Mechanisms of Deterioration of Nutrients

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Annual Report - Phase 2

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1. General Introduction

Phase II of this contract has been devoted to continuation of studies initiated during Phase I, which will lead to development of methods by which freeze dried foods of improved quality will be produced. In addition to continuation of Phase I studies, Phase II saw the initiation of a study into the technology of preparation of artificial food matrices.

As listed in the Phase I end items, studies have continued in the following areas:

1) The applicability of theories of flavor retention developed originally in mono- and disaccharide containing food liquids has been demonstrated for a number of food polymers, both proteins and polysaccharides.

2) Studies on the formation of structures during freeze drying have been continued for emulsified systems using the optical microscope having a special freeze drying stage.

3) The deterioration of organoleptic quality of freeze dried foods due to high temperature heating has been evaluated, and improved proceedures developed.

4) The influence of water activity and high temperature on retention of model flavor materials and browning deterioration has been evaluated for model systems and food materials.

5) A freeze dried food of high quality whose processing is based on results obtained in areas discussed

above has been prepared and sent to NASA/MSC as provided for in the contract schedule for Phase II.

6) Studies on production of a totally artificial food applicable to advance spacecraft food system needs has been initiated.

The scheme of presentation utilized in the Phase I Annual Report has been continued in this Annual Report. The research areas under investigation in Phase II are presented as separate sections as follows:

2. Studies using the freeze drying microscope

3. Browning of dried foods at high temperatures

4. Artificial gel structures for engineered space foods

5. Volatile retention during freeze drying

6. Freeze dried food products of improved quality.

A summary of the results of Phase II is presented as the last section (7).

2.   Studies using the freeze drying microscope

2.1 Introduction

The development of a freeze drying apparatus for mounting on a microscope stage for continuous observations at high magnifications of freezing and freeze drying behavior has been described in the Phase 1 Annual Report of this contract. A technical article based in large part on data presented in the Phase 1 Annual Report has since been published and is included here (as section 2.2) since it concisely describes the freeze drying apparatus and the methods of utilization. Section 2.3 presents an analysis of the freeze drying behavior of the ice/dry layer interface, as our observations had raised some question as to the nature of the moisture content gradient.

In conjunction with the project end items regarding food matrices, studies were initiated on the microscopic freeze drying behavior of various food components, relative to phase separation and matrix structure during freezing and freeze drying. These initial studies have concentrated on systems which could be more easily handled in the microscope, and generally consist of a non-volatile carbohydrate, a volatile or non-volatile organic liquid and water. These studies are discussed in sections 2.4 and 2.5.

2.2 The freeze drying microscope

Microscopic investigations of the freeze drying of volatile-containing model food solutions.

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MICROSCOPIC INVESTIGATIONS OF THE FREEZE DRYING OF VOLATILE-CONTAINING MODEL FOOD SOLUTIONS

INTRODUCTION

AQUEOUS SOLUTIONS of organic solutes are the basic fluids of biological systems, such as the extracellular and intracellular fluids of solid food materials. Extraction of these fluids from food solids results in organic-containing aqueous solutions of economic importance to the food industry. The behavior of these extracts during the freezing process is quite important in determining the final quality of freeze-dried products (Flink and Karel, 1970b; Flink and Labuza, 1972; Rulkens and Thijssen, 1972). This includes both the period of the lowering of temperature to a level at which the

solvent, water, starts to crystallize as ice, as well as during the continuation of the freezing process when the increased conversion of water to ice results in the eventual solidification of the aqueous solution as a relatively complex system of ice crystals, concentrated solute phase or phases, and perhaps even pure solute phases.

The flavors of food materials are composed of numerous organic compounds present at low concentrations in aqueous solutions. Most studies on flavor quality retention have dealt with measuring the before, during or after retention of suitable volatile organic compounds from

nonvolatile solute-containing aqueous solutions (Flink and Karel, 1970a,b; Thijssen and Rulkens, 1969; Rulkens and Thijssen, 1972; Chandrasekaran and King, 1972).

In a continuation of studies on the desorption of volatile organic constituents from aqueous carbohydrate systems during freeze drying (Flink and Karel, 1969, 1970a,b), microscopic analysis of the freeze-dried material showed changes which had taken place in the system during the freezing and freeze drying (Flink and Gejl-Hansen, 1972). Most notable were the partitioning of the carbohydrate nonvolatile solute (maltodextrin) into two fractions, and the appearance of the volatile organic constituent (hexanal) as liquid droplets. Flink and Gejl-Hansen concluded that the formation of the liquid droplets and their partitioning into the carbohydrate-rich phase were very important factors in accounting for the retention of the volatile organic compound during freeze drying.

To determine during which part of the freeze-drying process the partitioning occurs requires the development of a freeze-drying microscope stage on which the entire freeze-drying process could be observed. Various freezing and freeze-drying microscope stages have been reported in the literature (McCrone and O'Bradovic, 1956; MacKenzie, 1964; Chauffard, 1971; Freedman et al., 1972), though most studies using this equipment have dealt with investigations on conditions affecting the development and behavior of the ice phase (Luyet, 1968). Rey et al. (1966) have reported on microscopic observations of the solidification and freeze-drying behavior of organic mixtures and aqueous solutions of inorganic salts.

This paper reports on (1) the design of a new freeze-drying microscope stage capable of continuous observations at the high magnifications required for flavor retention studies and (2) the results of studies on the separation and retention of volatile organic compounds during freeze drying.

①	GLASS WINDOWS
②	
③	GLASS COVER SLIP
④	COOLING INLET AND OUTLET
⑤	
⑥	VACUUM PASSAGE
⑦	RUBBER O-RING
⑧	ALUMINUM RING
⑨	SAMPLE COVER SLIP
⑩	THERMOCOUPLE JUNCTION
⑪	GLASS CHIPS

Fig. 1—Microscope stage freeze-drying system with detail of sample holder.

EXPERIMENTAL

The freeze-drying microscope

The freeze-drying microscope is capable of

being used for continuous observation of freeze-drying samples at magnification up to 900×. This high magnification is essential when investigating the formation and separation of droplets within the nonvolatile solute matrix.

The freeze-drying microscope consists of three systems which can be considered independently: the microscope system, the freeze-drying system and the data acquisition systems.

The microscope system. The freeze-drying microscope is based on an Olympus Model EH microscope body. As this microscope is modular, it is "custom designed" by being equipped with the following special options:

1. Trinocular head to allow for both visual and data acquisition;

2. Wide field and flat field photographic eyepieces of 15× which allow magnifications at the limit of resolution;

3. A polarizing filter set used to evaluate crystallinity of the sample;

4. A dry 60× objective which can be used for high magnification (900×) analysis of the freeze-dried material;

5. A long working distance 40× objective that enables the distance between the sample and front surface of the objective lens to be 1.3 mm. This distance is an important consideration when designing the freeze-drying chamber.

The freeze-drying system. The freeze-drying system consists of a freezing and freeze-drying chamber specially designed and constructed in this laboratory for use with the Olympus microscope and the associated support equipment.

The freeze-drying chamber is a vacuum-tight optical system which is capable of being refrigerated (Fig. 1). Three glass windows set into an aluminum block provide for sample holding (2) and vacuum seals (1, 3). The block also contains an internal path for the flow of chilled refrigerant (4, 5) as well as a vacuum passage to the lower vacuum chamber (6). The glass sample holder has a large contact area with the aluminum block at the refrigerated end, giving a large heat transfer surface. A gap is left between the sample holder and one wall of the block permitting air and water vapor to flow to the lower chamber and out the vacuum line. The lower window is semi-permanently sealed to the block with Apeizon putty. The upper window, a 0.17 mm thick cover glass (45 × 50 mm) rests on a rubber O-ring (7) that is held in circular shape by an aluminum ring (8). The total thickness of the O-ring and cover slip is less than 1.3 mm, allowing use of the long working distance objective. Chips of a cover slip are placed at the extremities of the sample holder to support a cover slip for the sample at an approximate distance of either 170 μm (1 chip) or 340 μm (2 chips). This enables control of the sample thickness which is optically important.

A dry ice-alcohol refrigeration system is used for freezing and temperature maintenance during freeze drying (Fig. 2). A bottom-emptying flask maintains a flooded suction head on the centrifugal pump. Fluid is pumped to the freeze drier through a copper coil immersed in a dry ice-alcohol bath before being returned to the suction line reservoir. Temperature can be regulated either by on-off cycles of the pump, or by control of the pump speed.

A single stage rotary oil vacuum pump is used in conjunction with a CaSO₄ desiccant vapor trap to remove fixed gases and water vapor from the freeze-drying chamber.

