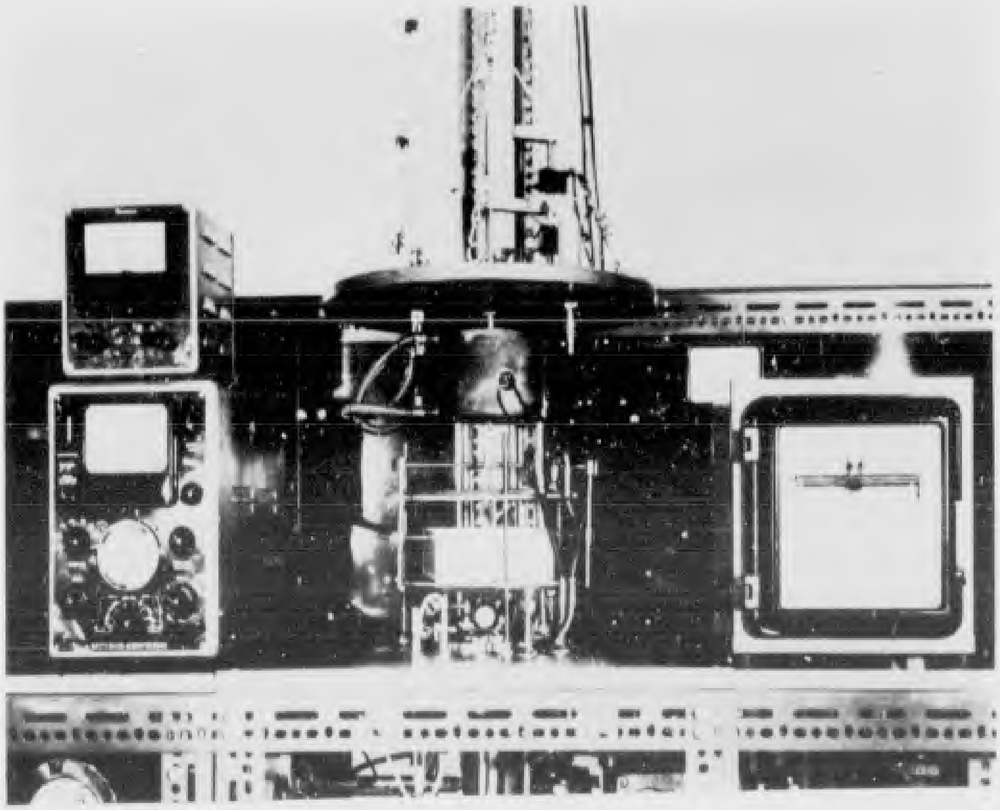


FIGURE 12. Laboratory freeze-dryer with temperature control and automatic balance.

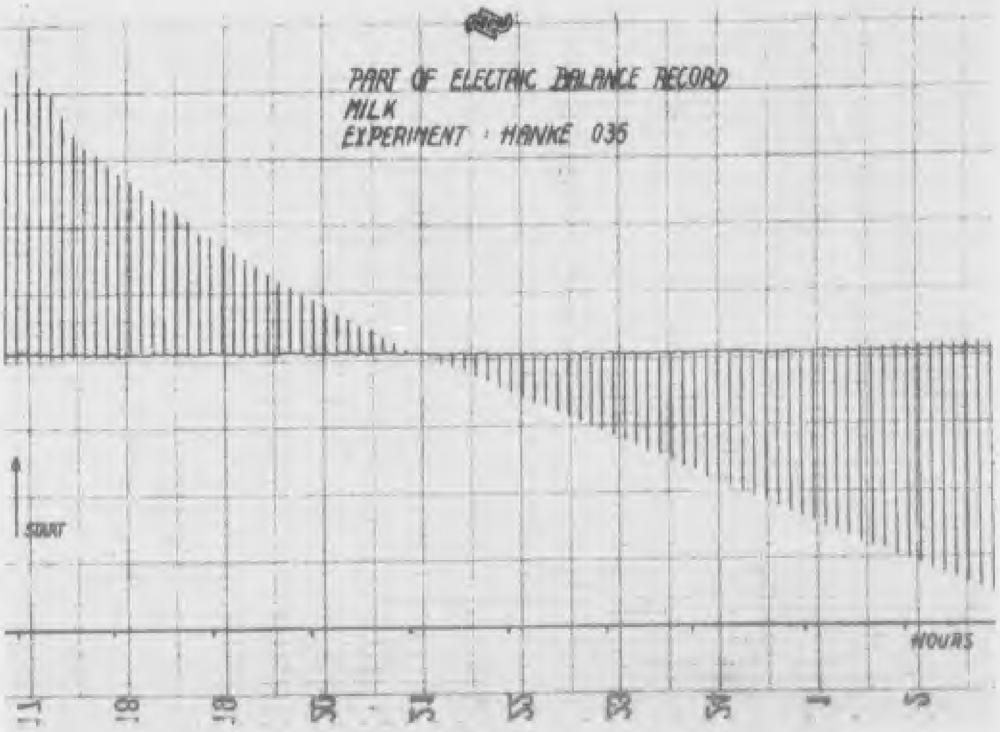


FIGURE 13. Example of measurement obtained from laboratory freeze-drier.

From this equation the pressure drop is independent (within the described circumstances) of the thickness of the already dried material. This result leads to the practical consequence that the operation pressure, once selected as optimum, can be kept constant throughout the whole process and must not be diminished as long as an actual ice surface exists. The calculated pressure drop for fish under the conditions specified is above 0.4 mm Hg which has been substantiated by measurements with the balance system described above. If additional heat is put into the ice by means of high frequency heating as shown in Fig. 14, the additional pressure drop caused by the additional flow of evaporated water is not independent of the thickness of the dried material.

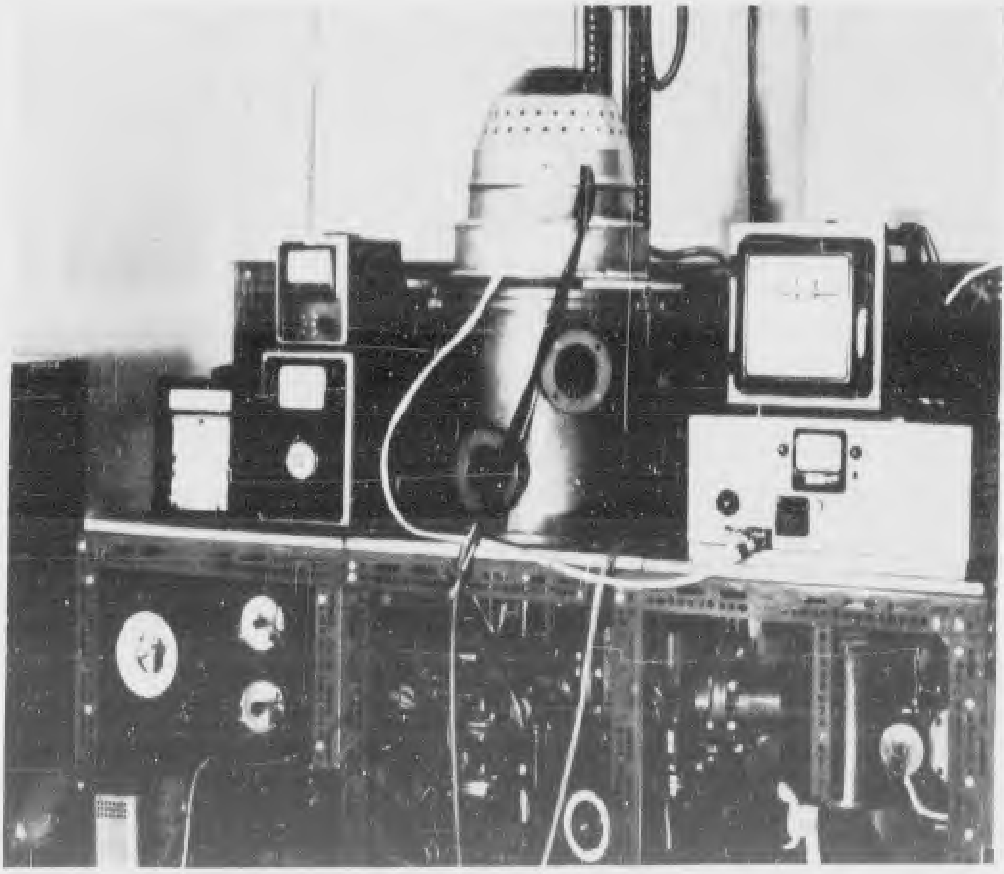


FIGURE 14. Laboratory freeze-dryer with microwave heating system.

Besides the economic and technical problems of high frequency heating itself, which cannot be a subject of this short paper, additional problems are raised in optimizing the steam ejectors or condensers. Thereby, the economy of the process is influenced a second time.

Having shown that the drying time for freeze-drying processes of various material can be calculated and predicted if ice and heating plate temperatures are carefully controlled, Fig. 15 gives experimental results of various products. Column 1 gives some results from experiments carried out in Aberdeen. Column 2 shows some

	Pressed heating plates[x]		Pressed heating plates (temp. controlled)		CQC - process	
	kg/m² · d	°C	kg/m² · d	°C	kg/m² · d	°C
Fish	37 (14mm)	60 - 120	40 (20mm)	70	105 (15mm)	100
Cauliflower	—	—	—	—	40	70
Carrots	—	—	45	70	45	70
Potatos	21	60 - 120	37	70	47	70
Meat	43 (15 mm, cubes)	60 - 120	—	—	60 (15 mm, slices)	80
Celery, leaves	28	60 - 120	—	—	50	80
Beans	30	70 - 120	—	—	—	—

[x] Aberdeen experiments

FIGURE 15. Throughput of different freeze-drying processes.

test-runs carried out in a pilot plant in which one of the heated plates was movable. The material to be freeze-dried was placed between this movable plate and a fixed one, and a variable pressure up to approximately 5000 kg/m² could be applied to these two plates by a pneumatic system. These tests were carried out with a simultaneous application of barometric temperature measurement and corresponding control of applied heat. Column 3 shows results without any movable plate or any pressure on the material. The products have been placed in trays optimized from the heat transfer calculations above and in accordance with the necessity of a practical operation. For example, for fish cut into slices of 20 mm thickness a load of approximately 30 kg/m² seems to be very near optimum. A drying time of 7½ hours is obtained, well in agreement with calculations. The results with cauliflower, under similar conditions, showed an increase in drying time of about 40%, again well in agreement with calculations.

In summation: If the ice temperature is measured and used to maintain controlled conditions during the freeze-drying process, the calculated results of the process, based upon some fundamental measurements, will be in good agreement with the observable results. Heat transfer coefficients are not improved by applying pressure to the material. Larger throughput per unit of heating surface can be obtained by optimizing load conditions based on the calculations.

SESSION NO. 5

IMPROVING PRODUCT REHYDRATION—BASIC BIOCHEMICAL AND BIOPHYSICAL APPROACHES

M. C. BROCKMANN, *Moderator*

THE INFLUENCE OF PROCESSING CONDITIONS ON THE REHYDRATION OF FREEZE-DRIED FOODS

W. R. SMITHIES

The ability to rehydrate rapidly is one of the most distinctive qualities of freeze-dried products and is a factor that demonstrates the superiority of freeze-drying over other methods of dehydration. The property of a material both to freeze-dry and subsequently to be reconstituted with water depends on the formation of an open porous structure free from impermeable barriers. Frequently, however, a product can be freeze-dried without difficulty but is of limited value because of its inability to rehydrate easily. Typical examples of this are wieners and whole raw mushrooms.

Meat Products

To obtain satisfactory products, as is well known, meat slices must be cut at right angles to the muscle fibers (1) and be relatively free from connective or fatty tissue. At the macroscopic level it appears that water penetrates the dried structure by capillary action aided by the ability of the muscle protein to be wetted. Occlusion of gas in the dried structure does not appear to restrict the rapid rehydration of freeze-dried meat prepared under good conditions. According to our standards, a freeze-dried raw meat slice should rehydrate in less than five minutes and taste panels be unable to detect gross differences from a frozen control sample.

Unsatisfactory processing conditions or prolonged storage may give a product that will require periods of up to one hour for rehydration and will be different in tenderness and juiciness from the control. The protein denaturation or migration of soluble solids or fat which takes place makes the structure less hydrophylic and so increases the time required for the water to penetrate the porous structure, besides affecting the muscle texture itself. Rehydration under vacuum increases the rate of absorption (1). When the product is finely divided in the form of small pieces or granules or prepared from a puree, rehydration is facilitated.

A simple method of measuring the ability to rehydrate is to determine the rate of uptake of water or rehydrating solution with time and to express the result either as a percentage of the original water content or as a ratio of the original dry weight. As absolute figures these have no value, but pieces from the same meat muscle can be compared in this way and the results can reflect changes in toughness and tenderness due to differences in processing or storing. Methods aimed at measuring bound water or water holding capacity have been proposed (2) but do not appear to be of much greater merit (3).

Some cooked meat products can be frozen by evaporation since water can migrate and evaporate easily (2), but raw meat is invariably frozen before the drying operation. In our experience, relatively rapid freezing using a dry ice-acetone mixture gives a product which rehydrates more slowly than is typical and which, after cooking, is tougher and drier than comparable samples frozen over a temperature range of −10° to −20°C.

At the commencement of the drying process, during loading and evacuation, it is essential that raw meat products are not allowed to thaw for this allows case hardening, the formation of a water impermeable barrier at the surface. For this reason it is very bad practice to lay samples for drying in direct contact with an unrefrigerated shelf. An advantage of radiant heating techniques (4) is that the tray on which the samples are supported is loaded between but not in contact with the heating shelves, greatly reducing the tendency to thaw.

When raw meat is dried under conditions where the frozen meat temperature exceeds about −10°C (the proportion of unfrozen water being relatively large), products are obtained which are of poor quality from the point of view of water uptake and texture (5). The claims made by Wang and co-workers (6) that pieces of meat freeze-dried first and then completely dried at higher pressures gave satisfactory products have not been substantiated in work on a large scale.

Dry surface temperatures exceeding 50°C also cause deleterious changes which can be detected by rehydration measurements or taste panels. In the case of pork, both raw and precooked, processing temperatures exceeding 40°C affect the quality. From observation of the material during drying, it is believed that the adverse effect is due to migration of fat.

The use of product ice temperatures and dried layer temperatures above the limits mentioned is thought to account for many of the claims that freeze-dried meat products suffer texture damage during processing.

Ground raw beef and pork patties are even more sensitive to processing conditions than meat slices. The fat content must be limited, the grind preferably coarse, and prolonged mixing avoided. Excessive mixing has a tendency to spread a water repellent layer throughout the sample.

In an oxygen-free atmosphere, freeze-dried meat products suffer only a slow change in quality over periods of several months, becoming gradually more insoluble and requiring longer time for complete rehydration. In order to detect small changes in texture, we have found it useful to rehydrate samples for only five minutes before cooking and presentation to a taste panel. Taste panel ratings relating to texture may then differ by 2 to 4 points on the 9-point hedonic scale whereas, if rehydration time is extended, the difference may be less than 2 points.

Air storage of raw or cooked freeze-dried meat at a temperature as low as 4°C can, in the course of two or three days, bring about spectacular decreases in the ability to rehydrate. On no account should samples be kept in this way. At as low a temperature as −20°C the rehydration ratio of samples of freeze-dried beef stored in air diminished significantly over a period of one month.

Fruits and Vegetables

In general, these products give rise to fewer problems than meat as long as thawing is avoided during processing. Fruits frozen in syrup or with sugar added are unsuitable for freeze-drying because thawing is then very difficult to avoid.

When skins are present as in peas, beans, and blueberries, they must be punctured to allow uniformly rapid rehydration. This puncturing may be achieved by mechanical means, by a preliminary blanching or cooking treatment, or by careful evaporative freezing.

Fruits and vegetables will tolerate processing temperatures as high as 70°C. The products will withstand prolonged storage in the presence of air without diminishing their ability to rehydrate. Loss of quality is associated rather with changes in flavor and color.

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EFFECT OF FREEZING RATES ON THE STRUCTURE OF FREEZE-DRIED MATERIALS AND ON THE MECHANISM OF REHYDRATION

B. J. LUYET

The existence of relationships between the freezing rates and the structure of freeze-dried materials and between that structure and the mechanism of rehydration would seem to be noncontroversial. Yet, as Rey remarks on page 38 of his recently published *Traité de Lyophilisation* (1): for many users of freeze-drying equipment, the only purpose of the initial freezing is to solidify the material; therefore there is no reason to attach much importance to that phase of the process. In this paper I will show that the rate of the initial freezing plays a fundamental role in the structure of the frozen material and in the following processes of vacuum desiccation and rehydration. The importance of this relationship had been strikingly brought to our attention by the results of studies conducted, during the last two years, in our laboratory on freeze-dried muscle tissue (the essential constituent of meat). The data obtained in those studies will be the *pièce de résistance* in the following discussion.