Dried compressed air is gently blown across the upper cover slip window to prevent condensation of environmental water vapor. The lower
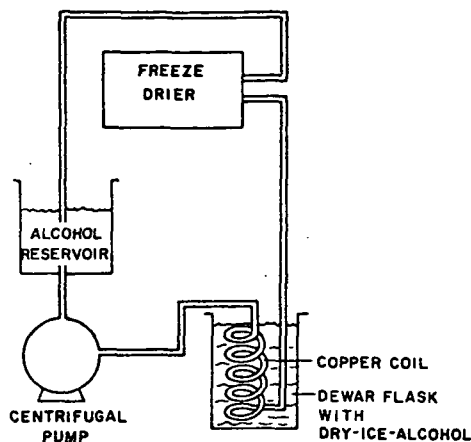
Fig. 2—Refrigeration system for freeze-drying microscope.

○ DROP OF VOLATILE
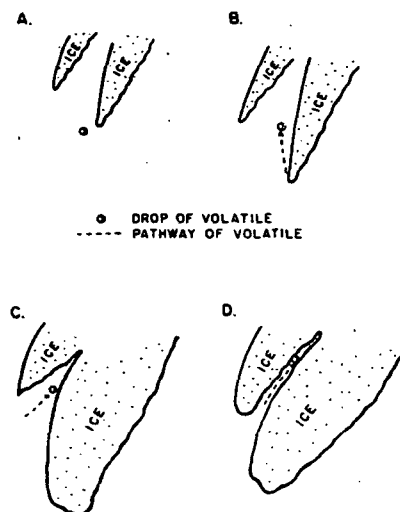----- PATHWAY OF VOLATILE

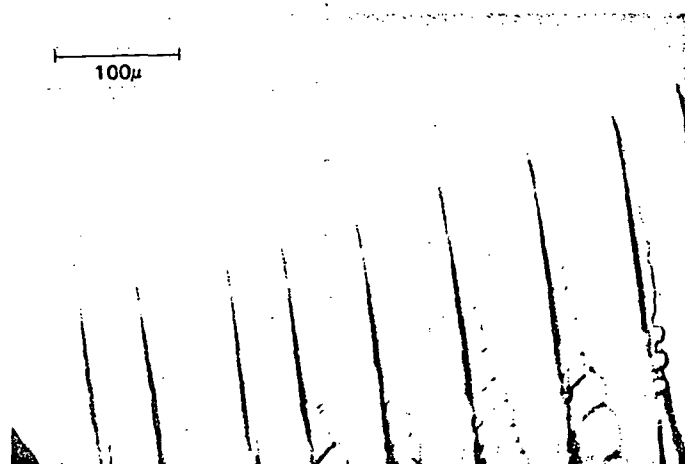Fig. 3—Path taken by hexanol droplet during freezing of aqueous maltodextrin solution.

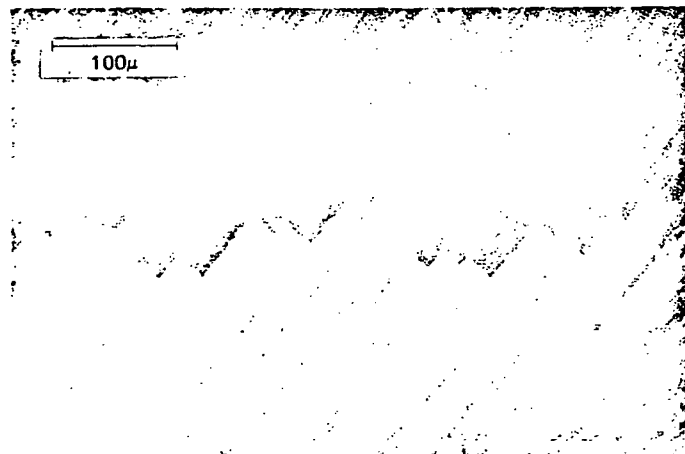Fig. 4—Ice dendrites during freezing of 3.3% maltodextrin solution (150×).

Fig. 5—Freeze-drying front in 3.3% maltodextrin solution (150×).

Fig. 6–Freeze-drying front in 3.3% maltodextrin solution (600X).

Fig. 7–Freeze-drying front in upper layer of 10% maltodextrin solution (shadow of lower region front is to left) (150X).

glass surface is within an insulation system and does not suffer condensation problems.

A carved balsa wood container is used for thermal insulation. This container is attached to the motion controls of the microscope stage.

Data acquisition systems. Evaluation of freezing and freeze-drying experiments requires the measurement of temperatures and pressures as well as documentation of visual observations. The following instruments supply this information.

Temperatures are measured by insertion of a microthermocouple junction into the samples (Omega Engineering Co., Stamford, Conn.). The junction diameter is 125 μm. The thin thermocouple wires (50 μm) are passed between the O-ring and aluminum block of the freeze dryer without loss of vacuum.

The system pressure is measured on the vacuum line with a thermocouple-type vacuum gauge having a range of 0-20 torr (Veeco Instruments).

Photographic records of typical visual observations are made with either a Polaroid ED-10 microscope camera or a 35 mm camera.

Visual observation is made via a closed circuit television system. This allows long visual observation periods without serious eyestrain and further, groups of people can observe and evaluate each experiment.

Methods

Preparation of model system. An aqueous solution is prepared according to a standardized procedure. This is especially important when utilizing components of limited solubility. The model system is either held at preparation temperature or chilled to 0°C by holding in crushed ice.

Preparation of microscope equipment. The dry ice-alcohol cooling system is prepared and the microscope stage connected to the cooling system. The freezing stage may be precooled to 0°C at this time, if desired. Dehumidified air sweeps the stage to prevent condensation of water vapor.

Sample freezing. The cooling system flow is adjusted to give the desired freezing conditions at the microscope stage. Freezing progress is followed either visually or photographically.

Subsequent steps prior to freeze drying. Upon the completion of the first freezing analysis, the sample can either be freeze dried or thawed and subjected to further freezing analysis. The latter is of interest when studying the resolubilization of the organic constituents or the influence of freezing history on subsequent freezing and freeze-drying behavior. Thawing is accomplished by stopping the coolant flow or additionally removing the heat absorbent from the lamp of the microscope optical system.

Sample freeze drying. Upon completion of the final freezing analysis, the chamber is evacuated and the frozen sample is freeze dried. Due to the small sample dimension, relatively rapid sublimation of the ice occurs. Following drying, the material can further be analyzed by more standard procedures as described by Flink and Gejl-Hansen (1972).

RESULTS & DISCUSSION

EXPERIMENTAL RESULTS can be divided into characterization of freezing and freeze-drying behavior in the micro-
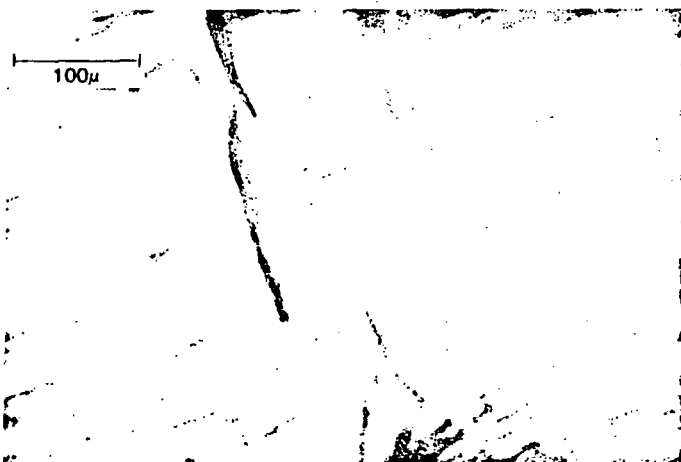
Fig. 8–Freeze-drying front in lower layer of 10% maltodextrin solution (shadow of upper region front is to right) (150X).
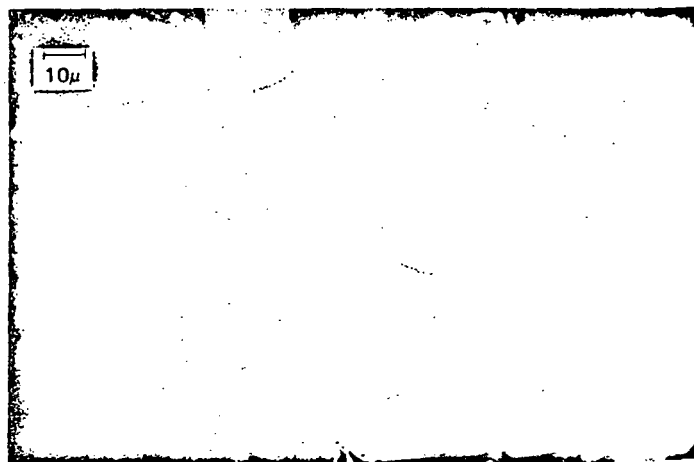
Fig. 9–Hexanol droplets at ice crystal grain boundries (600X).

Fig. 10—Hexanol droplets in freeze-dried matrix, 100 microns into sample (150×).

Fig. 11—Hexanol droplets in freeze-dried matrix; same view as 10, but at sample surface (150×).

scope, and studies on the freezing and freeze drying of aqueous solutions containing volatile organic compounds.

Freezing behavior

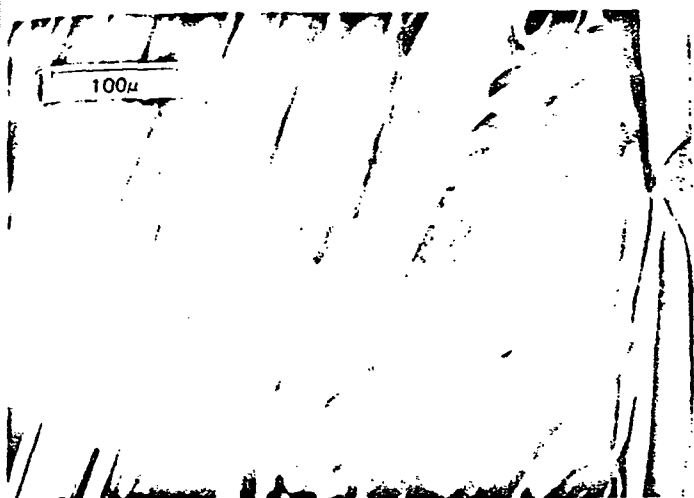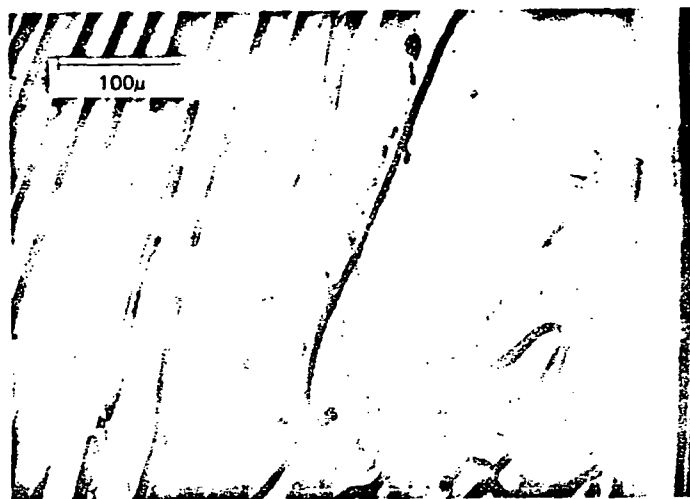Freezing of water and aqueous maltodextrin solutions (10%) from room temperature generally occurs in 2–10 min. Temperature measurements indicated that the maltodextrin solution commences freezing at approximately −5°C and is completed at −7°C (Fig. 13).

Fast freezing is characterized by solidification of the sample in less than 20 sec (initial appearance of ice to complete solidification). The ice structure appears as plates or sheets without fine structure (Fig. 9).

Slow freezing is characterized by a fast freezing of a small part of the sample closest to the chilled surface followed by dendritic growth of ice crystals over a period of 0.5–10 min (Fig. 4). Some samples which have been slow frozen in thin slabs undergo dendritic crystallization in two layers; the bottom of the sample crystallizes first as disordered dendrites (Fig. 8), the upper region crystallizes later as ordered dendrites (Fig. 7).

Freeze-drying behavior

Freeze-drying fronts (the moving interface between frozen and dried regions) recede into the sample from all four sides. Samples approximately 1 cm × 1 cm, and 0.155–0.3 mm thick under a cover slip require from 50–80 min to freeze dry.

Separate freeze-drying fronts are observed in each of the different ice crystal orientations (and thus solute matrix orientations). The fronts are not completely planar, with small variations occurring among crystals having the same orientation (Fig 5, 6), and larger varia-

tions for crystals of different orientation (at different vertical locations) (Fig. 7, 8).

Freezing and freeze drying of aqueous solutions containing volatile organic compounds

Aqueous solutions of maltodextrin (10% w/v) and hexanol (0.3% w/v) are used for studying the phenomena associated with freezing and freeze drying which are responsible for retention of the volatile organic compounds in the dried material. The initial solution contains some liquid droplets prior to cooling. During the freezing process, the hexanol solubility limit is exceeded and many droplets of hexanol liquid appear. Often these droplets of hexanol move relative to their initial location due to bulk liquid fluid flow associated with liquid density differences resulting from temperature gradients and fluid flow associated with

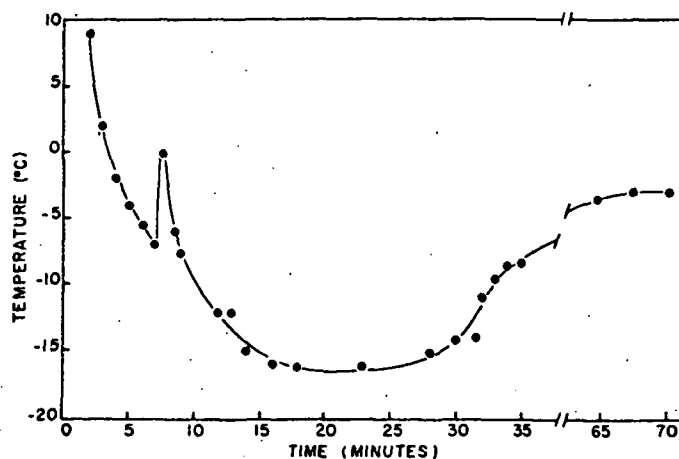*Fig. 12—Hexanol droplets in freeze-dried matrix; same field as 11 (600×).*

Fig. 13—Sample temperature during freezing and freeze-drying of droplet samples.

the growth of ice crystals. Figure 3 portrays the path taken by a hexanol droplet during the concentration steps associated with freezing. The extent of droplet movement is quite variable; in some cases it can be many droplet diameters, though in many cases it is little or none. This process results in the entrapment of the droplet in the interstitial solute matrix consisting of eutectic maltodextrin solution. Figure 9 shows these droplets at the ice grain boundaries of a completely frozen maltodextrin sample. These entrapped droplets of volatile (average diameter 2 microns) remain stationary during freeze drying and are found throughout the sample thickness in the dry amorphous matrix (Fig. 10 to 12). Similar behavior was observed in experiments at hexanol concentrations above (0.8% w/v) and below (0.1% w/v) that noted above.

The appearance of liquid droplets of alcohols in freeze-dried aqueous maltodextrin solutions has been related to the solubility of the volatile alcohols (Flink and Gejl-Hansen, 1972). Furthermore, the influence of molecular size, solubility and concentration on retention of the volatile following freeze drying has been demonstrated by Flink and Karel (1969, 1970a). A 0.5% (w/v) solution of a more

soluble alcohol, n-butanol, behaved similarly, though the droplets formed during freezing are much smaller, making them more difficult to observe.

While experimental work is continuing with the freeze-drying microscope, it appears obvious that for some typical volatile organic compounds of limited aqueous solubility, retention after freeze drying is in the form of liquid droplets which primarily develop during cooling and freezing and are entrapped in the interstitial matrix after freezing. These droplets of volatile compounds are locked into the dry material following the freeze drying step.

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Rulkens, W.H. and Thijssen, H.A.C. 1972. Retention of volatile compounds in freeze drying slabs of maltodextrin. J. Food Technol. (Brit.) 7(1): 79.

Thijssen, H.A.C. and Rulkens, W.H. 1969. Effect of freezing rate on rate of sublimation and aroma retention in freeze-drying. In "Recent Developments in Freeze Drying. Part 1. Thermodynamic Aspects of Heat and Mass Transfer." Bull. IIR Annex 1969-4, p. 99, International Institute of Refrigeration.

2.3 Observations of freeze drying fronts in the microscope

During observations of freeze drying processes, the sublimation front appears as a dark band of varying width. In an effort to determine changes occurring at the freeze drying interface, a study of the nature of the sublimation front was conducted. In particular, the dark nature of the band made observations difficult, and it was originally considered that the dark region could indicate some aspect of the changing moisture content.