The subject matter will be presented in three parts entitled, respectively: I. Effect of the Freezing Rates on the Structure of Freeze-Dried Muscle; II. Effect of the Structure Resulting from the Freezing Rates on the Mechanism of Drying of Frozen Muscle; III. Effect of the Structure Resulting from the Freezing Rates on the Mechanism of Rehydration of Freeze-Dried Muscle.

PART I: EFFECT OF THE FREEZING RATES ON THE STRUCTURE OF FREEZE-DRIED MUSCLE

It is known that, in general, when the freezing velocity increases, the size of the ice particles decreases and their number increases. Wang and associates (2) and Meryman (3), among others, have presented some data on the above relationship in muscle. It is also known that in several tissues, particularly in plant tissues, slow freezing causes the formation of extracellular ice; rapid freezing, the formation of intracellular ice. As the literature on that aspect of the problem was rather scant in the case of muscle tissue, we undertook an extensive series of experiments on the formation of ice in single fibers and in pieces of different sizes cooled at various rates. The results were studied with both the light and the electron microscope (4, 5). The factual information to be discussed in this section (Part I) comes from these papers and from still unpublished data.

As for the order of the presentation, I shall (A) examine the information from the literature on the structure of normal nonfrozen muscle, (B) describe the procedures that we employed for freezing at various rates and for observing the effects, and (C) give the results of these observations.

(A) *Structure of Normal Nonfrozen Muscle*

To picture a muscle at a scale which would permit an observer to visualize the separation of the molecules of water from the substrate in the course of freezing, we need to magnify it about a million times. At that magnification, the molecules of water, which actually measure 3 angstroms, would appear as particles about $\frac{1}{70}$ of an inch. These molecules (w in Fig. 1C) would be seen to surround and penetrate parallel rods of actin and myosin (ms in Fig. 1), the myofilaments, which would have a diameter of 0.1 to 0.4 of an inch and be spaced 1 to 2 inches apart. The myofilaments are arranged in bundles, the myofibrils (fl in Fig. 1), which would appear to be some 40 inches across. The myofibrils themselves are assembled into bundles 10 to 50 times larger, the fibers; these are enclosed in a membrane, the sarcolemma (sl). In cross-sections, the fibers would appear to be some 10 to 60 yards in diameter. The muscle itself consists of a large number of parallel fibers.

For us the problem is to know (1) where the ice crystals develop in the muscle upon freezing at various rates, (2) how large they are, (3) how many there are, and (4) how they affect the original structure.

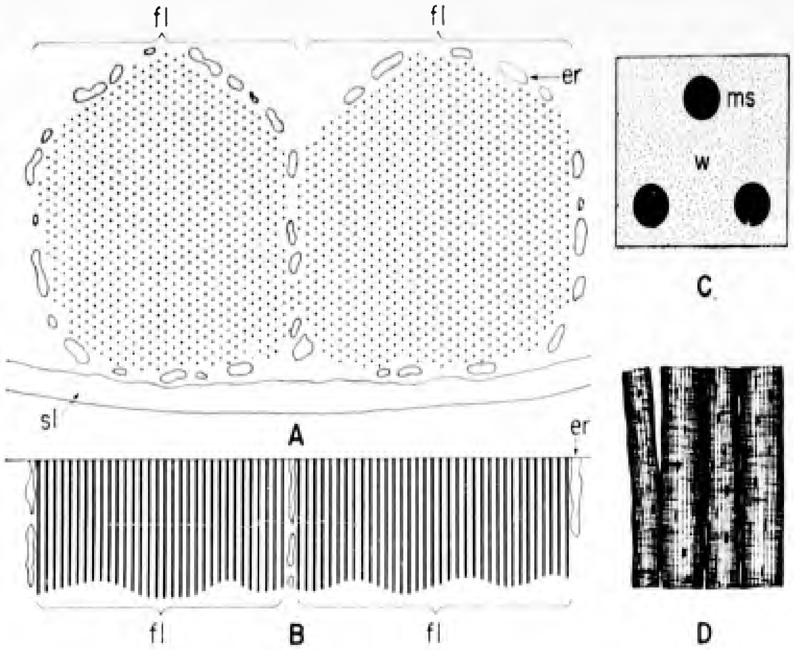

FIGURE 1. Diagrams representing the structure of a muscle fiber.

A. Cross section through a small fraction of a fiber showing two fibrils (fl) with their myosin filaments, the endoplasmic reticulum (er), and a small portion of the membrane surrounding the fiber, the sarcolemma (sl). × 45,000

B. Longitudinal section through the fraction of the fiber represented in A.

C. Portion of A enlarged 10 times to show the relative dimensions of the myosin filaments (ms) and the molecules of water (w).

D. Group of fibers drawn at a magnification 100 times lower than the magnification used for A and B.

(B) *Procedures for Freezing at Various Rates and for Observing the Effects*

Freezing Rates, Definition. In principle the rate of freezing, which may be conveniently expressed by the time spent at the freezing temperatures, could be varied over an unlimited range. To simplify the matter, we shall consider two principal freezing rates: one in which the material stays at the freezing temperatures about an hour; the other in which it stays there about a second. The two corresponding modes of freezing will be designated, hereafter, respectively, as slow and rapid. The former is that generally used for the freeze-drying of meat in industrial plants; the latter is that used in our experiments when a slice of tissue, $\frac{1}{12}$ of an inch thick, was frozen by immersion in an isopentane bath at −150°C. (It should be clear that I am not advocating such a high rate for the freezing of foods; I am only considering the principles involved.)

Freezing Procedure—*Slow Freezing.* The slowly frozen material we studied was meat prepared by the Quartermaster Food and Container Institute according to the regular freeze-drying procedures used in industry. A rib eye muscle of beef, about 4 inches in diameter, was frozen by exposure to an air current at a temperature of −29°C (−20°F). The temperature within the tissue remained between 0° and −10°C for about 2 hours.

Rapid Freezing. Discs, 2 mm thick and 17 mm in diameter, were cut, with the Stadie-Riggs microtome, from a piece of beef (usually eye of round) purchased at a butcher shop a few days after the killing. We then immersed these preparations in baths of isopentane at −150°C. For comparison, some preparations were frozen at −20° or −50°C. (Wang and associates, in comparable experiments, used larger pieces of meat.) The time the material stayed at the freezing point, as recorded in experiments in which a thermocouple was inserted between two discs of tissue each 1 mm thick, was of the order of a few seconds. (A copy of a record is reproduced in Fig. 2).

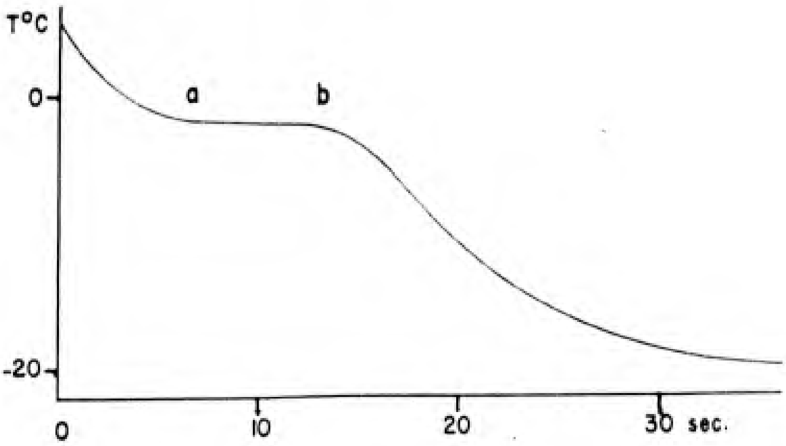

FIGURE 2. Freezing curve of a layer of muscular tissue 2 mm thick and 1 square inch in area, immersed in a bath of isopentane at −20°C.

Freeze-Drying—*After Slow Freezing.* The slowly frozen rib eye muscle was cut into slices about ¾ inch thick, and the slices were dried for 24 hours in a vacuum on the trays of a lyophilizer of the type used in industry. The temperature was allowed to rise gradually during drying.

After Rapid Freezing. The frozen discs were dried in a vacuum at −30°C for 3 days at a pressure of 5×10^{-5} mm of mercury. (The single fibers freeze-dried for electron microscopy were vacuum-dried at −60°C.)

Embedding and Sectioning—*For Study with the Light Microscope.* Pieces of the freeze-dried material, both slowly and rapidly frozen, were embedded in paraffin under a vacuum and sectioned with an ordinary rotatory microtome (Spencer, Model 820). To hold the fibers in place during sectioning, a layer of collodion was applied to the muscle-paraffin block before each knife stroke. Photomicrographs were then taken under two sets of conditions: (1) in polarized light and with the paraffin remaining in the preparation, and (2) in ordinary light and after removal of the paraffin with xylene. We used the former procedure when the preparations were to be photographed at low magnifications and the latter when they were to be photographed at high magnifications with an oil immersion lens.

For Study with the Electron Microscope. The freeze-dried material was embedded in methacrylate, sectioned with the Porter-Blum microtome to a thickness of about 1/30 of a micron, and stained with phosphotungstic acid.

(C) *Structure of Muscle Tissue Frozen at Different Rates*

Slowly Frozen Tissue—*General Characteristics.* The outstanding characteristic of freeze-dried tissue which had been frozen slowly is its relatively coarse, spongy structure. The tissue consists of a meshwork of fibers, aggregated into bundles (dark areas in Fig. 3), and of large interstitial cavities (clear areas). These cavities are the spaces previously occupied by ice with, in addition, the spaces resulting from the shrinking of the nonfrozen parts of the tissue during the dehydration which followed the sublimation of the ice (as discussed in Part II, below). Most of the cavities are deep pits formed by spears or columns of ice which had developed preferentially in the longitudinal direction of the muscle, pushing their way between the fibers and dehydrating them. The photographs in Fig. 3 give an idea of the size, shape, and position of the cavities and also of the relative proportion of water and dry matter in the tissue. (The size of the cavities varies considerably in pieces of tissue reported to have been treated in the same manner.)

At a higher magnification (Fig. 4, Photo 1) one can identify the individual fibers which compose the bundles. At a still higher magnification (Photo 2) the fibers are seen tightly packed against each other in the bundle, and their contents form a compact mass in which no cavities can be distinguished. An examination under the electron

FIGURE 3. Sections through pieces of freeze-dried muscle tissue embedded in paraffin and photographed in polarized light.
Photos 1 and 2: cross sections; Photo 3: longitudinal section.
Photos 1 and 3: sections from pieces with rather large cavities;
Photo 2: from pieces with rather small cavities. × 30

microscope of replicas of cross and longitudinal sections of such fibers did not reveal any porosity in them; holes of a diameter of 500 angstroms and fissures of a width of 250 angstroms would have been discernible.

After being freeze-dried, the pieces of tissues have about the same

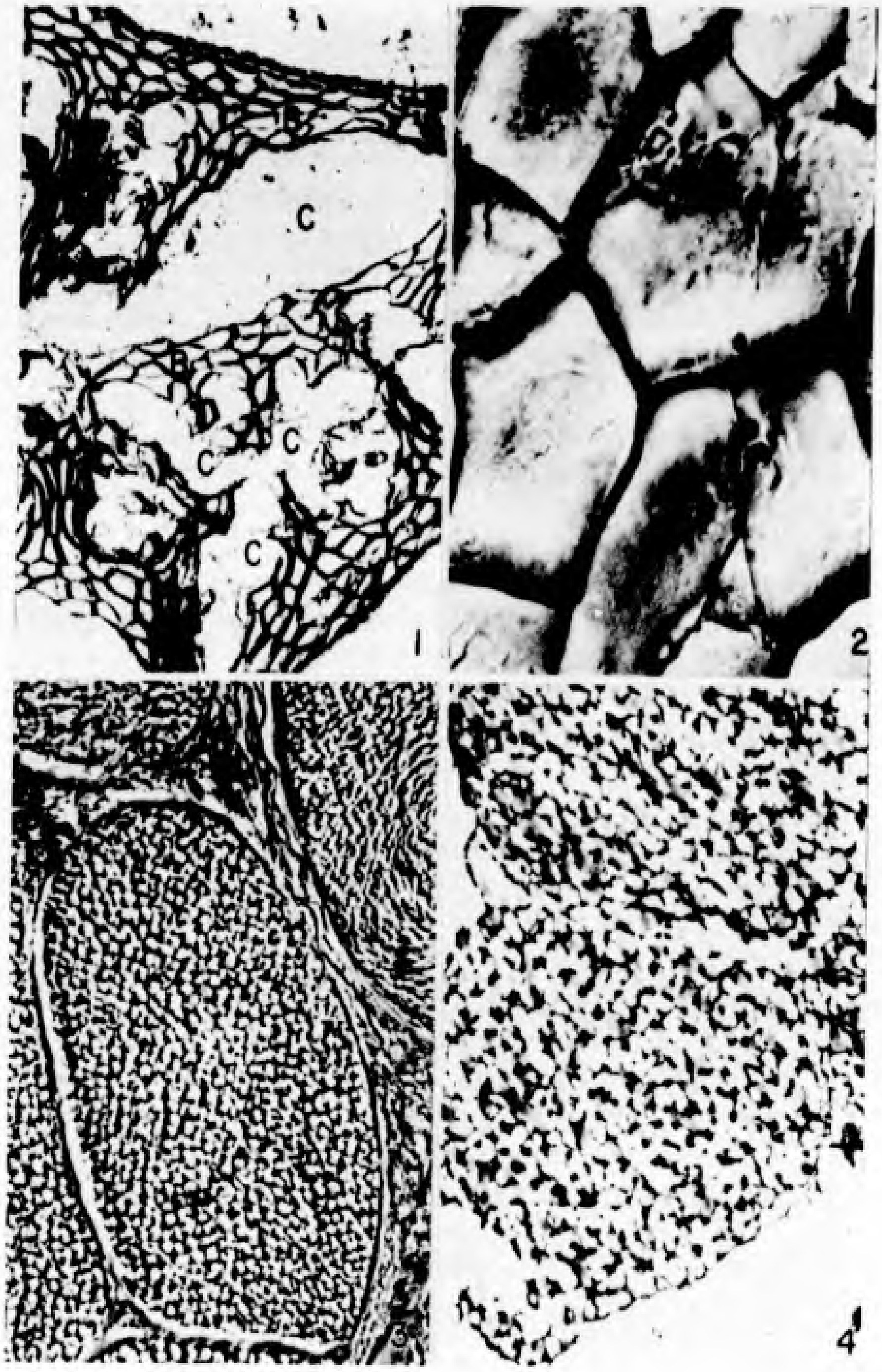

FIGURE 4. Cross sections through pieces of muscle tissue freeze-dried after slow freezing (photos 1 and 2), or after rapid freezing (Photos 3 and 4).

Photo 1 shows bundles B of muscle fibers, separated by cavities C now filled with paraffin. × 75

Photo 2: Enlarged view of some of the fibers shown in Photo 1. × 750

Photo 3 shows how the contents of a single fiber invaded by a large number of longitudinal spears of ice becomes separated by cavities now filled with paraffin. × 750

Photo 4: The same phenomenon as in Photo 3 seen at a higher magnification under the electron microscope. The cavities are now filled with methacrylate. × 1500 volume that they had in the frozen state, but the fibers have shrunken by the amount represented by the empty spaces. These shrunken, dry fibers have a very tough consistency; they could not be cut into thin sections for electron microscopy with the Porter-Blum microtome.

Dimensions. Several of the more or less cylindrical pits have a diameter of 100 to 150 micra; a few measure only 25 micra, and a few may reach 250 micra. Cavities in the form of crevasses vary in width from 40 to 400 micra. The cross sectional areas of the cavities, as measured by the weight of the paper covered by those areas in photographs of freeze-dried tissues, were found to represent 72% of the total.

Rapidly Frozen Tissues—*General Characteristics.* Freeze-dried tissues which had been frozen rapidly have a finely porous structure; the individual fibers themselves are porous, as is shown in Photographs 3 and 4 of Fig. 4. Numerous ice spears have been formed in the fibers during the initial freezing, and the spaces then occupied by the spears, now increased as a result of the shrinking of the nonfrozen contents of the fibers during drying (see Part II), appears as white specks in the pictures. The development of ice spears in a longitudinal direction within the fibers had been previously demonstrated by Rapatz and Luyet (4).