Two views of sublimation fronts for different malto-dextrin samples are shown in Figure 1. The observed width of the front was found to depend on the rate of movement of the front. The sharp front on the left was progressing at about 300 μm/min and had a width of about 2-3 μm, while the more diffuse front on the right (about 15 μm wide) was moving at less than 100 μm/min. Rates of movement of the freeze drying front are related to a balance of heat and mass transfer in the drying sample. A closer inspection of the dark band showed that rather than being a diffuse region, in actuality it was an ice-vapor interface at an angle to the optical axis. There was a sharp interface at the chamber bottom glass surface and another sharp interface at the chamber top glass surface, and it was possible at high magnification using

thin samples, to focus at selective places along the inclined interface. The fact that this interface is inclined to the optical axis means that light rays passing through the interface are refracted and/or internally reflected and thus the incline appears dark. The width of the dark band depends on the slope of the incline (and sample thickness) and the slope of the incline of the interface reflects the fact that the chamber cooling is entirely from the lower glass sample holder and thus there is a vertical temperature gradient in the sample. A low temperature at the sample-sample holder interface will give a slower drying at the lower level and thus the interface slope will decrease, resulting in a broadening of the diffuse dark band.

This behavior, while of interest, is not expected to effect the general validity of the observations made using the freeze drying microscope. Examples of the direct transferability of observations in the freeze drying microscope and the laboratory freeze dryer are the agreement of retention levels of ethyl acetate in freeze dried maltodextrin (see section 2.4) and similarity of physical appearance of samples freeze dried in both systems.

2.4 Retention of volatile organic compounds

Retention of volatile organic compounds was investigated in the freeze drying microscope for a complex food gel. This study was associated with the study reported upon in section 5.5 of this report. In addition to this study, an evaluation of the possible use of ^{14}C labelled volatiles as a means of quantitatively determining volatile retention during freeze drying under the microscope was conducted.

2.4.1 Retention of hexanol in a complex food gel

A complex food gel which is a commercial replacement for pectin served as the non-volatile solute at a solids content of 1%. The gel solids consisted of the following components:

locust bean gum	35%
guar gum	20%
Carrageenan gum	15%
Agar gum	30%

The 1% (w/v) gel was mixed with hexanol (0.2% w/v) and a droplet was placed on the stage of the freeze drying microscope. The liquid sample, prior to initiation of freezing contained a few hexanol droplets of $\leq 2\mu m$ which were observed to move about in the aqueous phase. Also present were undissolved gel particles of up to 100μm in maximum dimension.

Slow freezing conditions resulted in the growth of regular ice dendrites, first along the bottom surface of the microscope chamber (location of largest supercooling), and later at higher levels in the sample parallel to the chamber bottom. In all, five layers of regular ice dendrites formed. During the period of ice dendrite growth, hexanol droplets and insoluble gel grains were observed to be pushed away from the growning ice crystals and trapped in the interstitial solute phase. As this phase solidified (i.e. the temperature decreased), hexanol precipitated from solution as spherical inclusions in the solute matrix. (A similar phenomenon is graphically presented in section 2.2).

Following complete solidification of the gel vacuum was applied and freeze drying was commenced. Despite a satisfactory vacuum (0.4 torr) and ice front temperature (-12.5°C), the drying front moved very slowly ($\leq$100μm/min), with the highest rate occurring in the largest dendrites. Each layer of dendrites had a different rate of movement of the ice interface, probably due to a temperature gradient across the sample and possibly also due to differences in ice dendrite dimensions. One interesting phenomenon noted was the apparent spontaneous appearance of drying zone ahead of the drying front, much like dry islands in a sea of frozen material. It

could not be determined if this behavior might be due to
microscopic cracks in the frozen material, or from some
vertical drying fronts crossing dendrite boundaries.
In all cases, the freeze drying fronts in each dendrite
were quite diffuse and were often observed to advance
in "spurts", which might be interpreted as resulting
from sudden changes of the dry layer mass transfer coeffi-
cient due to cracking, or from evaporation of "liquid-
like" regions that were observed occassionally at the
sublimation front.

Examination of the dry matrix showed an "undamaged"
(at least on the gross scale) structure having a multitude
of small pores. The prevalence of small pores can explain
the slow drying rate, as it can be presumed that the
dry cake has a high mass transfer resistance. Even though
the dry layer mass transfer characteristics might be
expected to result in a high ice layer temperature, the
fact that the components of the complex gel mixture have
high collapse temperatures allowed drying to occur
without change in matrix structure.

When a comparison is made between freeze drying
of maltodextrin and the complex gel mixture, significant
differences and similarities can be noted. Separation of
components during freezing occurred in a similar manner
for both solutes. However, drying was more rapid with

maltodextrin samples, and the matrix pore size larger, even though the initial solute concentration has been 20% for the case of maltodextrin solutions and only 1% for the complex gel. Additionally, only in the case of the complex gel system has there been observations of isolated regions of drying occurring as "islands" in the dry matrix. For all these differences however, a comparison of volatile retentions from sections 5.2 and 5.5 shows that similar levels of retention are achieved for the two solute systems with either acetone or ethyl acetate as the volatile.

2.4.2 Quantitative evaluation of volatile retention in the freeze drying microscope using ^{14}C labelled ethyl acetate

An experiment was conducted in which the retention of ethyl actate (1%) in aqueous maltodextrin (10%) was determined for samples freeze dried in the freeze drying microscope and in the laboratory scale freeze dryer (Virtis 10-MRTR). An ethyl acetate solution of high ^{14}C activity ($\sim$10µc/ml) was used for the microscopic freeze drying since the total sample volume was 20µl. A lower ^{14}C activity ethyl acetate solution was used for retention of the conventionally freeze dried samples. While sample size, geometry, freezing and freeze-drying conditions

differed, efforts were made to treat both samples as
similar as possible. For example, in the crucial area
of freezing rates, the time to freeze the sample was
a matter of minutes in both cases. Considering the
unavoidable differences in process conditions, the observed
retentions (laboratory freeze dryer - 27.4%; microscope
freeze dryer - 23.9%) were considered to be in good
agreement. This quantitative agreement in volatile
retention gave further indication that the microscope
observations can be related directly to freeze drying
behavior in conventional samples.

2.5 Freeze drying behavior of emulsified model systems

Food materials, and complex systems simulating food materials often contain non-voaltile, water insoluble components, for example oils and related lipid substances. The influence of these materials on the freeze drying behavior of food systems, and the interaction of the water insoluble solutes with the water soluble components is of interest relative to the successful production of the freeze dried system and subsequent stability in storage. For example, the way in which an emulsified lipid phase interacts with the matrix forming solute may be significant in determining lipid oxidation rates during storage, or ease of rehydration prior to use. It has been shown, for example, in the Phase 1 Annual Report (and related here in 2.2) that organic volatiles of limited solubility form droplets which are pushed by the growing ice crystals and are incorporated into the solid matrix during freezing. These entrapped droplets are retained during freeze drying and give a stable product. In the case of the volatile organic compounds studied at that time, all droplets which were not incorporated are presumably volatilized and lost. With emulsified systems of non-volatile lipid, the fate of the droplets during freezing and freeze drying is not known. It can be conceived that some droplets are incorporated into the

solid matrix, though the fate of the non-incorporated droplets is subject to question. These may exist on the solid surface as droplets, or they may spread on the matrix surface following the passage of the freeze drying front. These differences are of potential significance relative to a number of areas affecting subsequent product quality. With these and other related problems in mind, a study on the freeze drying behavior of emulsified systems using our freeze drying microscope was initiated.

Aqueous emulsions, which consisted of a non-volatile water soluble matrix former, a non-volatile water insoluble oil and water, were prepared using a high speed Sorvall Ommi-mixer. These emulsions can be divided at present into three groups:

triolein - maltodextrin

1-bromonaphthalene - maltodextrin

triolein or 1-bromonaphthalene - Avicel

As most of the studies to date have been conducted with maltodextrin-triolein, the behavior of this system will be described in some detail.

2.5.1 Triolein-maltodextrin

Triolein-maltodextrin emulsions are prepared by dissolving the maltodextrin in the water, then adding

the oil and emulsifier and homogenizing for 1-1 1/2 minutes in the high speed mixer. A typical composition is: maltodextrin (10%), Triolein (3.7%), Tween 80 (0.4%) and Tween mos-100 vs (0.2%). The composition yields a phase volume relative to water ($\phi_{o/w}$) of 4%. Samples have been investigated with phase volumes of 1-10%, and with one (Tween 80) or no emulsifier.

It should be noted that at the higher phase volumes (above 4), there was a tendency for a small portion of the oil to cream following homogenization. Samples taken for microscopic observations were always taken from the bulk liquid, though this would mean that the actual phase volume would be slightly lower than initially presumed.

In the liquid state prior to freezing, the emulsion had an essentially uniform oil droplet size of about 1μm, though a few larger droplets of up to 12μm diameter were noted. The droplets were well dispersed, with no observed tendency to cluster.

During either fast or slow freezing, small oil droplets are trapped in the maltodextrin matrix; those droplets larger than about 3μm are not entrapped but rather located at system interfaces such as ice-maltodextrin or sample-glass (freeze dryer chamber walls). As the phase volume increased a larger proportion of the small

droplets is not incorporated into the maltodextrin matrix.