The rapidly frozen fibers are neither shrunken nor deformed appreciably; they have a relatively soft consistency, and one experiences no particular difficulty in cutting some 30 slices per micron from them for electron microscopy.

Dimensions. The diameter of the pits which have an approximately cylindrical shape is of the order of 2 micra.

Tissues Frozen at Intermediate Rates. Intermediate rates of freezing were obtained by immersing the pieces of tissue in isopentane baths at −50° and −20°C, instead of −150°C. The width of the cavities, as measured in photographs of cross sections of the freeze-dried material, increased with decreasing rates (Table 1).

TABLE 1

Size of Cavities in Muscle Tissue Frozen at Various Rates*

Designation of Series:	**Rapid**	**Interm.**	**Interm.**	**Slow**
Thickness of piece of tissue, mm	2	2	2	100
Temperature of cooling bath, °C	−150°	−50°	−20°	−29° **
Duration of freezing, sec.	1/4	3/4	8	1 to 2 hr **
Width of cavities, micra	2	5	10	150

* The rate of freezing is expressed by its reciprocal, that is, by the time the specimen remains in the range of freezing temperatures, at the "freezing plateau" of the curve.

** From data supplied by the Quartermaster Food and Container Institute.

Tissues Frozen Very Rapidly. To obtain higher rates of freezing than that studied so far under the designation rapid freezing," we used thinner discs of tissue. Fig. 5 shows four electron micrographs of longitudinal and transversal sections of muscle fibers, obtained when discs, 2 mm thick (used for a standard of comparison) and 0.2 mm thick, were frozen by immersion in isopentane at −150°C. The width of the cavities varies from about 2 micra in the less rapidly

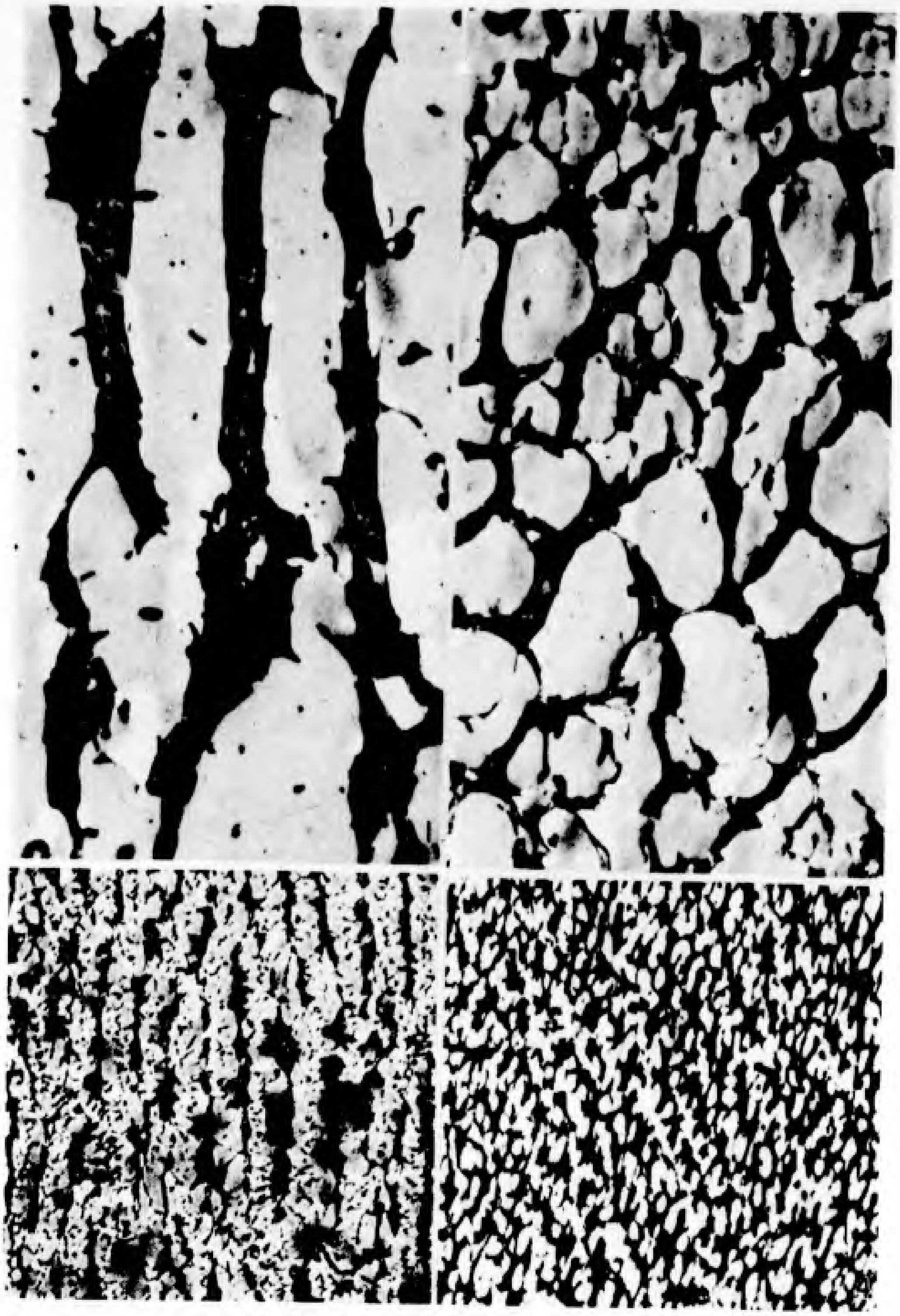

FIGURE 5. Electron micrographs of longitudinal sections (Photos 1 and 3) and cross sections (Photos 2 and 4) of rapidly frozen muscle discs 2 mm thick (Photos 1 and 2) and 0.2 mm thick (Photos 3 and 4). × 4,400

frozen fibers of the thicker discs to about 0.5 micra in the more rapidly frozen fibers of the thinner ones.

The freezing rate was increased still further by immersing single muscle fibers in isopentane at −150°C (5). This was done with fibers teased from frog's muscle. The electron micrograph of the structure resulting from such treatment (Fig. 6) shows a great difference between the outside of the fiber where cooling was very rapid and the inside where it was slower. The diameter of the approximately

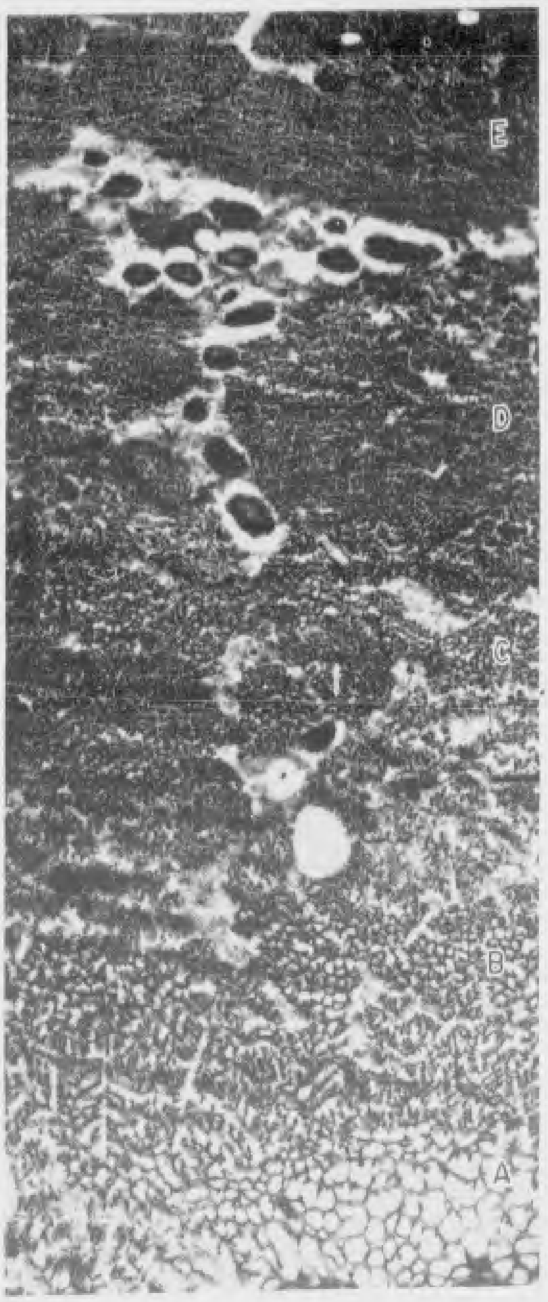

FIGURE 6. Electron micrograph of a cross section through a portion of a freeze-dried frog muscle fiber after rapid freezing in isopentane at −150°C. Notice the gradual decrease in the size of the cavities at positions from A to D, that is, when one approaches the edge of the fiber. In region D, the arrangement of the myofilaments is slightly disturbed; in region E, it appears about normal. × 14,200

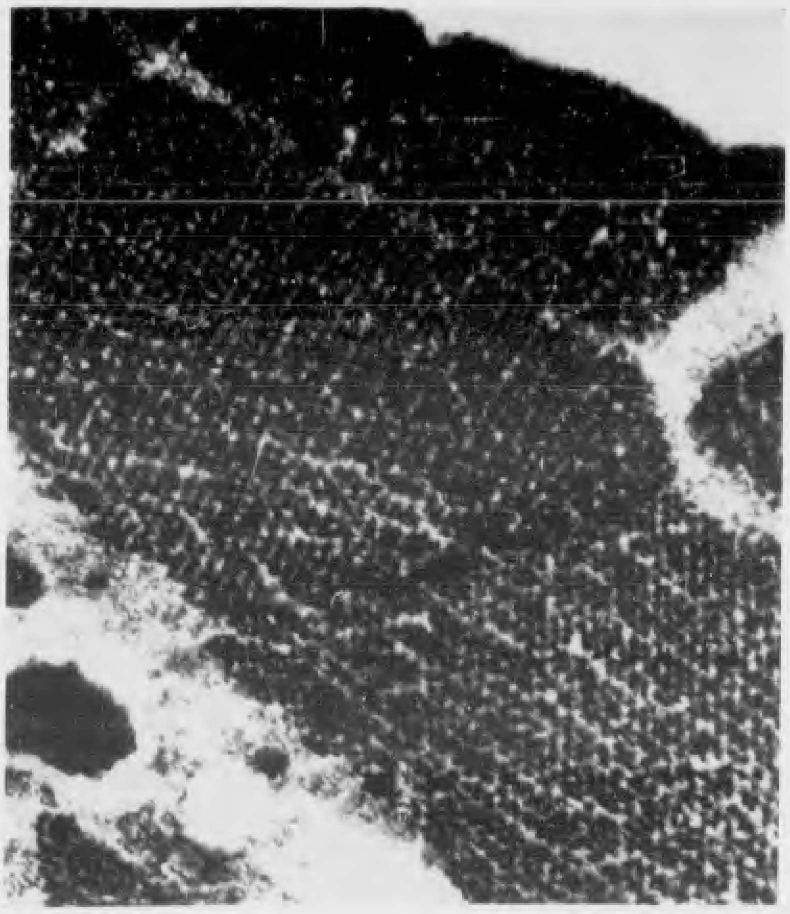

FIGURE 7. Enlarged view of part of the micrograph shown in Fig. 6. (Part about half-way across, at level E.) × 55,000

circular cavities varies from about 2300 angstroms in the inner region at level A to some 850 to 450 angstroms at levels B and C, respectively, further out toward the border of the fiber. The level D appears to be approximately the limit beyond which we are not certain that any ice has been formed. Further on, at the level of E, the photograph shows a regular array of myosin filaments which seem undisturbed. (A portion of level E is shown enlarged in Fig. 7.) Since the myofilaments have a diameter of about 100 angstroms and are some 150 angstroms apart, pieces of ice which would leave them undisturbed should not measure more than 150 angstroms. Thus, a gradual increase in freezing velocity permits one to obtain ice particles of gradually decreasing size until a point is reached at which no ice can any longer be ascertained.

PART II: EFFECT OF THE STRUCTURE RESULTING FROM THE FREEZING RATE ON THE MECHANISM OF DRYING OF FROZEN MUSCLE

Principle of Freeze-Drying and Analysis of the Process

The process of freeze-drying is based on the principle that the water of an aqueous system can be separated from the nonaqueous component by freezing and that the ice thus formed can be removed by sublimation, leaving a dry substrate.

This concept of freeze-drying, however, oversimplifies the picture. The process consists of three stages: (1) An initial freezing which withdraws some water from the specimen and, therefore, constitutes a partial dehydration; (2) A sublimation of the ice formed, which is a mere removal of the water already withdrawn and does not involve a further dehydration; (3) A withdrawal of the remnant nonfrozen (noncrystallized) water, which, of course, constitutes a further dehydration of the specimen. In the proper sense of the words, this third stage should not be considered a part of the freeze-drying process since it consists in the withdrawal of nonfrozen water; it is merely a drying process. Some authors call it "secondary drying." In order to emphasize the fact that it is not a true freeze-drying process, I proposed to call it "pseudo freeze-drying" (6).

To gain a better understanding of the various aspects of freeze-drying, let us now compare it with plain drying. Firstly, in plain drying, the escaping water molecules have only one way out, the surface of the piece of tissue; thus, to leave the tissue, they have to travel all the way from where they are to the surface. On the contrary, in freeze-drying, each crystalline nucleus formed in the course of the initial freezing becomes a center where the molecules of water converge from the neighborhood. The piece of tissue is then partitioned into numerous small "domains" from each of which the molecules can more readily escape toward the ice. Secondly, a consequence of the mode of withdrawal of water in plain drying is a nonuniform state of dehydration through the piece of tissue, in particular the formation of a dry shell near its surface while the inside may still retain a high water content. The compactness of this shell may prevent any further drying or lengthen enormously the drying time. In freeze-drying, the nonuniformity of concentration is limited to the spaces between the particles or spears of ice. Thirdly, plain drying results in a shrinking of the whole tissue and in a considerable overall disturbance of the structure. Freeze-drying does not cause a reduction in volume, and the disturbances that it may bring about are within the domains delineated by the crystallization units.

Effect of Initial Freezing on the Subsequent Stages of Freeze-Drying

We shall now examine how the structure resulting from the initial freezing (stage 1) of the freeze-drying process affects stages 2 and 3.

Initial Freezing and the Sublimation Stage. From what has been said, it appears that the characteristic of the freeze-drying process, as opposed to plain drying, is the division of the specimen into domains and that, the smaller and the more numerous are the domains, the better preserved is the original structure. The role of the second stage in freeze-drying is now to remove the core of ice from each domain by sublimation. The ease with which this can be done will depend on the size and configuration of the ice masses, in particular, whether and to what extent they offer large surface areas for vacuum sublimation and are interconnected to provide channels for the circulation of the departing water vapor. Since the size and number of crystallization centers depend primarily on the rate of the initial freezing, as was shown in Part I, the role of the initial freezing on the sublimation stage of the freeze-drying process is evident.

The advantages gained in reducing the size of the freezing domains and the size of the ice particles in them may be lost when a certain minimum size is reached because of the impossibility of removing the water molecules from these ice particles without letting the surrounding walls collapse. The minimum effective size of the domains and the rate of initial freezing which controls size are to be determined in each particular case.

Initial Freezing and Removal of Nonfrozen Water. A frozen aqueous medium may contain nonfrozen (noncrystallized) water (1) because its temperature has not been brought low enough to cause the crystallization of all the freezable water, (2) because it contains water (bound water) which does not freeze at any temperature, or (3) because it has been cooled so rapidly that some of its water remained amorphous or in a state of incomplete crystallization. In any of these three cases, the remaining water will be removed slowly by diffusion and the remaining framework may be continuously collapsing and shrinking. This is the drying process which I called pseudo freeze-drying.

We experienced the collapsing of the framework and the shrinking of the specimen in our studies on the freeze-drying of very rapidly frozen gelatin gels. Gels which contain crystallites smaller than the wavelength of visible light (and are, therefore, transparent) shrink upon being freeze-dried, whereas gels with crystallites of the order of a micron (which are opaque) do not shrink or shrink less (7).

A point which is often overlooked in the study of freeze-drying of proteins is the large proportion of bound (nonfreezable) water that they contain; gelatin gels may hold as much as 35% by weight (8). Thus, in the freeze-drying of gelatin gels, the initial freezing (stage 1) may transform the bulk of the moisture into ice until the water content is reduced to about 35%; the ice thus formed is then sublimed (stage 2); the remaining nonfrozen water is removed by mere drying (pseudo freeze-drying, stage 3). The empty spaces in the completely freeze-dried specimen apparently consist of the spaces occupied by ice (stage 1) and of the spaces resulting from the additional shrinking which occurs in the pseudo freeze-drying (stage 3).