Freeze drying behavior appeared to depend in part on phase volume. With a phase volume of 1%, a good vacuum (0.3-0.4 torr) was obtained and drying proceeded with a sharp interface. The sample structure does not undergo any observable structural changes, but it was noted that droplets not incorporated into the matrix are sometimes moved with the passage of the ice front. Figure 2 of this section, shows a 1% triolein emulsion following freeze drying. The breaks which can be seen in some of the matrix walls are due to the method by which this cut was made. In this figure, many free oil droplets are observed adhering to the pore walls. In addition numerous oil droplets appear to be located within the maltodextrin matrix, though a rough estimate of droplet size indicates that these may have increased inside the wall.

When the phase volume is 4-5%, it proved difficult to maintain the chamber vacuum below 1 torr. The sublimation front was not sharp and the interface appeared to have a "liquid-like" nature. This interpretation is based on the observations that as the sublimation front progressed, unincorporated oil droplets appeared to be mobilized and transported a few micrometers in a direction opposite to the movement of the front (i.e. into the dry

layer). These transported droplets would then be deposited
as a film on the dry matrix. Drying occurs with a loss
of matrix structure, the degree of structure loss depend-
ing on how liquid-like the front appears. With matrix
breakdown occurring, the possibility exists for release
and transport of the droplets entrapped in the matrix.
An examination of the dry matrix of these samples shows
regions of a glassy nature (surface dissolution) with a
characteristic speckled surface appearance presumably
due to deposits of oil. It is not unreasonable to
consider that oil droplets dispersed at the sublimation
front increase transport resistance to water resulting in a
higher sublimation front temperature and partial melting.
The "liquid-like" nature of the front, partial matrix
collapse and the inability to maintain a high vacuum
in the chamber may be due to this phenomenon.

The system of 10% Triolein, 10% maltodextrin,
0.4% emulsifier and water was homogenized for 30-45
seconds and droplet sizes of 1-8μ were obtained. The
high oil content made it difficult to distinguish
separate droplets in the homogenate and the fast freezing
conditions gave a very opaque sample in which only few
details could be seen. However, ice crystals and larger
fat droplets in the surface region were observed. Drying
proceeded very slowly and due to the presence of multiple

drying fronts no observations of drying process could be made.

In an attempt to improve droplet contrast, the oil phase was stained with a fat soluble dye (Sudan Black B). Staining did not improve liquid emulsion sharpness, however, when frozen, much better contrast was obtained between the oil and other solid phases, so that many fat droplets could be clearly distinguished. During drying, the sublimation fronts were quite diffuse and the fate of fat droplets during passage of the sublimation fronts was difficult to establish, though examination of the dried sample indicated that the eutectic solid was freeze dried without observable collapse.

The dried material was also observed with crossed polarizers in the microscope's light path. Since triolein is polymorphic and some percentage of the oil will be in the solid state, birefringence would be expected. It was found that the oil deposits and emulsions were visible with crossed polarizers as regions of 1st order grey interference color. This means that the fat has a low degree of birefringence and thus crossed polarizers alone will not be capable of giving improved contrast. Any further utilization of cross polarizer techniques will require adaptation of the microscope for use with a gypsum plate or quartz wedge.

Rehydration behavior of freeze dried emulsions was investigated with samples having an initial oil phase volume of 1%. Water was added to the edge of the dried material and dissolution of the matrix observed under the microscope. At the initial addition of water, the matrix begins to dissolve and an irregular surface pattern of oil appears on the water. As more water is added, agglomerates of oil droplets float in the sugar solution, eventually splitting to single spherical droplets as the water concentration increases. In samples where drying proceeded with a good vacuum and sharp interface, the droplet size is essentially unchanged from the initial emulsion. For samples in which the freeze drying was difficult, droplet sizes following rehydration are larger than in the initial emulsion. These differences can be explained by higher probability of coalescence at interfaces.

2.5.2. 1-bromonaphthalene-maltodextrin

A model system based on 1-bromonoaphthalene as the oil phase was investigated, since its high refractive index of 1.656 (for comparison water is 1.33, maltodextrin is 1.51 and triolein is 1.456) relative to the aqueous phase should result in a higher visual contrast of the droplets.

A system of 10% 1-bromonaphthalene, 10% malto-
dextrin, 0.4% emulsifiers and water was prepared as in the
triolein samples. The homogenate showed an abundance
of very distinct oil droplets of sizes $\leq 1\mu m$. However,
the numerous small oil droplets and small ice crystals
which were present uniformly throughout the fast frozen
samples, resulted in a very diffuse image due to the
high degree of light scattering.

When the homogenate was frozen slowly to give
growth of large ice dendrites, the freezing process
could be followed and similar behavior as has been
observed previously for non-soluble alcohols was noted (i.e.
growth of ice dendrites in one or more layers, entrapment
of oil droplets between ice crystals, migration of oil
droplets in the concentrated solute phase until the
latter solidified a few minutes after the surrounding
ice was formed). By observing the Brownian movements
of the oil droplets, the changes in viscosity of the eutec-
tic phase could be detected.

Figure 3 shows a frozen sample with oil droplets
in the solute matrix between ice crystals. Some "out
of focus" droplets (white spots), which are above the
dendrites (i.e. in another plane of focus) can also be
seen. During the freezing process it was observed that
some oil droplets were deformed, probably due to 3 factors:

a) pressure from growing ice crystals

b) shearing forces on droplets during their
    translational movement in the viscous eutectic
    phase

c) slow solidification leading to changes of
    the spherical droplet to a non ideal shape.

These observations show that the irregular appearing
fat deposits previously observed in freeze dried matrices
may not only be a result of a "liquid-like" sublimation
front, but may in some cases be already formed during
freezing.

Figure 4 shows freeze dried maltodextrin which can
be seen to have small 1-bromonaphthalene droplets entrapped
in the matrix (within circles labelled with 2). Additionally
there are some deformed droplets of larger diameter
observable (arrows numbered 1), though it is not possible
to determine if these are present on or in the matrix.

2.5.3 Triolein or 1-bromonaphthalene-Avicel

Avicel, a microcrystalline cellulose, has been
used in some studies as the carbohydrate matrix former.
The sample composition for initial tests was chosen to be
that used earlier in this laboratory for lipid oxidation
studies (though those were conducted with methyl linoleate
as the lipid phase). Sample compositions of either

3% triolein, 16% Avicel and water or 1.5% 1-bromonaphthalene,
8% Avicel and water, were emulsified in a high speed mixer.
In the liquid state these samples have a markedly different
appearance from the maltodextrin samples due to the
presence of the insoluble cellulose microcrystals. Also
present is a component of the mixture which is difficultly
resolvable in the microscope and presumably is broken
microcrystal units.

In Figure 5 (left) is shown a liquid Avicel sample
with the large crystalline units embedded within a "sea"
of the smaller, more difficulty resolvable material.
During freezing, dendrites are not observed and the resultant
solid material is shown in Figure 5 (right). Here it can
be seen that the freezing process has resulted in a
compaction of the microcrystalline units into islands
between the ice crystals which appear to be completely
open. The freeze drying is shown in Figure 6 at low
magnification (150x) and in Figure 7 at a higher magnifi-
cation (600x). A straight, but diffuse front can be seen
in these figures, and no appearance of structure change
is noted. In Figure 7, the leading edge of the freeze
drying front (which is advancing to the left) is denoted
by the arrow (1). Avicel grains with droplets of oil on
the surface are denoted by two (2) and a free oil droplet
in the dry matrix is denoted by the arrow (3). The
bridging effect of the small Avicel grains results in a

dense matrix structure (quite opaque) and open holes.
Following disruption of the dry matrix by grinding, chunks
of Avicel were suspended in microscope immersion oil
and investigated for 1-bromonaphthalene retention.
Figure 8 shows such an island of Avicel, which appears
to contain large microcrystalline units in an overall
matrix. An enlargement of the encricled area (Figure 9)
shows a thinner section of the Avicel matrix. It may be
expected that the entire island is composed of such plates
much like a "house of cards." The means by which these
plates are held together is not discernible. In Figure 9
can be observed many 1-bromonaphthalene droplets. Many
very small droplets can be seen inside or perhaps on
the Avicel platelet. Larger droplets (labelled 1) are
definitely observed to be present on the plate surface
and to be distorted from the spherical shape. In parti-
cular, one oil deposit which is apparently being presented
edge on, has a quite distorted appearance.

The microscopic observations of freeze drying
behavior of the various emulsified systems indicates
that simple expectations and explanations of product
properties will not be possible. Oil phases may exist
in freeze dried emulsions as entrapped droplets, distorted
droplets on the matrix surface and presumably also as thin
surface films. Freeze drying behavior also appears to

be affected by the presence of an emulsified phase,
especially when that material is present at a substantial
phase volume. The transference of these observations
in the freeze drying microscope to the larger scale
laboratory freeze dryer will be necessary to more fully
appreciate the implications of the observations made to
date.