The role of the initial freezing (in particular, its rate) in controlling the amount of moisture that remains unfrozen and which undergoes pseudo freeze-drying is obvious and does not call for further elaboration.

Application of the Principle of Three-Stage Freeze-Drying to Muscle Tissue

In the case of muscle tissue slowly frozen (stage 1), the ice, which originally occupied the major portion of the large channels and crevasses now seen in Fig. 3, was sublimed (stage 2). The remaining bundles, which still contained nonfrozen water, were merely dried, that is, their water was removed by diffusion and they shrunk further to the size shown in Fig. 3. All together, the tissue lost its moisture in three operations: first, by the transformation of most of it into ice (initial freezing), then by the removal of that ice (sublimation), and finally by the withdrawal of the remnant nonfrozen water (pseudo freeze-drying).

In the case of rapidly frozen muscle, the "domains" around the ice phase were much smaller and much more numerous. Each individual fiber contained many "domains," but the principle of freeze-drying in three stages remained the same. The spears of ice which developed in each fiber during the initial freezing (stage 1)—spears which formed the cores of the cavities now shown in cross sections in the electron micrograph of Fig. 6—were sublimed (stage 2). The myofilaments and other solid components of the fiber, already reduced to more compact aggregates by the initial freezing, underwent further shrinking because of the removal of the nonfrozen water (pseudo freeze-drying, stage 3). The cavities accordingly increased in size.

Cavities measuring about 450 angstroms, as at level C in Fig. 6, are well defined. Cavities of smaller dimensions may not be formed. Thus, as was already said, we cannot ascertain that there are cavities in the interstices at level E in Fig. 6. Since these interstices measure 150 angstroms, the lower limit of the size of the cavities may be tentatively set at a few hundred angstroms in the conditions of the experiments. The absence of cavities might be due either to the absence of original ice particles or to the collapsing of the walls of the cavities in the sublimation and the postsublimation stages of the freeze-drying process.

The existence of a critical size for the stability of the cavities may render useless any attempt to obtain smaller ice particles in the initial freezing.

PART III: EFFECT OF THE STRUCTURE RESULTING FROM THE FREEZING RATE ON THE MECHANISM OF REHYDRATION OF FREEZE-DRIED MUSCLE

The general conclusion of what has been said is that the rate of the initial freezing determines to a considerable extent (1) the size and the number of cavities in the freeze-dried material and (2) the amount of tissue which remains unfrozen and which will shrink when subjected to pseudo freeze-drying. Our next task is to examine how the rehydrating fluids (water only, in the work reported here) penetrate these diverse structures. A distinction should be made between rehydration of whole pieces of tissue and rehydration of single fibers. The former will only be considered in the case of slowly frozen tissues, where penetration through large cavities poses special problems; the latter needs to be considered in both slowly and rapidly frozen material.

(A) *Methods for the Study of Rehydration*

Rehydration of Whole Pieces of Tissue. A strip of slowly frozen, freeze-dried muscle tissue, thin enough to be examined by transparency (less than 1 mm), was strung vertically on a wire frame held by a micromanipulator between a horizontally-placed microscope and a bright light source. Water for rehydrating the strip of tissue was placed in a small container, underneath, on a stand adjustable for height. The strip was then lowered into the fluid by means of the micromanipulator, and/or the container was raised to meet the strip. Examination then could be made of the path followed by the water in penetrating the tissue.

Rehydration of Single Fibers. Fibers of slowly and of rapidly frozen muscle were teased from the freeze-dried tissue and fastened to a glass slide by threads of soft methacrylate. The methacrylate then hardened and held the fibers in place. Two spacers of masking tape were attached to the glass slide on either side of the fiber, where they served as shoulders to support a cover glass fastened to the slide.

The fiber, thus held between slide and cover slip, was then flooded with water, under the microscope. Its changes in volume were measured either directly with an ocular micrometer or after they had been recorded on film. To illustrate the changes, photographs were taken before, during, and after rehydration, usually under dark field illumination. Variations in opacity were estimated by visual observation.

(B) *Observations on the Mechanism of Rehydration*

We shall first examine the main obstacles encountered by the rehydrating fluids, namely: water-repellent surfaces, impermeable membranes, and trapped air bubbles. Then we will consider the mode of penetration of the fluids through the freeze-dried material.

(a) *Obstacles to the Penetration of Rehydrating Fluids*

Water-Repellent Surfaces. From the point of view of the wettability of the surfaces, one may distinguish three cases: (1) Sometimes, the surfaces (external or internal) of the pieces of freeze-dried tissue are covered with a glossy coat which is not wetted by water. (2) At other times, no such coat is visually observable although the surfaces are not readily wetted. The addition of surface active substances such as alcohol or "Tween" (polyoxyethylene sorbitan monolaurate) increases the wettability. (3) In still other cases, some tissues or regions in a tissue do not seem to show any reluctance to absorb water.

Impermeable Membranes. Often one sees the moisture invade a strip of tissue to a certain border line, rather sharply delineated. A closer examination reveals the presence of membranes at such boundary lines. These membranes were identified as parts of the perimysium and the endomysium. They could be isolated by removal of the fibers attached to them. The degree of impermeability seemed to vary considerably from one membrane to another.

Trapped Air Bubbles. A major obstacle to the invasion of the cavities by the rehydrating fluids is the presence of trapped air bubbles. The mode of formation of these bubbles will be examined in the next section. In a series of quantitative determinations, the amount of entrapped air was found to represent 9% of the total volume of the pieces of tissue when the pieces, held with the fibers in a vertical position, were immersed slowly into the rehydrating bath; and 26% when the pieces, held with the fibers in a horizontal position, were immersed rapidly into the bath.

(b) *Mode of Penetration of Rehydrating Fluids*

Rehydration of Whole Pieces of Tissues. The fact just reported of the expulsion of most of the air, upon immersion in the rehydrating fluid of a piece of tissue with fibers in the vertical position, indicates that water moved freely through some of the large cavities in slowly frozen tissues. To know to what extent capillarity is involved in this phenomenon would require further investigations.

In the case of smaller cavities in slowly frozen tissue, small enough to be contained in the field of the low power microscope (25x), we could often observe that the moisture would spread around the cavities but not through them. This encirclement resulted in the formation of air bubbles and in their enclosure in the cavities. Sometimes, we could see the moisture travel along one of the longer sides of an elongated cavity, turn around the narrow end, and return on the other side to enclose the air bubble. Thus, in such cases, water does not invade cavities or channels by making sudden thrusts through them by capillarity.

Rehydration of Single Fibers—*In Slowly Frozen Tissues.* (1) The fibers from slowly frozen tissue, which have shrunken to about one third of their original volume (Fig. 8, Photo 1), reabsorb water at such a rate that in 3 seconds their diameter increases by 80% of its total increase. (2) Water does not ascend through a fiber held in a vertical position with its lower end immersed in a dish underneath. (3) The fibers, which are quite transparent in the dry state, as they contain no cavities (Photo 1), and which become fully transparent immediately after the application of water, show, about one minute later, a veil of opacity (Photo 2) which increases to a slight, fine-grain fog in about 10 minutes. (Photo 3 does not permit a proper evaluation of that increase in opacity).

Rapidly Frozen Tissues. (1) Upon being flooded, the porous fibers from rapidly frozen tissues (Fig. 8, Photo 4), which did not shrink

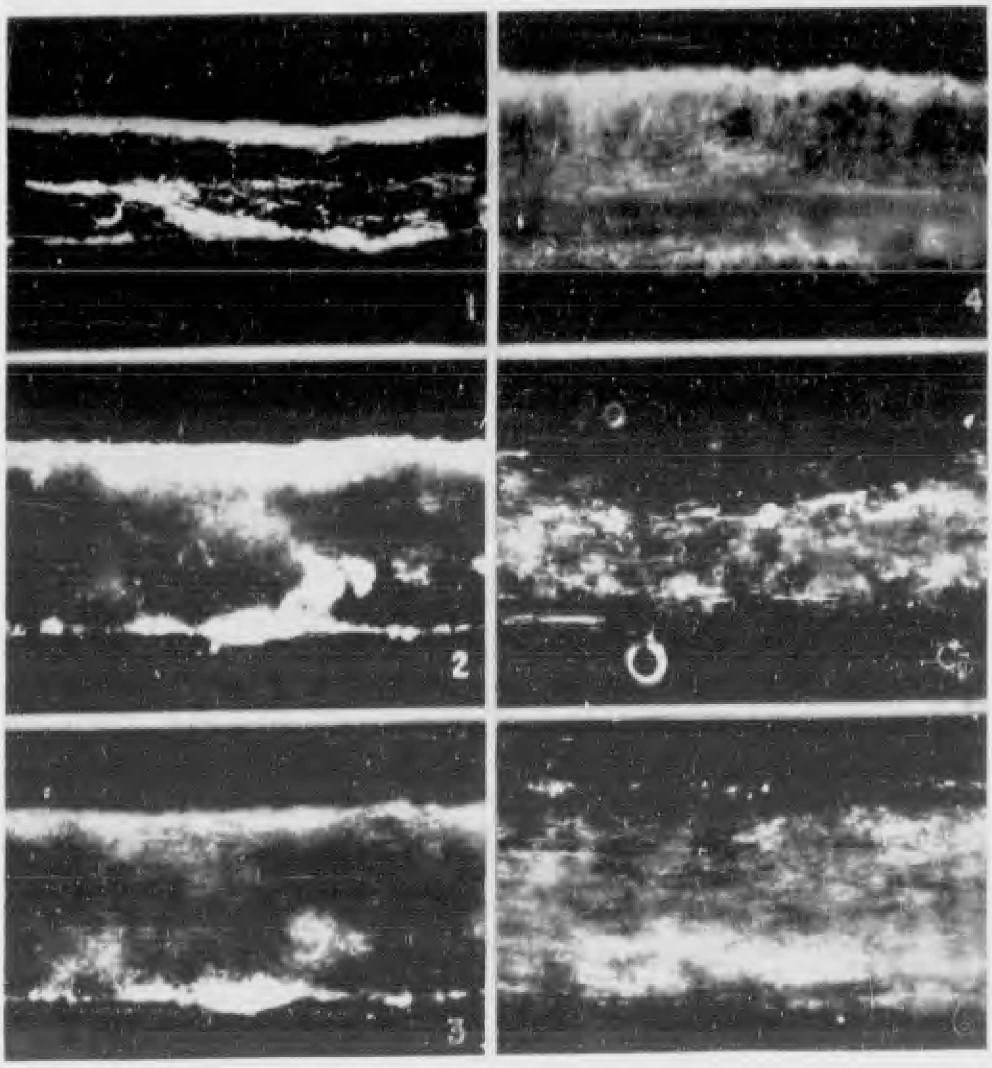

FIGURE 8. Rehydration of freeze-dried muscle fiber.
Photo 1: Single fiber from a slowly frozen tissue in the dry state. × 160
Photos 2 and 3: the same fiber one minute after immersion in water (Photo 2) and 20 minutes later (Photo 3). × 160
Photo 4: Single fiber from rapidly frozen tissue in the dry state. × 190
Photos 5 and 6: the same fiber one minute after immersion in water (Photo 5) and 20 minutes later (Photo 6). × 190

in the course of freeze-drying, absorb water and swell about as rapidly as the fibers from slowly frozen tissues; most of their increase in diameter takes place during the first few seconds after flooding. (2) Although they have a porous structure, the fibers from rapidly frozen tissues behave in the same manner as the compact fibers from slowly frozen tissues when held in a vertical position with their lower end in contact with water in a dish underneath; water does not rise in them. (3) Immediately upon being flooded, the fibers which were completely opaque in the dry state (Fig. 8, Photo 4) become transparent, except in places occupied by channels which contain elongated air bubbles that reflect and disperse the incident light (Photo 5). Some of these bubbles are soon dissolved, others are expelled and aggregate into relatively large bubbles (Photo 5). The transparency originally acquired upon application of water gives way to a gradually increasing, fine-grain fog (Photo 6), as was described in the case of fibers from slowly frozen tissues.

GENERAL CONCLUSIONS ON REHYDRATION

The facts reported lead to the following tentative conclusions, or suggestions, on the mechanism of rehydration of freeze-dried muscle:

(1) The process consists of two stages: (a) The penetration of water through the cavities in the tissues, either the large ones formed between the fibers in slow freezing or the minute ones formed within the fibers in rapid freezing; (b) The penetration of water through the solid parts of the tissues, either when the entire fibers consist of compact material as after slow freezing, or when only the framework of porous fibers constitutes the solid parts as after rapid freezing.

(2) In both the slowly and the rapidly frozen tissues, under the conditions reported, water seems to penetrate more freely through the solid parts than through the cavities. Most of the water to be reabsorbed by single fibers enters them during the first three seconds after they have been flooded. Having imbibed water, the solid parts apparently swell into the cavities, and the entrapped air is either dissolved or expelled. The air in the very small cavities, in the rapidly frozen tissues, is rather rapidly disposed of; the air in the cavities of intermediate size (that is, the smaller of the cavities in the slowly frozen tissues) may constitute a major obstacle to the completion of rehydration; the air in the very large channels, in slowly frozen tissues, may be expelled at once by the onrush of the flooding water.

(3) The mechanism by which water readily penetrates single fibers in the diametral direction, and does not seem to be able to enter them longitudinally, calls for further inquiry.

(4) The gradual increase in opacity following rehydration has been examined with particular care in the hope that it might furnish a significant clue to the changes which take place, at the molecular level, upon readmission of water. But our factual information on the mode of binding of the molecules of water to those of the substrate is too fragmentary to permit, at present, a fruitful attempt at an interpretation of the results reported.

On the general problem of the relationship (1) between the rate of the initial freezing and the size and location of the cavities in the tissues and (2) between the size and location of the cavities and the mechanism of rehydration, the facts discussed speak for themselves.

Acknowledgments

I wish to express my gratitude to my associates, assistants, and laboratory technicians who did the experimental work on which this study is based. I am indebted, in particular, to Mr. M. Persidsky and Mr. M. Ross for the photomicrographs of freeze-dried tissues, to Dr. L. Menz for the electron micrographs, to Mr. L. Gelencser for the study of the rehydration of single fibers, and to Dr. A. MacKenzie who conducted the work on entrapped air, on rehydration of whole pieces of tissues, and on electron microscopy by the replication method.

Most of the experimental data presented were obtained in the course of a research supported by the Quartermaster Research and Engineering Command, Contract No. DA 19-129-QM-1413. Figs. 6 and 7 are from a work supported by a grant from Oscar Mayer and Co., Madison, Wisconsin.

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REHYDRATION CHARACTERISTICS OF FREEZE-DRIED PLANT TISSUE

WAYNE J. MCILRATH, ELIAS D. DEKAZOS, and KARL R. JOHNSON

In this discussion, remarks will be restricted to work carried out with Swiss chard leaf blades. All of the material was taken from plants grown in a complete nutrient solution by the water culture technique under greenhouse conditions at Chicago, Illinois. Unless otherwise indicated all experimentation was performed using disks of fresh leaf tissue 1/4 inch in diameter. Care was taken to exclude major vascular elements in cutting the disks.

One of the first aspects investigated was the influence of the drying method on the degree of rehydration. Of the numerous techniques tried, freeze-drying produced material which not only rehydrated the most rapidly but also to the highest degree (Fig. 1). Of the several freeze-dry methods used those in which the tissues were frozen the most rapidly yielded the best quality product with respect to the degree to which it rehydrated. Ultra-rapid freezing in liquid nitrogen was superior to freezing with an ethanol-dry ice mixture.