<u>List of figures</u>

1) Freeze drying fronts in maltodextrin samples (600x)

   (left - front width 2-3μm; drying rate ∿300μm/min)

   (right- front width 15μm; drying rate ∿100μm/min)

2) Freeze dried systems of triolein (1%), maltodextrin

   (10%) and emulsifier (0.4%) 600x

3) Frozen sample of 1-bromonaphthalene (10%) and malto-

   dextrin (10%) (600x)

4) Platelet of freeze dried maltodextrin containing

   1-bromonaphthalene (600x)

   (#1 - distorted oil droplets on surface)

   (#2 - entrapped small droplets within circles)

5) Avicel (microcrystalline cellulose)(8%) and

   1-bromonaphthalene (1.5%) emulsion (150x)

   (left - initial sample)

   (right - frozen sample)

6) Freeze drying front in Avicel (8%) and 1-bromonaphthalene

   (1.5%) emulsion (150x)

7) Freeze drying front in Avicel (8%) and 1-bromonaphtha-

   lene (1.5%) emulsion (600x)

   (#1 - leading edge of freeze drying front)

   (#2 - Avicel grains with oil droplets)

   (#3 - free oil droplet)

8) Cluster of freeze dried Avicel containing 1-bromonaph-
   thalene (150x)

9) Encircled area of Figure 8 showing oil droplets and
   Avicel platelet
   (#1 - distorted large oil droplets on platelet surface)

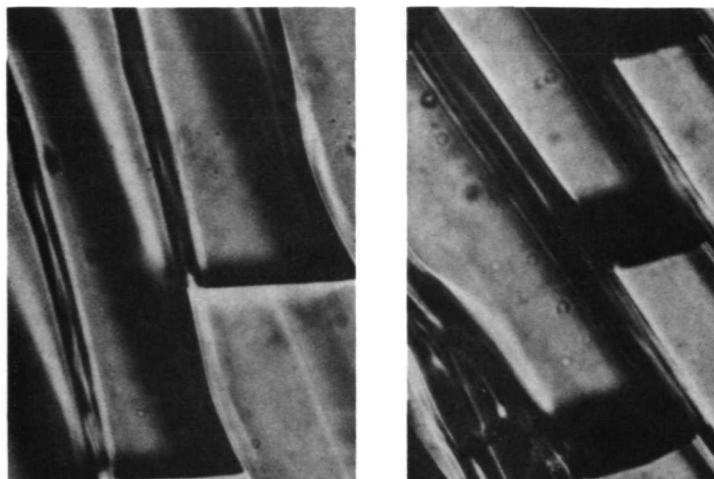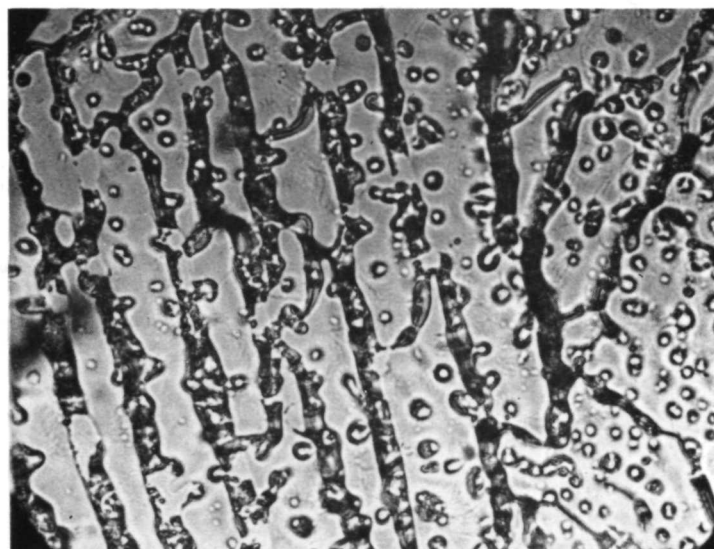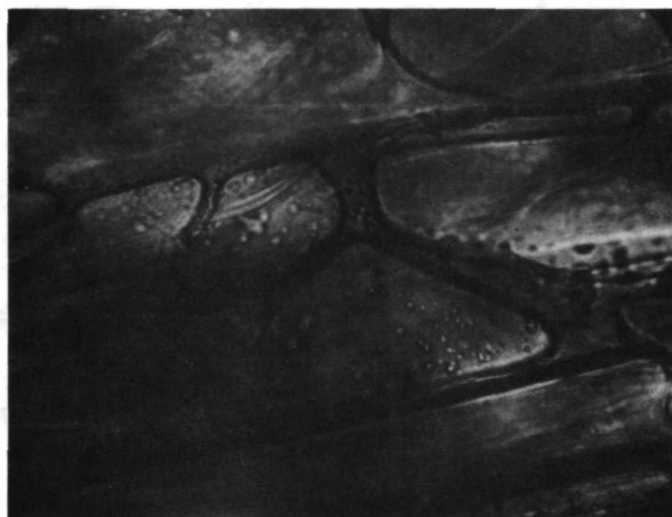Figure 1

|—————|
10 µm

Figure 2

|—————|
10 µm

Figure 3

|————|
10 µm

Figure 4

|——|
10 μm

Figure 5

|——|
100 μm

Figure 6

|——|
100 μm

Figure 7

|——————|
10 μm

Figure 8

|——————|
100 μm
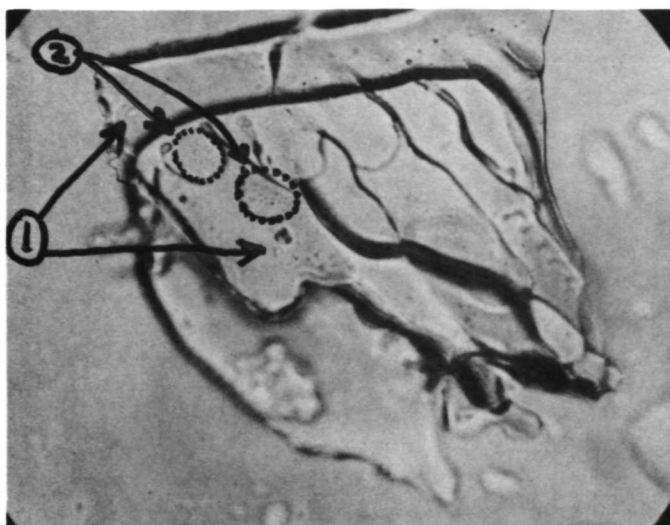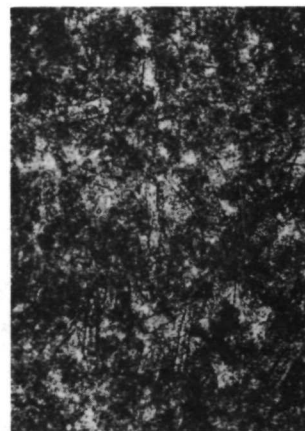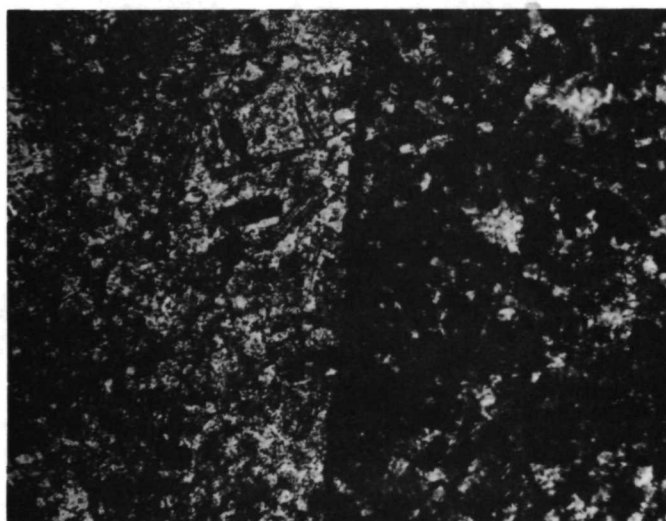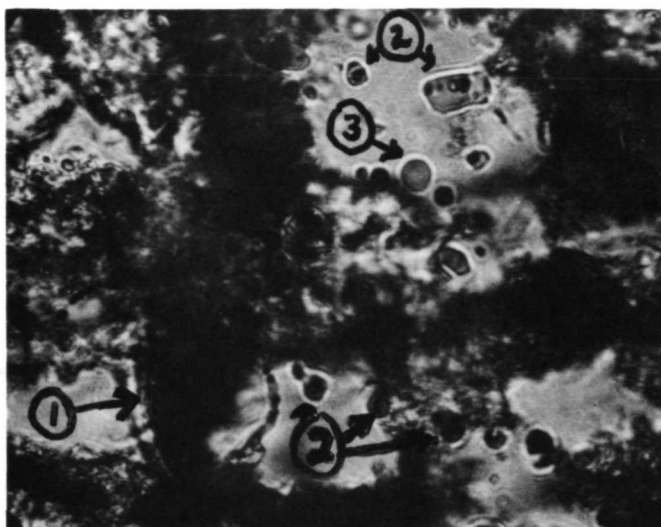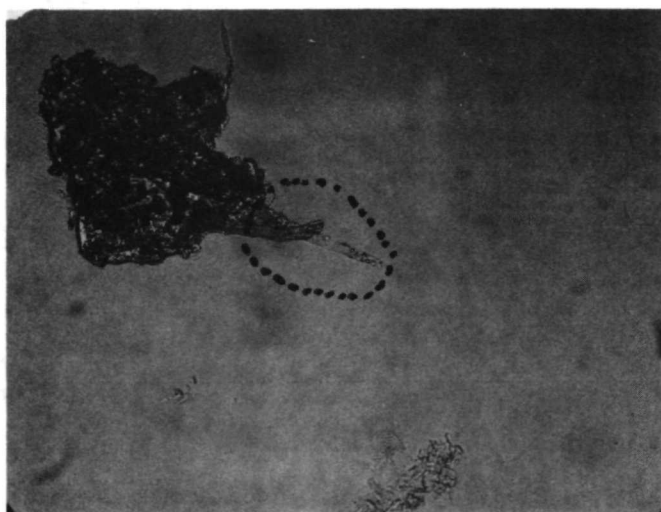
Figure 9

|———|
10 μm
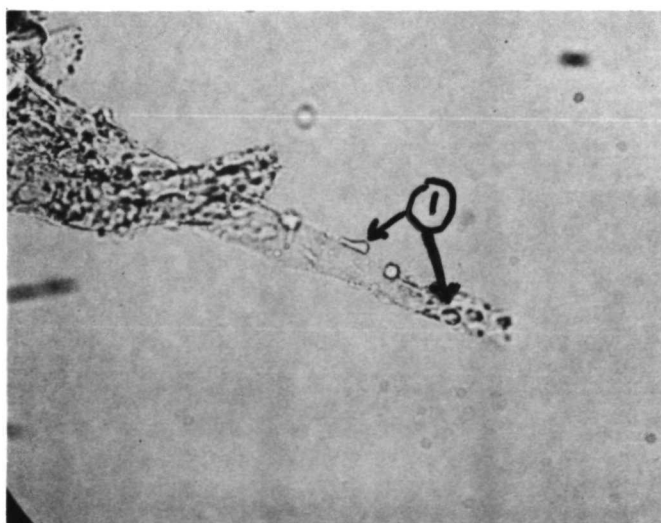
3.   Browning of dried foods at high temperatures

3.1   Introduction

In a continuation of work reported upon in the
Phase I Annual Report, we have investigated the high tempera-
ture susceptibility to browning of two dried food products,
nonfat milk and whole egg. As was noted in the Phase I
Annual Report, exposure of food materials during freeze
drying to high temperatures, especially in the second
(desorption) phase of freeze drying, will result in
nutritional and organoleptic deterioration. Since the
time of the Phase I Annual Report, the temperature problems
associated with the Skylab launch indicate the wider need
for information regarding high temperature stability of
dehydrated food products during storage. Determination
of the kinetics of product deterioration at temperatures
above those expected for any "reasonable" mishap, will
allow actual product conditions during high temperature
storage to be computed by interpolation of the high tempera-
ture data and the more normal storage studies rather than
by extrapolation proceedures. It is fortuitous that the
same range of heating temperatures is applicable to
both the study of product deterioration during the desorp-
tion phase of freeze drying and the high temperature storage
stability of dehydrated products.

To test the applicability of the concepts learned
with model systems, which were reported in the Phase I

Annual Report, nonfat dried milk was chosen. This product

is easily available and represents a highly susceptible

system. Further, analytical proceedures for evaluating

brown color formation in dried milk are well known.

As a result of the Skylab temperature problem, it became

apparent that dehydrated eggs were particularly susceptible

to high temperatures, and this product was immediately

included into the program. In this case, analytical

techniques are not so well defined, and some effort has

been expended in developing a quantitative technique for

evaluating brown color which can correlate with qualitative

visual differentiation of treated samples.

3.2 Nonfat dry milk

3.2.1 Methods

Commercially available spray-dried nonfat milk powder is used as the raw material. This is generally reconstituted to 20% solids and aliquots of the solution pipetted into 50 ml erlenmeyer flasks. These are frozen in liquid nitrogen and freeze dried with the platens at ambient temperature. Following freeze drying, the samples are humidified for 24 hours at 37°C using saturated salt solutions of constant water activity. A water sorption isotherm for the freeze dried milk powder is shown in Figure 1. The flasks are then tightly sealed with rubber stoppers and heated in air at the desired temperatures.

Measurements of oven and sample temperatures indicated that the desired temperature conditions were re-established in the oven within 15 minutes after loading (to within 2°C). At this time also, the samples were in thermal equilibrium with the oven. During removal of samples, the oven shows a 2°C drop in temperature but returns to the set temperature within one minute. Samples remaining in the oven showed no change in temperature during this period. Sample weights are monitored at all steps of the process so that sample moisture contents may be determined gravimetrically.

In tests to be described in section 3.2.2 browning behavior for air heating was compared with heating in a manner in which the milk powder was in an environment of reduced pressure. Individual methods used in those studies will be presented in that section.

Measurement of the brown color was conducted spectrophotometrically on an aqueous extract of the milk powder. The preceedure is as follows:

1) Add 20 ml distilled water to 2 g of dry sample in a 50 ml erlenmeyer flask.

2) Add 2.5 ml of a freshly prepared 10% trypsin solution to each flask and stopper.

3) Hold each sample for 1 hr in a 45°C water bath, with shaking.

4) Following incubation, add 2 ml of a 50% Trichloroacetic acid solution to each sample.

5) Add about 0.1 g of Celite Filter Aid to each sample.

6) Filter the samples through either S+S #576 or Whatman #42 ashless filter paper.

7) Any solutions which contain suspended material should be centrifuged.

8) The clear solutions are read at 450 nm using a treated trypsin solution as the blank.

9) Browning value is calculated as $\dfrac{O.D._{450} \times 100}{\text{dry sample weight}}$

3.2.2 Vacuum vs Air Heating

The Phase I Annual Report details the development of brown color in a glucose-glycine-avicel model system by placing dry samples in an evacuated oven and heating at high temperatures for various periods of time. It was desired to heat treat samples which have been humidified to various water activities by equilibrating over saturated salt solutions. These humidified samples must remain at their moisture content throughout the drying process. Several tests were conducted to develop methods by which water loss from the samples could be prevented during heating in vacuum.

Initial tests with the glucose-glycine-avicel model system, which were conducted in petri dishes sealed with plastic tape, gave widely differing results. An evaluation of the sample moisture contents for the vacuum heating showed that sizable loss of moisture had occurred. Vacuum heated samples also showed large scatter presumably due to widely differing moisture contents.

In subsequent tests nonfat milk was the material studied. Petri dishes were previously shown to be unsuitable, and erlenmeyer flasks were not suitable in vacuum, but could be used heat samples in air with only a small loss of water (32% R.H. samples decrease to 5.5% moisture from the initial value of 6.0%).

A comparison of air and vacuum heating of the spray dried non-fat milk at three relative humidities using the stoppered erlenmeyer flasks, showed that heating the dry powder in a vacuum at 90°C is equivalent to heating in air.

To evaluate the effect of presence of air during heating at higher water activities, samples were frozen, freeze dried, and humidified in glass ampoules. The humidified samples were sealed either in air or under a vacuum. In some tests, browning in vacuum sealed ampoules was compared with air heating of stoppered flasks.

The results indicated that rates of browning at each moisture content are not greatly different for samples heated in ampoules and samples in stoppered flasks, though some lag period in heating in flasks is observed, probably due to thermal equilibration effects.

Comparative tests presented in Figures 2 and 3 using sealed ampoules showed that there was no difference in browning behavior when the ampoules were sealed with vacuum or containing air. There was no loss of water from the ampoules during the heat treatment. These results indicate that the chamber pressure during freeze drying will not have a substantial direct effect on browning development in the drying ice-free layer. It was also established that pertinent information about temperature and water-dependence of browning could be obtained by tests in stoppered flasks.