Histological examination of freeze-dried tissue showed that although the protoplasts in some of the mesophyll cells retained their normal orientation with the cell walls, others appeared to be separated from the walls as though extra-cellular ice formation had occurred. Examination of the fine structure of such cells with the electron microscope also revealed abnormalities in structure. Major changes in chloroplast

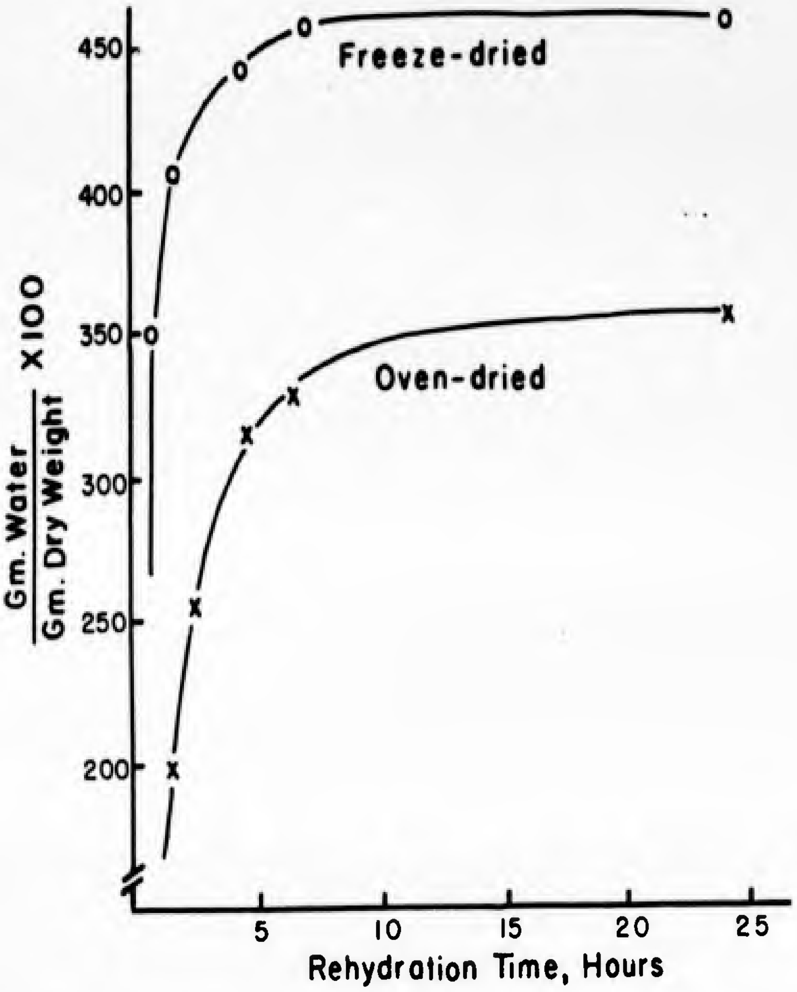

FIGURE 1. Rehydration of Swiss chard leaf blade tissue dried by freeze-dry and vacuum oven techniques. Tissues rehydrated in distilled water at 21°C.

structure, for example, were incurred in the freeze-drying process (Fig. 2).

Although it was observed that the magnitude of water uptake per unit of dry weight was much greater in freeze-dried tissue than in that dried by other methods, it was not known whether the forces binding the water were similar or of equal magnitude in the various tissues. Several experimental techniques were tried in an attempt to elucidate this point. One of these involved a study of the absorption of water by tissues maintained at various relative humidities (Table 1). In such experiments it was found that freeze-dried tissue absorbed a greater amount of moisture at a given relative humidity than tissue dried in other ways. This moisture also constituted a higher percentage of the total that was absorbed when the tissue was submerged in liquid water.

Another technique involved studies of the uptake of water by tissues from NaCl solutions of various osmotic pressures. In these experiments it was observed that freeze-dried tissue absorbed a greater portion of its moisture against very high osmotic pressures than did tissue dried in the vacuum oven (Table 2). In a solution with

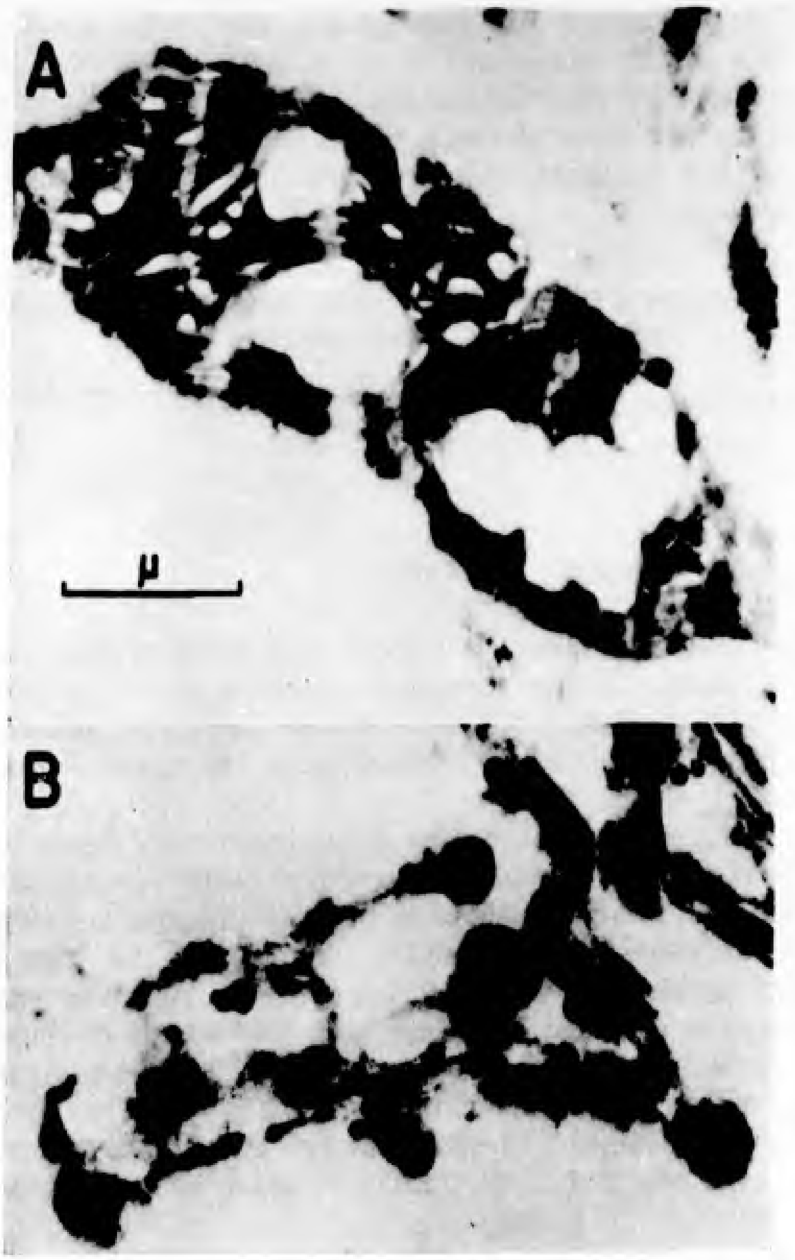

Figure 2. Electron micrograph of chloroplasts in Swiss chard leaf tissue: A, from fresh tissue; B, from freeze-dried tissue.

TABLE 1

Uptake of Moisture by Swiss Chard Leaf Disks at Various Relative Humidities. All Disks Taken from the Same Leaf.

Dehydration technique	**Relative humidities to which leaf tissues subjected prior to being placed in water***				
	94.0%	**70.4%**	**91.2%**	**100%**	**Water**
			$\frac{\text{Gm. water}}{\text{Gm. dry weight}} \times 100$		
Freeze-dried	39.6	42.8	88.5	423.0	559.1
40°C Vacuum Oven-dried	13.3	17.5	52.0	270.6	403.2

* Tissue maintained at each relative humidity until its water content reached equilibrium (24 hours) at 21°C before being transferred to the next higher humidity.

an osmotic pressure of 375 atmosphères freeze-dried tissue took up about 79% of the total water it had a capacity to absorb whereas vacuum oven-dried material absorbed only about 67%. The difference between the two types of tissue in the percentage of total moisture absorbed from a solution with an osmotic pressure of 15 atmospheres was much less.

TABLE 2

Rehydration of Swiss Chard Leaf Disks in NaCl Solutions of Varying Osmotic Pressures. Tissues in Solutions for 24 hrs.

Dehydration technique	**Osmotic pressure, Atmospheres**		
	0	**15**	**375**
		$\frac{\text{Gm. water}}{\text{Gm. dry weight}} \times 100$	
Freeze-dried	474.3	421.8	373.6
40°C Vacuum Oven-dried	373.0	318.8	251.8

These facts would seem to indicate that freeze-drying resulted in less disturbance in the structural characteristics and hydrophilic properties of the tissue. The latter data probably also indicate that if osmosis played a role in the rehydration of the tissues it was a very minor one.

Since there was less shrinkage in the freeze-dried tissue the possibility existed that a portion of the greater water absorption by such material could be accounted for on the basis of larger capillary spaces within the tissue. In an attempt to determine if this were true, rehydrated tissues were subjected to a force of 1000 times gravity for 30 minutes in a special centrifuge head. The results of these experiments showed that although freeze-dried tissues did contain a greater portion of their total moisture in a loosely-bound condition, they nevertheless retained a greater amount of water per unit of dry weight after centrifugation than did tissues dried by other techniques (Table 3).

TABLE 3

Moisture Lost by Rehydrated Swiss Chard Leaf Disks Subjected to a Centrifugal Force of 1000 Times Gravity for 30 Minutes. Samples Were Taken from Five Different Leaves.

Fresh wt., mg.	**Dry wt., mg.**	**Water absorbed, mg.**	**Water absorbed, Gm. dry wt.**	**Water remaining after centrifugation, mg.**	**% of water loss**	**Water remaining Gm. dry wt.**
		Freeze-dried Tissues				
22.04	2.81	18.3	6.63*	8.6	52.8	3.12*
		40°C Vacuum Oven-dried Tissues				
23.80	3.70	12.3	3.31*	9.2	25.0	2.48*
		100°C Oven-dried Tissues				
25.30	3.68	4.2	1.14*	3.6	13.7	0.98*

* Freeze-dried tissue statistically different from other series at 1% level.

The suggestion might be made that the greater rehydration of the freeze-dried material was correlated with the maintenance of the free polar groups in the tissue in such a state that they did not lose their water-binding capacity. The tissue shrinkage incurred with certain methods of drying may have resulted in the polar groups being drawn together so that groups on adjacent molecules mutually satisfied each other; upon rehydration such groups would no longer be free to orient water molecules. It seems logical to assume that if polar groups bond with each other upon shrinkage of the tissue more of these bonds should be broken by rehydration at elevated temperatures with a concomitant increase in the degree of rehydration. To check this assumption dried tissues were rehydrated at 21 and 55°C. As was anticipated, rehydration at the higher temperature resulted in an appreciable increase in the degree of rehydration (Table 4). This observation would presumably support the assumption that the 55°C temperature resulted in the greater liberation of polar groups to a functional role in the binding of water. A fact which is not clear, however, is why such temperature treatment should result in as great an improvement in rehydration of freeze-dried material as in oven-dried tissue. If it can be concluded that with the most shrinkage there was also the greatest elimination of water-binding sites, one might assume that such tissues would show the greatest improvement in rehydration with higher temperatures. Such was not the case.

In an attempt to determine the importance of the basic structure of the leaf tissues to rehydration, various cellular components were isolated by differential centrifugation (Fig. 3) and their rehydration characteristics examined.

TABLE 4

Effect of Temperature on the Rehydration of Swiss Chard Leaf Disks Dried by Various Techniques

	Temperature	
Dehydration technique	**21°C**	**55°C**
	$\frac{\text{Gm. water}}{\text{Gm. dry weight}} \times 100$	
Freeze-dried	733.3	900.0
40°C Vacuum Oven-dried	554.2	623.1
100°C Oven-dried	260.9	304.3

When the structure of the chard leaf was destroyed in the isolation of the various constituents, it was found that none of the components rehydrated to a level comparable to that of the intact tissue (Figs. 1 and 4). It was also noted that once the leaf tissue was fractionated into the various components the method of drying had very little effect upon the rehydration characteristics of the component. For example, fraction 3 (supernatant) dried at 100°C was found to rehydrate as well as that dried either by the vacuum oven or freeze-

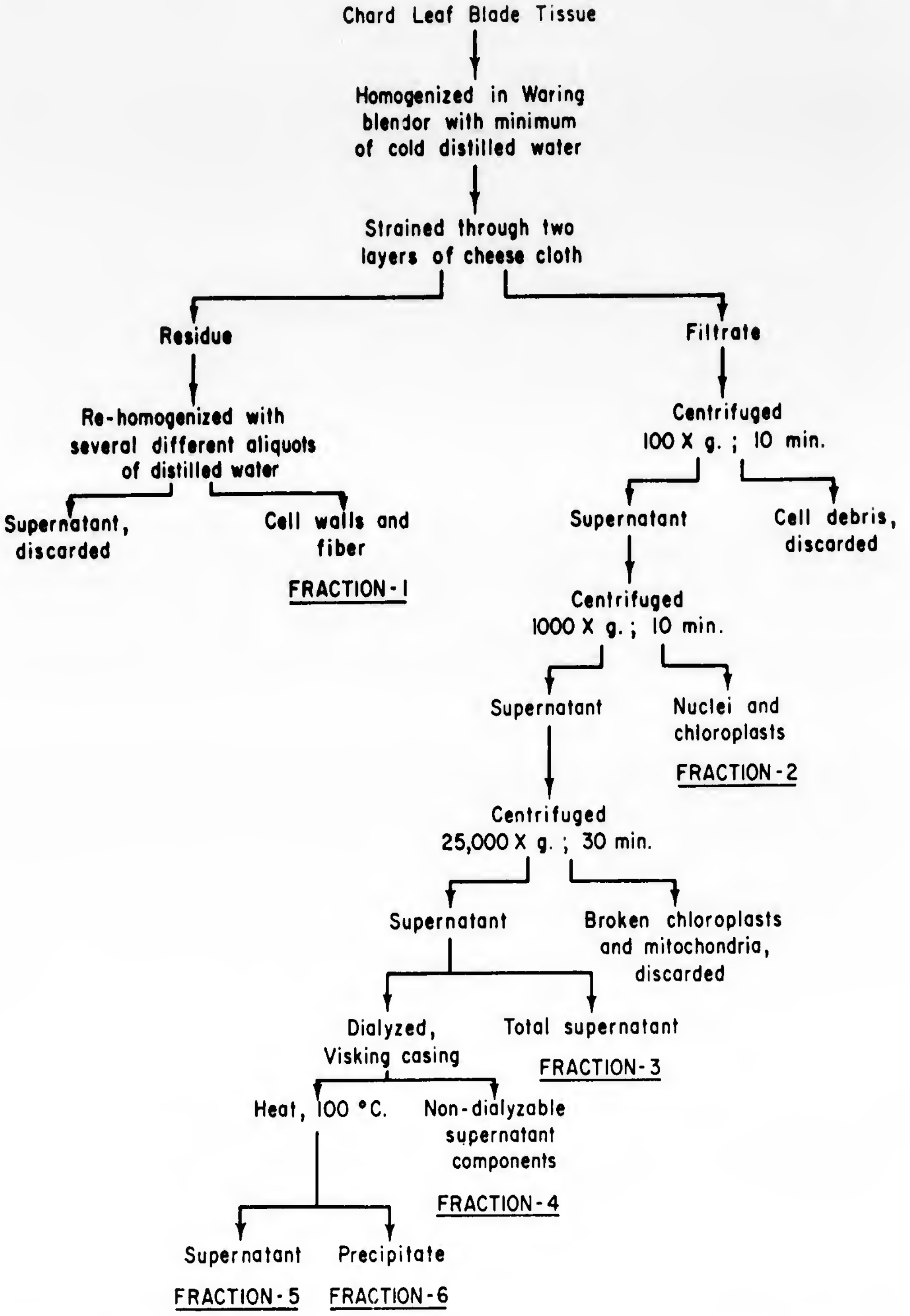

FIGURE 3. Procedure for isolation of cellular components from Swiss chard leaf tissue.

dry technique. These facts would appear to indicate that maintenance of the integrity of cellular structure during the drying process is important in determining the degree to which Swiss chard leaf tissue will rehydrate. Minimum disruption during drying is positively correlated with a high degree of rehydration.

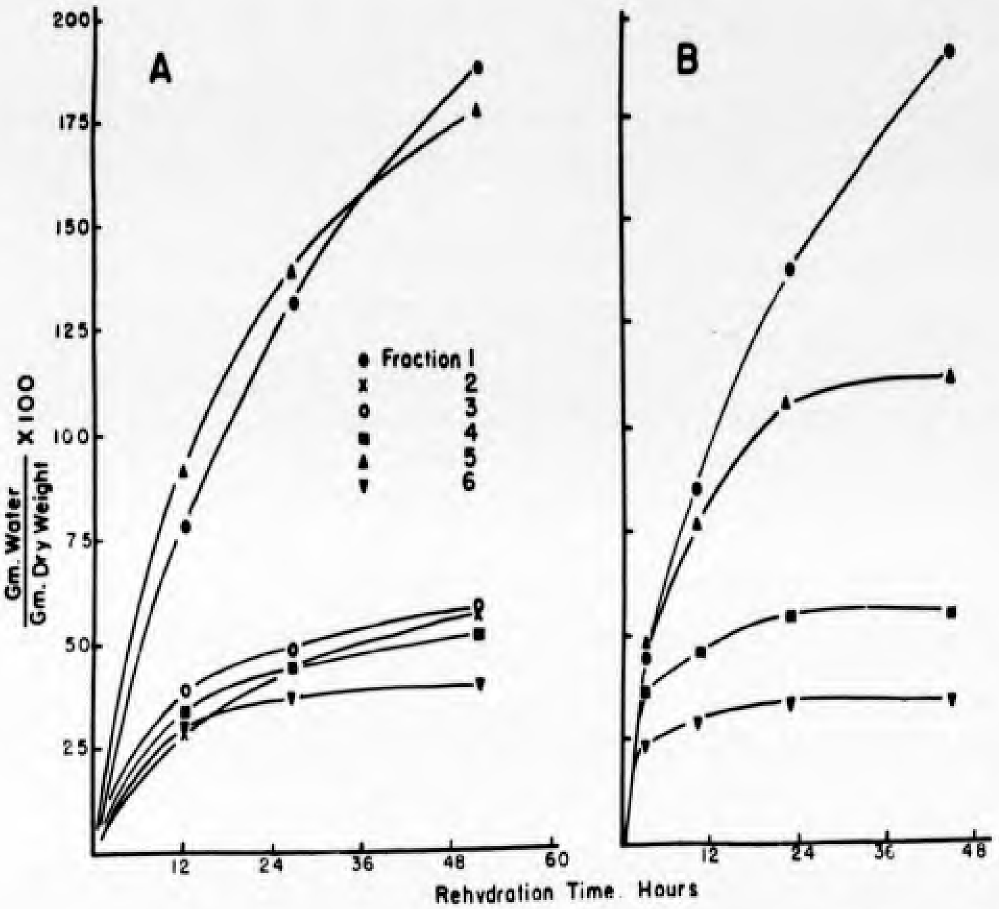

FIGURE 4. Rehydration of cellular components in an atmosphere of 100% relative humidity: A, fractions dried in a vacuum oven at 40°C; B, fractions freeze-dried. See Fig. 3 for composition of fractions.

A COMPARISON OF THE EFFECTS OF FREEZING AND DRYING ON THE REHYDRATABILITY OF FREEZE-DRIED BEEF

L. J. N. COLE

The purpose of this paper is to discuss the changes at the molecular level resulting from freezing and freeze-drying. It is reasonable to assume that any deleterious effect which is brought about by freezing and subsequent drying can reflect itself in the ability of the product to take up and retain water. It would be advantageous therefore to be able first to measure the degree of change which may result from these treatments and secondly to understand the nature of the changes in the over-all assessment of their effect on the quality of reconstituted freeze-dried beef.

Deatherage and Hamm (1, 2) have attempted to characterize the changes resulting from freezing and from freeze-drying by applying three criteria. These criteria are (a) water holding capacity by the press and tube methods, (b) buffering capacity of the water and trichloroacetic acid extract of beef, and (c) the dye binding capacities of free acidic and basic groups.

Initially these workers sought to investigate the reasons for the changes which produced a freeze-dried product that was tougher and less juicy after reconstitution than was the original frozen control. This led to a study of heated meat (3) and later, using different methods of freezing and freeze-drying, to a comparison of frozen and freeze-dried meat products. They found that there was no similarity in the type of change brought about in heated meat with that occurring in frozen and freeze-dried meat.

A comparison of fresh and frozen beef showed that over a range of pH there were very small differences to be found in the ability of the meat to bind water. Similarly, the buffering capacities of fresh and frozen meat followed closely related curves over a range of pH.

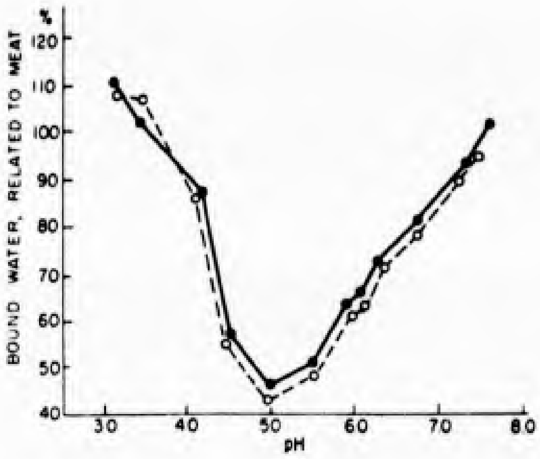

FIGURE 1. The relation between pH and water-holding capacity of fresh (●) meat and the same meat after freezing and thawing (○).

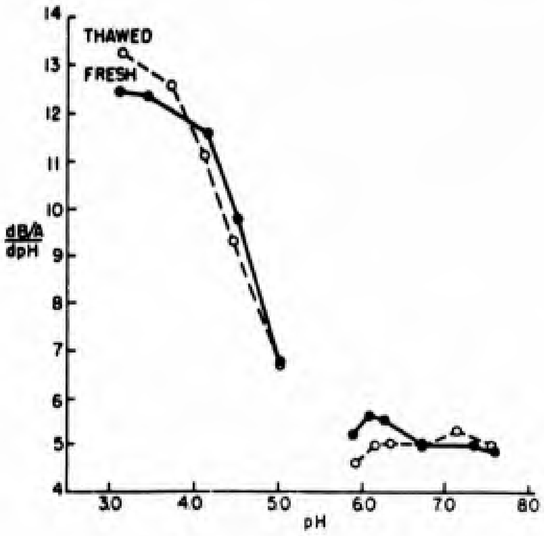

FIGURE 2. Relation between quick freezing and the buffer capacity of (●) fresh and (○) quick frozen and thawed meat.

With freeze-dried meat, however, the picture was not the same. In general, fresh meat exhibited a greater water holding capacity than freeze-dried at the natural pH of meat (5.6). Where this capacity was measured over a range of pH, freeze-dried meat was found to exhibit greater water binding capacities than fresh at pH greater than 6.5, but less at pH less than 6.5. The minimum for both products was at pH 5.0, where differences in the water binding capacities were greatest.

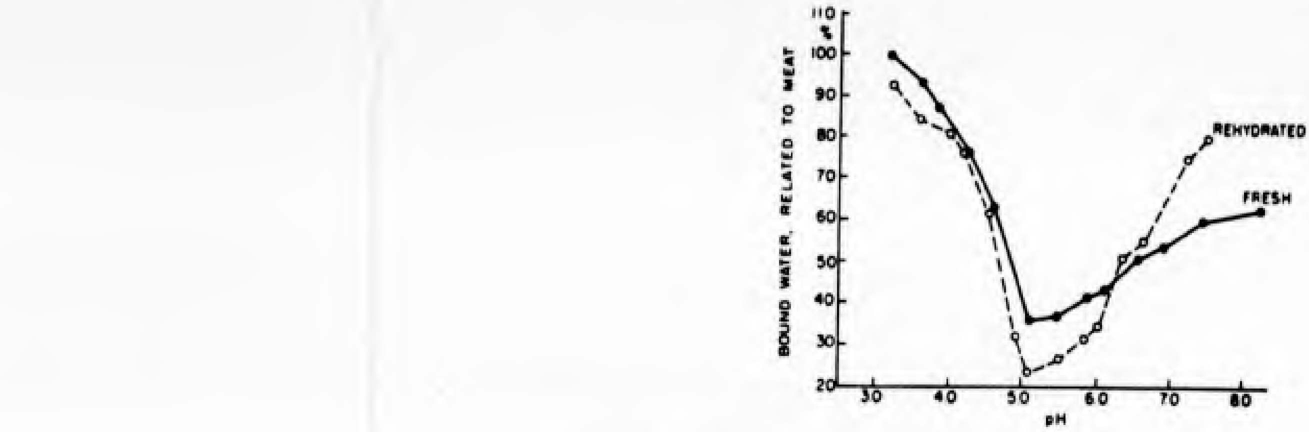

FIGURE 3. Influence of pH on the water-holding capacity of fresh (●) and rehydrated (○) meat.

There are differences also between fresh and freeze-dried meat in their buffering capacities. This difference is most marked between pH 6 and 7, where for both water extract and structural proteins freeze-dried meat exhibits a higher buffering capacity. The assumption is that more free acid groups are liberated on the basic side of the iso-electric point.

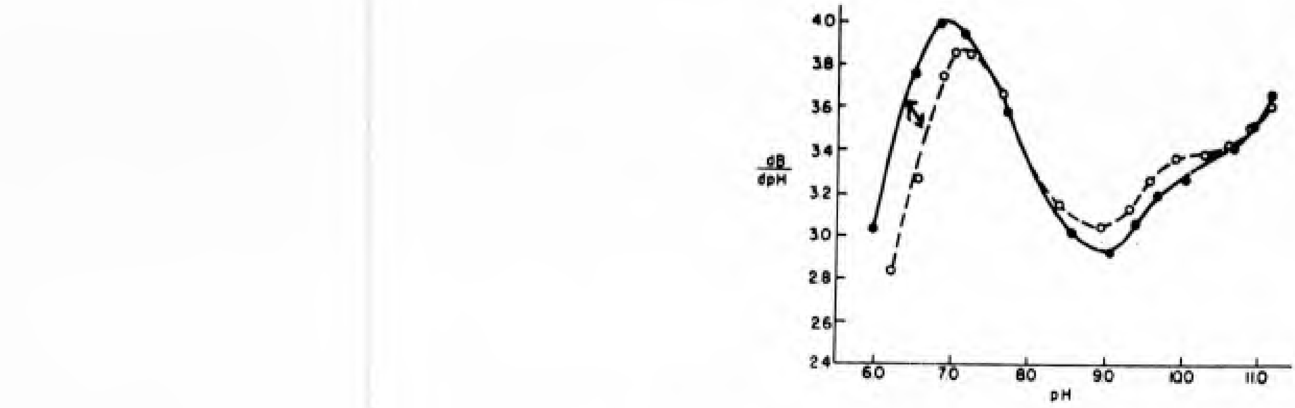

FIGURE 4. The influence of pH on the buffer power of the water extracts of fresh (●) and rehydrated (○) meat. Basic range.

The results obtained from the dye binding proclivities of free acids and basic groups support this assumption. More acidic groups are bound by the alkaline dye safranine with freeze-dried meat than with

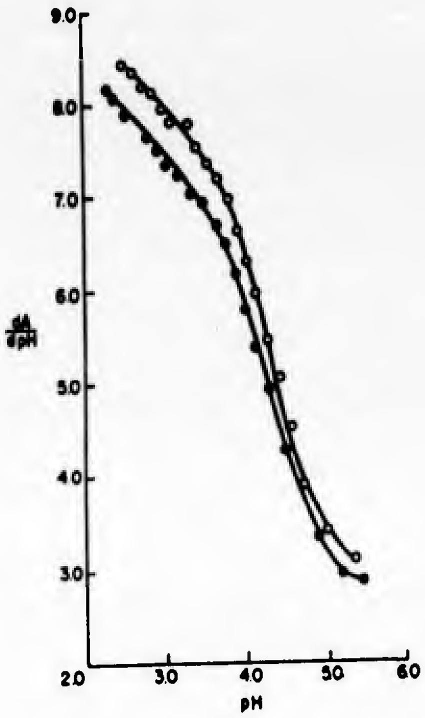

FIGURE 5. The influence of pH on the buffer power of the water extracts of fresh (●) and rehydrated (○) meat. Acid range.

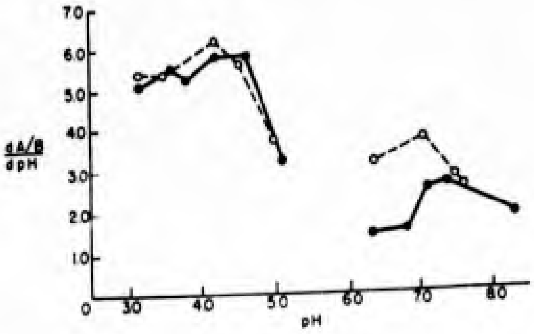

FIGURE 6. The influence of pH on the buffer power of the structural proteins of fresh (●) and rehydrated (○) meat.

fresh. With frozen meat, however, there is no appreciable difference from fresh.

TABLE 1

Influence of Quick-Freezing and Thawing on the Amount of Acidic and Basic Groups of the Muscle Proteins *

Treatment	Equivalents acidic groups per 10^4 g protein	Equivalents basic groups per 10^4 g protein
Fresh	15.5 (0.8)	13.1 (0.2)
Thawed	15.8 (0.6)	13.3 (0.2)

* The figures in parentheses are the standard deviations.

TABLE 2

Influence of Freeze-Drying on the Number of Acidic and Basic Groups of the Muscle Proteins *

Treatment	Equivalents acidic groups per 10^4 g protein	Equivalents basic groups per 10^4 g protein
Fresh	14.7 (0.6)	13.0 (0.2)
Rehydrated	16.0 (0.6)	13.2 (0.3)

* Standard deviations shown in parentheses.

From the foregoing results, Deatherage and Hamm have concluded that a reasonable hypothesis to account for freeze-drying changes would be that the removal of water gives rise to a decreased number of protein groups available to bind water after reconstitution. They suggest further that drying results in a more closed protein structure, due NOT to the formation of irreversible bonds such as disulphide, peptide, or ester type but to the salt and/or hydrogen bridge type which can be reversed at high or low pH.

At the Defense Research Medical Laboratories we have not found freeze-dried beef produced in the standard way (4) by radiant heating to be inferior in quality to the frozen control. It may be thought *a priori* that freeze-dehydration would be harsh enough to cause marked changes in the protein of beef. Less refined biochemical techniques, however, such as salt solubility have failed to point up any differences. As a result, we have had to resort to more subtle techniques in order to demonstrate such differences. In earlier work (5) during which frozen controls were used, we were able to distinguish very little difference as a result of freeze-drying when electrophoretic patterns of the 0.15 ionic strength (μ) extract (sarcoplasmic protein) were compared. Adenosine triphosphatase (ATP-ase) activities of the extracted actomyosin gave no clue, but an attempt at an electrophoretic separation of this fraction did indicate some deteriorative change.

Subsequently, a more intensive and extensive investigation has been made of the ATP-ase activities of the actomyosin extract (contractile protein) of fresh, frozen, and freeze-dried beef. It was found in every instance that stimulation of the enzyme by 2,4-dinitrophenol is less for freeze-dried and frozen than it is for fresh meat extract.

These extracts were prepared in two ways (5): one by extraction with 0.53μ solution at pH 8.3, the other by prior removal of the sarcoplasmic proteins. The method of preparation did not affect the result. These experiments were carried out on the Semitendinosus (S.T.) and the Longissimus Dorsi (L/D) from beef carcasses, some of which were aged before excision of the muscle. There appeared to be no change in the dinitrophenol effect as a result of aging, nor was there any significant difference between frozen and freeze-dried extracts.

We have also had a look at the subcellular granular fraction

TABLE 3

Comparison of DNP Stimulation of Actomyosin ATP-ase from Fresh, Frozen, Freeze-Dried Beef Activities Expressed in $\mu gP/min/mg\ N$

	Precipitated Actomyosin	
	Activity Without DNP	% Stimulation with DNP
S.T. Aged 29/3/60		
Fresh	35.5	104.2
Frozen	57.2	37.2
F. D.	40.7	9.8
S.T. Not Aged 29/3/60		
Fresh	39.9	71
Frozen	58.4	7.0
F. D.	43.1	55.0
S.T. Aged 7/1/60		
Fresh	48.7	53.6
Frozen	64.1	14.2
F. D.	61.9	3.3
S.T. Aged 16/11/59		
Fresh	36.5	41.0
Frozen	32.0	32.2
F. D.	52.1	15.5
L/D Aged 29/3/60		
Fresh	44.2	57.5
Frozen	53.1	5.8

	Indirect	
	Activity Without DNP	% Stimulation
S.T. Aged 7/1/60		
Fresh	60.2	62.0
Frozen	66.2	45.7
F. D.	44.6	41.2
S.T. Not Aged 7/1/60		
Fresh	54.0	53.2
Frozen	47.6	25.6
F. D.	43.1	35.0
S.T. Aged 29/3/60		
Fresh	54.1	61.2
Frozen	45.1	7.5
F. D.	55.6	1.6
S.T. Not Aged 29/3/60		
Fresh	54.0	53.2
Frozen	47.7	30.2
F. D.	43.1	35.0
L/D Aged 29/3/60		
Fresh	46.1	74.0
Frozen	52.3	20.2
L/D Not Aged 29/3/60		
Fresh	41.2	126.5
Frozen	55.5	11.5

obtained from fresh, frozen, and freeze-dried beef. Normally this might be compared to a sarcosomal fraction because it is prepared in a similar fashion (6). We have been unable, however, to detect any intact mitochondria in our preparations. We have therefore followed the example of Baird and Perry (7) and referred to this material as granules. In addition to electron microscopy, we have attempted to apply to these granules the criteria in standard usage in the field of mitochondrial study. These granules behave in a manner similar to muscle mitochondria. They exhibit phosphate cleavage activity in the presence of adenosine triphosphate (ATP) and magnesium. Except in isolated instances no stimulatory effects could be elicited in the presence of dinitrophenol at concentrations between 10^{-4} and 10^{-5} M.

TABLE 4

Mg^{++}-ATP-ase Activities of Granules from Sarcoplasmic Protein Extract of Fresh, Frozen and Freeze-Dried Beef

	pH of Medium	Activity (μgp/min/mg N)
Fresh L/D		
−DNP	7.2	5.4
+DNP	7.2	12.2
−DNP	7.2	6.5
+DNP	7.2	4.8
Frozen		
−DNP	7.2	2.9
+DNP	7.2	2.5
−DNP	7.5	2.5
+DNP	7.5	3.2
Freeze-Dried		
−DNP	7.5	2.9
+DNP	7.5	2.5

In all three conditions, fresh, frozen, and freeze-dried, the resultant granules possessed the ability to swell in hypotonic salt solution (0.1M KCl-Tris, pH 7.4). Swelling was increased in the presence of Ca^{++} and, with addition of ATP, was reversed in a manner resembling the behavior of mitochondria.

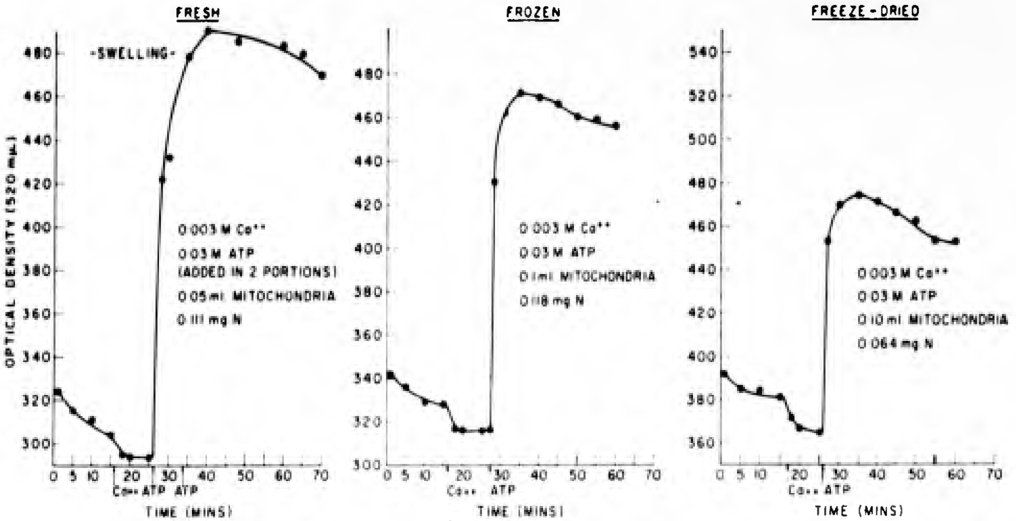

FIGURE 7. Reversal of hypotonically swollen granules from fresh, frozen and freeze-dried beef. Medium contained 0·10 M KC1-Tris buffered at pH 7·4. Additions were 0·003 M Ca^{++} and 0·030 M ATP.

Fig. 7 shows swelling and reversal curves of granules obtained from the same muscle (L/D) in the fresh, frozen, and freeze-dried states. The curves are generally representative of the type of osmotic behavior exhibited by the granules; however, the response shown by those from freeze-dried beef was not as great in some instances. As a rule the amount of granular material recovered from freeze-dried samples was less than from frozen, which in turn was less than that recovered from fresh meat. There appears to be a rough correlation, as might be expected, between optical density and concentration of granules in terms of nitrogen. Reversal of swelling appeared to be taken to its maximum by .03M ATP since further additions at this concentration had no appreciable effect; however, there were instances where single additions of ATP at .08M did produce a greater response.

We have been able also to demonstrate the presence of L-glutamic acid dehydrogenase in the granules from fresh, frozen, and freeze-dried beef. Although reliable quantitative measurements have so far proven illusive, the establishment of the retention of potency of this enzyme in subcellular granules after drying adds further support to the thesis that changes introduced as a result of freeze-drying are quite mild.

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SESSION No. 6

CONFERENCE SUMMATION

GEORGE F. STEWART, *Moderator*

MINIMIZING PRODUCT DAMAGE—BASIC BIOCHEMICAL AND BIOPHYSICAL APPROACHES

H. T. MERYMAN

It is obvious at the close of this conference that freeze-drying of foods has reached a stage of development at which the engineering design of equipment for large-scale production and the economics of the process as a whole have become major considerations. But there are hazards generated by a preoccupation with production, many of which have been all too evident over the last three days.

Production is concerned only with the quality of the product and the method and economics of its preparation. Production is not concerned with basic or even applied research. It assumes this ground to have been covered and bases its actions accordingly. Admittedly, there is substanial experimentation done at the production prototype stage. Equipment must be tested, altered, and adjusted to produce the most satisfactory relationship between input and output, a process often erroneously referred to as research. I feel that this is more than a semantic nicety and that many of the growing pains of the food drying industry may be traced to misconceptions regarding the nature and relative functions of research, technology, and testing.

If I were asked to define basic research I would say that it was research leading to an understanding of a natural phenomenon. In turn I would define applied research as the attempt to manipulate the phenomenon toward a useful purpose according to predictions or expectations based on that understanding. In both of these definitions, understanding is the key word. Applied research in the absence of an understanding of the phenomenon itself can at best be only empirical and at worst, random trial and error. It is only when one leaves the field of science and moves well into technology that a complete understanding of the basic phenomena may not be necessary; where competent and creative work can be done based on facts and formulae used simply as tools. But here flexibility is sacrificed since an empirical formula is, like a wrench of fixed size, unadjustable to meet changing needs.

When an industry becomes, as the food drying industry is becoming, production oriented, this lack of concern for a basic understanding of the system as a whole becomes a danger. Standard procedures become inviolable as though no alternative were possible; the engineering problem has been defined and the accent is on technique. This is entirely justified when the research homework has been satisfactorily done and the rules of the game are based on a sound foundation. When they are not, no amount of empirical testing of a production prototype will reveal the nature of a basic error in understanding of the principles involved.

There is a mental device used in research known as the black box. Whenever a process is inaccessible to examination, the black box becomes a useful concept. One assumes the causative events to be the input to the box and the results to be the output and then speculates upon what the contents of the box would have to be in order to create such a relationship between cause and effect. This is a legitimate and useful device, but its utility exists only when the interior of the box is invisible. Much of the discussion of the freeze-drying of foods that we have heard during this conference has been in black-box form in which alterations of the conditions of drying are correlated with the acceptability of the final product, and, from the data, inferences are made regarding the relative importance of various aspects of the drying process. The most notable example has been the discussion of the relative importance of heat transfer into the specimen and vapor transfer out as rate limiting events. On the one hand, this aspect was examined through the use of theoretical analyses of models which at best must be highly idealized and at worst can be completely unrelated to reality. On the other hand, data was presented in which variations of heat input or absolute pressure were correlated with product quality in classic black-box experimental fashion. Yet heat transfer through a frozen specimen and vapor transfer through a dried shell are easily subject to experimental measurement.

It is obviously of tremendous practical importance to know whether heat or vapor transfer is rate limiting, to know whether one should bend every effort at delivering heat to the drying boundary with a possible compromise in vapor transfer or vice versa. But it should be equally obvious that the answer cannot be easily obtained simply by varying heater plate temperature or absolute pressure in an intricately interdependent system.

During some of the discussions I fear that I was guilty of introducing the term "driving force" in analyzing the heat and vapor flow mechanism. Out of context this is a meaningless phrase. What is the driving force in an automobile? Is it the wheel on the pavement, the gasoline, the spark, the foot on the throttle, or the payments to the finance company? One must understand the complete interrelationship of the whole before the significance of any part can be placed in its proper relationship. Failure to do so can be positively misleading. For example, experiments have been reported in which the efficiency of rehydration is correlated with the rate at which the product was frozen. Taking this data in isolation one has no alternative but to accept the fact that one rate of freezing results in a dried product which rehydrates much better than that frozen at other rates. Even though this optimum rate might be inconvenient or undesirable for other reasons, one is stuck with it in order to obtain adequate rehydration. It took Professor Luyet to look inside the muscle and give us an understanding of rehydration and to demonstrate the importance of internally-trapped air. Only when one begins to understand the mechanism of rehydration does he gain the flexibility of thinking intelligently about the problem and devising means of attaining the end without undesirable and unnecessary compromise.

A second subject deserving of comment is the danger of technological isolation. One of the problems that we all have is that of discriminating between fact and theory. Facts are a property of nature—they are the possession of no one man nor of his specialty field. They demand respect and acknowledgment regardless of their origin. Theories, on the other hand, are the creation of man who tends to develop a proprietary interest in them to the extent that he is often tempted to prefer them to conflicting fact. This is particularly tempting when the conflicting fact has origin in some other discipline. There are many other applications of freeze-drying besides food, in fact the history of freeze-drying lies almost entirely in the field of histology, vaccines, blood plasma, microorganisms, and pharmaceuticals. Many of the answers that have been asked for and the measurements that have been lacking at this conference can be found in other biological literature. At length we have heard described the apparently insoluble food drying problems of physical distortion, chemical alteration, inefficient rehydration, and poor storage characteristics. Yet freeze-drying has been used with great success to prepare microscopic histological sections. Greaves has reported the preparation of proteins and microorganisms by methods that permit storage without alteration for years. Whole animals, dried intact as museum specimens, have failed to show rancidification of fats after eight years in atmosphere. These are all facts and are unavoidable. All of the problems of product change experienced in the freeze-drying of foods have been overcome in some other field by some special variation of technique.

The burden of my comments is thus twofold. Food is a biological substance composed of fat, carbohydrate, and proteins and as such differs only in detail from biologicals and pharmaceuticals. The fact that these latter materials are prepared by expensive techniques is no justification for turning one's back on the lessons to be learned. These lessons teach the attainability of perfection and one should only compromise with perfection knowingly.

To attain such knowledge, however, requires understanding of the system as a whole as well as in part, an understanding achieved by careful analysis of the mechanisms involved at each step and by experiment. The real problem is that the food drying field is not really ready for technology. Many basic principles are still cloudy. A great deal of both basic and applied research is yet to be done. Food freeze-drying is still a long way from being a simple problem in vacuum technique or heat transfer. It is still a problem for scientists, not technologists, and for experimental research, not testing. The real breakthrough in food drying will come from the intelligent understanding that is born of research. Industries say they cannot afford to do this kind of research but I question whether they can afford not to.

INCREASING PRODUCT STORAGE LIFE—BASIC DETERIORATIVE REACTIONS

D. M. DOTY

You know, there are a couple of disadvantages in being at this point on the program, especially when you have to follow someone as lucid as the previous speaker. Also, I think that anyone who attempts to summarize even one phase of a conference such as this is placed in the same position as the young man on his 21st birthday, when he inherited a harem—he knew what to do but he didn't know where to begin.

However, I would like to summarize rather briefly some of the facts which came out not only in the formal presentations, but also in the discussion which related to the basic reactions that tend to reduce the storage life of freeze-dried foods. I would hope that in this area, at least, we looked on the inside of the little black box to some extent.

First, I think that we need to establish what criteria we use for stating that a reaction is deteriorative. I think that some of the disagreements at this conference arose from the fact that we were not all using the same criteria for determining whether or not a given reaction that might occur during freeze-drying or storage was deteriorative. We heard a great deal of discussion on enzymatic activity. This activity may indirectly determine whether or not a product has deteriorated, but it is certainly not a direct measure if one uses organoleptic characteristics. If we can, for the moment, limit ourselves to characterizing those reactions as deteriorative which do have some influence on the ultimate acceptability of foods, then we can perhaps classify them, as did Dr. Olcott, into oxidative and non-oxidative reactions that occur during the storage of freeze-dried products. With that classification, we can characterize the various basic chemical reactions and begin to understand what ones we have to worry about in various kinds of food.

We have heard a lot about lipid oxidation. Despite the fact that it may be possible to prepare materials containing relatively large amounts of fat in a way that they do not oxidize during storage, it is a general observation that lipids do oxidize in the presence of oxygen. The reason for this reaction in foodstuffs, as contrasted to the whole animals which were mentioned, may be that the fat has been modified or has been liberated from its normal placement and perhaps brought in contact with reactants that stimulate oxidation. Certainly lipid oxidation is a very real problem, particularly in some food products such as fish, as was indicated by Dr. Olcott. We would be remiss if we did not mention the fact that in some foods oxidation will be a much greater problem than in others. Certainly fish are very susceptible, after freeze-drying, to oxidation of fat. Beef is not as susceptible. If we are talking about a food with a very low fat or lipid concentration, then it is not usually a serious problem.

The oxidative changes in pigments are certainly important, both in terms of the acceptability of a product by the ultimate consumer and also in terms of the nutritional values in some types of foods. There was some discussion about the nature of heme pigments in meat immediately after freeze-drying. It seems clear that the heme pigments are present after proper freeze-drying in the unoxidized form and become oxidized during storage if not properly stored.

Another type of oxidative reaction, which was discussed in some detail by Dr. Connelly, involves the protein denaturation which results from formation of -S-S- bonds. Thus some of the protein denaturation is truly an oxidative reaction.

What are some of the factors which influence the oxidative reactions? First, the product itself. In some products the oxidative reactions are extremely important; in others, of little importance in terms of storage. We have heard continuously throughout this conference that the method of processing definitely influences the susceptibility to oxidation of the finished product. We must never forget that processing is certainly a second factor. Obviously, if we are talking about oxidation, we are talking about the presence of oxygen, at least in some cases, so that any time we find a product is susceptible to oxidation we should be extremely careful about the presence of oxygen (the third factor). It was mentioned by Dr. Olcott and others that one can do much to prevent the presence of absorbed oxygen in freeze-dried products by breaking vacuum with an inert gas such as nitrogen so that we do not have appreciable levels of oxygen in the product prior to storage.

The fourth factor which influences oxidative changes is the presence of moisture. Mr. Salwin, in his presentation, discussed carefully and very clearly the influence of moisture on oxidation in some food products. He pointed out that if his theory was valid (and his data very clearly showed it to be) then different moisture levels are optimal for different products. These varied (according to his values) from six per cent for starchy products down to zero per cent for high sugar foods. Therefore, if one is attempting to prevent oxidation during storage, one should give careful attention to the ultimate moisture level to which products are dried.

Another factor in connection with oxidative reactions is the presence of antioxidants. We heard some discussion as to whether or not these could be effective in preventing oxidative reactions. It is undoubtedly true that they could prevent oxidation if we could get them to the site that would be essential for preventing oxidation. In other words, could we get them in contact with the fat? In many cases, we cannot. In this connection we need to define an antioxidant in terms of its particular use. We need, when we begin to talk about an antioxidant, to define what we are talking about and how it may ultimately act in the product itself.

Let us turn to the second type of reaction which occurs in stored products and see if we can summarize some of the things which have been brought out in this conference.

Under non-oxidative reactions, one of the most important ones mentioned time and again was protein denaturation. Dr. Connelly discussed this aspect in some detail and outlined what influence this might have on the storage life of freeze-dried products, particularly meat and flesh proteins. He pointed out that a more stable actin-myosin bond was formed and more intermyosin bonds were formed in the meat proteins both during freeze-drying and during storage after freeze-drying. I would like to emphasize again that ATP-ase activity has been shown to differ somewhat. As Dr. Cole pointed out, this activity may not be a true measure of the deteriorative reactions from the standpoint of texture or organoleptic criteria.

A second type of non-oxidative reaction, and one which came in for a lot of discussion, is the so-called browning or Maillard type of reaction. We should emphasize here that this type of reaction may not always be undesirable. We may classify it as deteriorative, but it may not be undesirable. It was clearly pointed out that some products apparently were improved in flavor by the reaction. This improvement is not surprising since we now suspect that some of the characteristic food flavors are the result of this type of combination. Dr. Olcott emphasized that the rate of the browning reaction, which usually is undesirable, may be reduced by low moisture content, reduced pH, and by separating the reactants from one another. It should be clearly indicated here that while this reaction is apparently quite general—this type of reaction occurs in all types of food products—the reactants may vary and, therefore, the final products may vary.

In summarizing the factors which may influence the non-oxidative effects that occur during storage of freeze-dried products, we first have to consider the type of product because, certainly, this will be of primary importance. In the non-oxidative type of reactions, the moisture level of the finished products becomes extremely important in preventing deteriorative reactions. Also, the reactants which are present for the browning reaction will certainly influence the type and speed of non-oxidative reactions.

It is true that this conference has not been designed primarily to bring out the practical implications of these various facets, but I think we would be remiss if we did not indicate how these various more or less fundamental reactions are related to the factors which must be considered in properly storing and preserving freeze-dried foods or products.

I am sure that the following points are clear to all of you—that in order to decrease the deteriorative actions during storage, we must be particularly careful about packaging conditions, whether we use inert gas or a vacuum, and we must consider the product in terms of whether it is one which is subject to deteriorative oxidative or non-oxidative reactions. If it is true that the first limiting factor in storage life is the browning reaction, which is non-oxidative, then it is of little value to prevent oxidative reactions and set up conditions conducive for browning reactions.

Another very important consideration is temperature. We all know that any reaction is dependent upon temperature. While we may not be able to do much about storage temperature, we can recognize how important it is in preventing deteriorative changes in final product.

Last of all we must carefully consider the moisture level for preventing deteriorative reactions in the final freeze-dried products or combinations thereof.

IDEAS FOR ACCELERATING THE FREEZE-DRYING PROCESS—BASIC PHYSICAL AND ENGINEERING PRINCIPLES

EDWARD SELTZER

Our panel started with Dr. Carman, who presented a basic discussion of molecular distillation and ordinary evaporation, showed the distinctions between them, and indicated that molecular distillation could apply in freeze-drying only if one got to very low temperatures, such as −80°C. The conditions regarded as molecular distillation did not need to hold for ordinary water evaporation, namely, for low molecular weight material, such as those actually obtained in freeze-dehydration. He developed some equations which will go into our Proceedings and can be more readily studied with some care and attention than at the blackboard.

This presentation served as a very good basis for succeeding business. Mr. William Mink of Battelle Memorial Institute then presented his views developed from available literature about some factors controlling the rate of freeze-drying and about the major factors related to potential barriers, mass transfer and heat transfer. His conclusion was that in most cases heat transfer was a limitation—that mass transfer was not within consideration in practical freeze-drying except when heat transfer was at such a rapid rate that, at a certain range, the mass transfer barrier curve intersected the heat transfer curve and then mass transfer added to the limitation of freeze-dehydration. He had prepared some mathematical models which summarized his views of the mechanisms.

This paper was followed by one by Dr. Marshall and Dr. Lambert. These two gentlemen took the view that the two mechanisms were not separate and that there needed to be a combined expression because the boundary between mass transfer and heat transfer was not sufficiently well known. Now, these men had worked directly with several food materials and had direct experimental evidence. Mr. Mink's report was based on information from other people's published literature. Drs. Marshall and Lambert's literature will now be published in our Proceedings and will help people such as Mr. Mink in providing a far better basis for setting up mathematical models. Of course, this interaction is one of the very great accomplishments of a meeting of this type.

Moreover, Drs. Marshall and Lambert tended to coincide in many fundamental respects with the principles as presented by Dr. Carman. I think here also we will have to see the equations in the Proceedings to understand how closely they agree.

We proceeded from there to a talk by Mr. Albert Leatherman on dielectric heating and its possible applications to freeze-drying. This was essentially a presentation of the fundamentals of dielectric heating without actual experimental evidence related to freeze-drying, the presentation of the common equation, and a discussion of energy sources and potential difficulties in working in certain vacuum ranges or pressure ranges.

Our concluding paper by Mr. B. O. M. Gall and Mr. Roger LaPlante was on methods of application of microwave energy to industrial processes. These men performed the good service of showing us what kinds of magnetrons or other types of microwave sources were available and of describing the techniques that were being used to apply them to materials on moving belts and to cover all of the zones so that the material would be uniformly treated. Even though the applications were not specific to freeze-dehydration, this paper presented knowledge of available equipment and work being done by some progressive and outstanding companies that can now be used in the area of freeze-dehydration.

IMPROVED INSTRUMENTATION FOR FREEZE-DRYING OPERATIONS—EXPLORING NEW APPROACHES

CLINTON O. CHICHESTER

In order to intelligently design a unit operation, it is imperative that the basic phenomenon of the process be understood. In our case, we are dealing with the sublimation of water from a biological support under conditions of low total pressure. The process is thus not a unique one, at least from an engineering standpoint. The design unknowns in food lyophillization are concerned primarily with the physical parameters of the support. Data, as well as theoretical analyses of the over-all system as discussed by Dr. Marshall in his paper, are available to the engineer. If the engineer can be provided with the physics of the movement of water vapor through the support, in this case the food, the entire system is amenable to analysis. As we have seen from the papers presented and in the discussions following, there is general agreement as to the mechanism of water transport and heat conduction. Data have been presented by a number of the participants to define, at least partially, the characteristics of the materials we are dealing with from an engineering standpoint. There is no need to suggest that all of the data which could be of use has been collected, but it should be stressed that sufficient data and analysis of basic mechanisms are now on hand to be able to understand the process of freeze-drying and to allow the designing of units on a sound engineering basis.

The over-all problem in the acceleration of lyophillization is centered about the transfer of thermal energy in a vacuum. We have seen in the analysis of all of the engineering-oriented participants unanimous agreement that a limiting factor in obtainable drying rates at the present state of the art is transfer of the energy of sublimation from a reservoir to the ice interface. It has been suggested that if the rate of energy transfer could be increased many-fold some difficulty might be anticipated in providing sufficient vapor removal, but under present conditions heat transfer is rate limiting. In all cases encountered in conventional or accelerated freeze-drying, the process involves a balance between the outward flow of vapor and the inward flow of heat. Vapor movement from the ice phase takes place both by hydrodynamic flow, as a result of a gradient in total pressure difference, and by diffusion, resulting from a gradient in the partial pressures of water. If the total pressure in the vacuum chamber is small compared to the vapor pressure of the ice within the material, diffusive transport is negligible compared to hydrodynamic flow. At higher total pressures, there is a negligible pressure gradient and transport is by diffusion. Because diffusion coefficients vary inversely as the total pressure, vapor transport rates drop off rapidly as the pressure is increased.

As a possible method of increasing the rate of heat transfer, it has been suggested that dielectric heating be utilized since this process is one of the very few which does not require that the thermal flow of energy take place through the dried material. Dr. Decareau presented information on some pilot scale runs using microwave methods to supply the energy of sublimation. In these experiments, the total input power was relatively low, yet the rate of drying was significantly higher than that encountered in contact plate freeze-dryers. In an analysis of the economics of the process, Dr. Decareau's data showed that the cost of using microwaves was approximately comparable to the use of conduction heating in a contact type dryer. If, however, he used a combination process, such that the major portion of the water was removed by conventional methods which have moderately high initial rates of evaporation and then utilized microwave energy to sublime the remaining ice, the unit costs were considerably below either of the other examples.

Previous speakers indicated that the economics of dielectric heating were becoming more favorable. High power magnetrons are being manufactured at the present time and employed in industrial processes. If the usage of these tubes becomes more wide-spread, considerable economies may be realized and thus be reflected in lower costs in the design of microwave freeze-dryers.

In any process of freeze-drying, it is particularly important, if maximum speed is to be realized, that the processing be discontinued the moment the product is dry. Ideally, one would desire all portions of a large quantity of product to dry uniformly so that the process could be discontinued as soon as the bulk water content was reduced to the desired point. Unfortunately, this is not the case and in most practical situations there is non-uniform drying. The process thus must be continued until the slowest drying unit is completely dried. If this is not done and semi-dried products are included, it is quite possible to destroy the quality of the entire batch. Dr. Kann discussed four methods for determining the end point of the drying cycle. Dr. Oetjen also contributed to this discussion. At the present time, it would appear that either the vapor pressure rise method or the pressure depression method of determining the end point of drying is most amenable to immediate use. The vapor pressure rise suffers from the disadvantage of being affected by leakage into the drying chamber. The vapor pressure depression method does not suffer from this disadvantage, but requires the installation of some additional equipment.

Two methods which appear promising but require additional research are the use of a mass spectrometer to determine the water vapor content of the drying chamber and the use of scanning infrared units for the detection of water in the dried product.

Dr. Oetjen discussed various methods of increasing drying rates in conventional dryers. His data demonstrate that it is desirable to operate at the maximum permissible ice temperature within the product. Considering the unit operation as a whole, the use of higher pressures and higher temperatures promote higher rates of water vapor flow. These factors, of course, must be balanced against the product quality desired and certainly must take into consideration the transport of the energy of sublimation. In analyzing the factors to be considered, Dr. Oetjen showed methods of evaluating the thermal conductivity and the permeability of dried materials. The results of these experiments confirmed those which have been previously published and extend the available design data for freeze-drying units.

In discussions following the various papers, Drs. Carman, Sunderland, Harper, Morgan, and others were in general agreement as to the fundamental mechanism of energy and vapor transport in freeze-drying. Additional work is needed to clarify the relative importance of the different transport mechanisms in the over-all process. Of particular significance in this regard is the reported observation that there is an optimum pressure for maximum rate of drying. Additional work is also required on the over-all process in order to investigate the possibilities of utilizing alternate energy supplies such as dielectric energy, ultrasonic energy, and radiant energy. Combinations of these sources of energy with refined designs of the over-all process may contribute materially to the acceleration of freeze-drying. The effect of freezing rates on the physical characteristics of the products as well as the effect of freezing temperatures and drying temperatures on product quality and drying rate appear to require substantial investigation.

IMPROVING PRODUCT REHYDRATION—BASIC BIOCHEMICAL AND BIOPHYSICAL APPROACHES

M. C. BROCKMAN

In introducing this session it was pointed out that the speed with which freeze-dried foods attained an acceptable level of hydration represents a major military advantage for products dried in this manner. Recognition was also given to the fact that too frequently rehydration results in a product characterized as dry, spongy, tough, or otherwise of decreased acceptability.

The first speaker, Dr. Smithies, provided a general picture based on his broad experience with the preparation, the rehydration, and the quality evaluation of freeze-dried meats, both raw and cooked, as well as fruits and vegetables. Attention was directed to specific practices which have been observed to produce favorable or unfavorable effects on the reconstituted product. It was encouraging to note that sliced meat, freeze-dried according to a defined procedure, can be rehydrated in five minutes to yield a product which is difficult to distinguish from its frozen control.

The next speaker, Dr. Luyet, described freeze-drying as a three stage process. The first stage, freezing, results in a separation of water from the tissue in the form of ice crystals; from the standpoint of the tissue this separation is, in reality, dehydration. In the second stage, ice crystals are caused to sublime; this represents removal of water which has already been separated from the tissue and, in a strict physical sense, is not dehydration. The third stage is characterized by evaporation of residual water. Since residual water is from non-crystalline sources, its removal cannot be regarded as true freeze-drying. In specific cases shrinkage or collapse of structural components is associated with the withdrawal of this unfrozen or residual water.

As pointed out by Dr. Luyet, significant structural differences are observed between slowly and rapidly frozen muscle. Moreover, differences in the speed of freezing are reflected in the rate of freeze-drying.

in the structure of the freeze-dried muscle, and in its behavior on rehydration. Single fibers from both rapidly and slowly frozen muscle rehyrate very rapidly when flooded with water.

In the rehydration of freeze-dried muscle tissue, water penetrates either through large channels formed by ice crystals during slow freezing or through the minute cavities formed within the fibers during rapid freezing. This is followed by an inhibition of water by the tissue solids. Recognition was given to the lack of information on the binding of water by tissue solids. Several obstacles to the penetration of water were noted, such as water repellent surfaces which interfere with wettability, impermeable membranes which resist transmission of water, and trapped air which may prevent entry of water into dry tissue cavities.

On the basis of a series of experiments performed on vegetable tissue, mainly Swiss chard, Dr. McIlrath found that freeze-dried tissue has a greater water uptake than comparable tissue dried from the non-frozen state and that more of this water is bound, as indicated by the water retained after vigorous centrifugation. When the structure of a leaf is destroyed and the cellular components subjected to rehydration, the level of rehydration is far below that obtained by the intact tissue. This observation points to the relationship of basic structure to the attainment of normal levels of tissue rehydration.

The last speaker, Dr. L. J. N. Cole, reviewed the published results of investigations undertaken to throw light on the nature of certain adverse changes which have frequently been associated with freeze-dried meats, such as decreased water-holding capacity, incomplete rehydration, and increased toughness. Studies have included measurement of the water binding and buffer capacities at different pH levels, dye binding by acidic and basic groups, protein solubility in various solutions, electrophoretic behavior of protein fractions, and activity of specific enzymes. Many of these experimental approaches fail to identify significant changes which can be attributed to freeze-drying. Other studies point to the possibility that drying brings about reversible changes which result in a more closed protein structure. Recent experiments conducted at the Defense Research Medical Laboratories on the ATP-ase activity of extracts from fresh, frozen, and freeze-dried beef have failed to reveal consistent differences. The addition of 2,4-dinitrophenol, however, produces a marked stimulation of a ATP-ase activity in extracts from fresh beef; comparable extracts from freeze-dried beef show significantly less stimulation. An interpretation of this interesting observation remains to be developed.

In summarizing this session involving phenomena related to rehydration, it is appropriate to recall that speakers at other sessions have called attention to additional factors which influence the rate or the level of rehydration. Among the considerable list of such factors are the chemical and physical effects of a high concentration of soluble material in the unfrozen water, mechanical damage to cells from freezing, chemical reactions involving the unfrozen water, and damage resulting from rehydration by immersion in water.

This session has emphasized a fact solidly supported by experience, namely, that a freeze-dried food imprints its history and in one way or another its history is reflected in its rehydration pattern. The technology of freeze-dried foods has advanced, essentially on an empirical basis, to the point that a considerable variety of meats, fruits, and vegetables can be dried and reconstituted with, at most, minor changes. There remains, however, a substantial void in our knowledge of the nature of the processes involved, of the requirements for attaining a reversible process, and of the means for controlling the all critical operations by objective methods.

NATIONAL ACADEMY OF SCIENCES
NATIONAL RESEARCH COUNCIL

The National Academy of Sciences-National Research Council is a private, nonprofit organization of scientists, dedicated to the furtherance of science and to its use for the general welfare. The Academy itself was established in 1863 under a congressional charter signed by President Lincoln. Empowered to provide for all activities appropriate to academies of science, it was also required by its charter to act as an advisor to the Federal Government in scientific matters. This provision accounts for the close ties that have always existed between the Academy and the Government, although the Academy is not a governmental agency.

The National Research Council was established by the Academy in 1916, at the request of President Wilson, to enable scientists generally to associate their efforts with those of the limited membership of the Academy in service to the nation, to society, and to science at home and abroad. Members of the National Research Council receive their appointments from the President of the Academy. They include representatives of the Federal Government, and a number of members at large. In addition, several thousand scientists and engineers take part in the activities of the Research Council through membership on its various boards and committees.

Receiving funds from both public and private sources, by contribution, grant, or contract, the Academy and its Research Council thus work to stimulate research and its applications, to survey the broad possibilities of science, to promote effective utilization of the scientific and technical resources of the country, to serve the Government, and to further the general interests of science.

ADVISORY BOARD ON QUARTERMASTER RESEARCH AND DEVELOPMENT

Recognizing the need for independent scientific advice on his research and development program, The Quartermaster General, in 1943, requested advisory services and for this purpose established a formal contract with the Academy-Research Council. To fulfill the terms of this agreement, the *Committee on Quartermaster Problems* was organized by the Academy-Research Council under the Division of Engineering and Industrial Research. In 1948, the scope of the Quartermaster advisory activity was broadened, and the committee was reorganized as the *Advisory Board on Quartermaster Research and Development.*

The objective of the Advisory Board on Quartermaster Research and Development and its committees, by providing scientific and technical advisory services to the Quartermaster Research and Engineering Command, Natick, Massachusetts, is to aid the Quartermaster Corps in the most efficient achievement of the Corps mission—protecting, feeding, and supplying the combat soldier in any future emergency.

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