3.2.3 Influence of water activity and temperature

3.2.3.1 Quantitative measure of browning

The time and temperature behavior of browning of freeze dried milk powder has already been shown in Figures 2 and 3 for samples prepared in glass ampoules. The same basic behavior has been observed for samples heated in stoppered erlenmeyer flasks (Figures 4-6). The influence of powder structure was also considered by comparing product sensitivity of the original spray dried powder and freeze dried powder.

It was demonstrated that freeze dried samples will brown more rapidly than the original spray dried powder. This difference is seen to increase with increasing water activity at constant temperature (Figure 4) and with increasing temperature at fixed water activity (Figure 5). These observations can be explained by considering the differences in powder microstructure for spray dried vs freeze dried and the influence that this might have on water activity and heat transfer properties. Microscopic observations of the powders showed a fundamentally different structure and surface. The spray dried powder exists as hollow spheres with a glassy surface. The rapidly freeze-dried powder exists as a compact solid with numerous small pores. The appearance of the surface of the freeze dried samples is like a smooth velvet. The glassy

surface appearance of the spray dried powder probably indicates a high sugar content in the surface, which may result in a lower level of water sorption prior to heating. This would tend to give lower levels of browning with the spray dried. In addition there may be differences in browning due to differences in heat transfer between the freeze dried samples with many small pores and the spray dried which have few points of contact between spheres.

Reproducibility of degree of browning depends on being able to duplicate all the steps of the process, but most importantly, the temperature of the oven. Good reproducibility for two experiments heated at 100°C is evident from data in Figure 6. In most experiments temperature was regulated less rigorously and thus variations were observed.

Browning as function of heating time is shown in Figure 7 for 32% RH and Figure 8 for 11% RH samples. In Figure 8 the effect of heating at 100°C and 110°C on dry samples is also shown. At lower temperatures browning of dry samples is negligible over the time period studied. For straight line portions of the browning curves linear regression was used to calculate the slopes (rates of browning) and the correlation coefficients. Rates as functions of temperature are shown in Figure 9. As was

reported in the Phase I Annual Report for the model system, there exists a critical temperature above which there is a rapid rise in brown color formation. This critical browning temperature can be seen to depend on moisture content. Using an Arrhenius plot (Figure 10) the energy of activation of browning was calculated. For the three water activities 0, 0.11 and 0.32, activation energies of 47, 53 and 33 kcal/mole, respectively, were determined. The values for the dry samples are somewhat higher than the value reported in the Phase I Annual Report (19 kcal/mole) for the model system.

An interesting phenomenon noted but not investigated as yet, is the indication that browning at very high temperatures _may_ proceed rapidly to some level and then undergo a sudden decrease in rate, and that the level achieved may depend on the temperature.

3.2.3.2 Analysis of browning

Visual organoleptic evaluation of powders have been conducted for samples at 3 humidities (0,11 and 32%) which were heated at 90°C for periods of 1 to 6 hours. The 15 samples were ground and sieved to give an identical surface appearance and then presented as a group to an untrained panel of judges for ranking in terms of color. The sample which was judged to be the limit of acceptability was also noted. The results are presented in Table 2

with samples not statistically different (at the 5% level) having the same lower case letter. The notation of the sample code refers to sample humidity and hours at 90°C for the heat treatment. The average limit of acceptability was judged to be between samples 11/4 and 11/3 (i.e. 11/4 and all darker were not acceptable).

Following this test, some of the samples having high and low extremes of browning were removed and the remaining heat treated samples were presented for acceptability judgements on an individual basis. Following analysis of these results, the average acceptabilty limit was found to have moved toward the darker end and lie between 11/6 and 32/2. Following the completion of visual organoleptic studies quantitative evaluation of brown color of the samples was measured and this information is also presented in Table 2. This data shows that when samples having a wide range of brown colors (and specifically when "pure white" samples are present) a browning value of about 4.5 is the limit of acceptability. However, if samples are presented independently of each other (i.e. judgments made without reference to one another) a browning value of 8.4 is judged to be the limit of acceptability. Thus, samples having browning values between 4.5 and 8.4 were now judged to be acceptable.

A similar test was conducted using both spray dried and freeze dried samples at 11 and 32% RH and heated at 70, 80 or 90°C for 0 to 6 hours. Two judges only were

used, but these were more experienced than the untrained
panel. There was a lesser range of browning values,
and in particular for the freeze dried, none between the
highest acceptable (3.68) and lowest unacceptable (5.69)
browning values. For the spray dried powder the highest
acceptable browning value was 3.73 while the lowest
unacceptable was 3.83 as has been noted earlier. Micro-
scopic observations of the freeze dried and spray dried
powders reveals a fundamental difference in surface
structure. The visual effect of this difference is a
distinct yellow appearance of the spray dried powder,
in contrast to the "pure white" of the freeze dried powders.
Thus, unheated spray dried powders were judged among the
unacceptable samples when presented with heat treated
freeze dried powders. Instrumental analysis however
placed them among the lowest in browning values. It is
due to these structural effects that the browning value
for the limit of acceptability of the spray dried powders
is lower than for the freeze dried.

In another example of material structure and dry
powder appearance, sample colors for different preparation
methods have been evaluated for their influence in
browning studies. Using spray dried nonfat milk powder
as the initial raw material, the following sample prepara-
tion conditions give the following sample colors:

Mixing	Color of powder
high speed blender	"pure" white
hand or low speed blender	off white
initial spray dried powder	yellowish cast

The "pure" white sample has the same (or slightly higher) browning values than the fresh powder.

Concentration also had an influence on appearance. Concentrations above the normally used 20% have an "off white" color whether mixed at high or slow blending speed. The difference observed for the various preparation schemes are apparently related to the degree of incorporation of air bubbles into the liquid. Conditions which favor the formation of numerous highly reflecting surfaces, such as many small bubbles in thin films etc, result in a white color and in the case of milk a high visual acceptability. In spite of differences in appearance the degree of browning as measured spectrophotometrically are similar for all unheated samples, and when reconstituted all samples had the same color.

Visual comparisons may be used along with the data for heating time at various temperatures and water activities to define allowable processes in which degree of browning will be within the range of acceptable values.

3.3 Freeze dried whole egg

3.3.1 Methods

Fresh eggs were gently stirred with a fork to make as homogenous mixture as possible without air incorporation or foaming. The egg mixture is frozen in erlenmeyer flasks using liquid nitrogen, freeze dried and then the freeze dried egg is stored over drierite in a refrigerator since fluorescence occurs rapidly at temperatures above ambient (Figure 12). Fluorescence implies formation of browning reaction intermediates.

Evaluations of browning in the heat treated egg is conducted on a chloroform extract of the egg and on an aqueous KCl extract of the previously chloroform-extracted material. The proceedure is given below:

1) Weigh exactly 3.00g of freeze dried egg into a 50 ml erlenmeyer flask.

2) Extract with 35ml chloroform for 15 min with agitation.

3) Suction filter taking care to collect all clear filtrate.

4) Wash sample with 2-10ml portions of chloroform.

5) Repeat steps 2-4 and combine the filtrates.

6) If the filtrate is unclear, complete 2-5 before refiltering the entire combined filtrate.

7) Solution absorbance is measured at 390 nm, using chloroform as a blank.

8) Browning value for chloroform extract is calculated as:

$$B.V. = \frac{O.D._{390} \times 100}{3.00g}$$

9) The material collected on the filter paper after step 5 (or 6) is dried in air.

10) Weigh exactly 1.00g of the dried material into a 50 ml erlenmeyer flask.

11) Extract with 25ml of a 10% KCl solution for 15min with agitation.

12) Gravity filter (No. 589 filter paper) into a 100ml volumetric flask with a small amount of asbestos fiber at the apex of the filter paper.

13) Rinse solids with 3-20ml portions of 10% KCl solution.

14) After sample is well drained, filtrate to mark with 10% KCl solution.

15) If solution is cloudy, centrifuge before continuing.

16) Measure fluoresence against a 0.200 µg/ml quinine sulfate solution set at 50%.

Fluorimeter settings:

Exict λ = 365nm   Exict slit = 40

Emm λ = 450nm   Emm   slit = 14

Filter = 39

3.3.2 Evaluation of browning of freeze dried whole egg

During the SKYLAB mission, freeze dried eggs were found to be particularly susceptible, and it was decided to include them in the program in Phase II. Brown color formation was studied in freeze dried raw whole egg. It was necessary to first evaluate an analytical technique. It would be most desirable to obtain a quantity which can be correlated with visual evaluation of product discoloration. A standard method for deterioration of quality of dehydrated egg products during storage is the Standard Fluroescence Technique of Pearce and Thistle [Can. J. Res. 20D:276(1942)].

This technique is generally used at temperatures of normal or slightly accelerated storage conditions, and there was no information on its applicability to the higher temperatures. Initial results showed that while the visual brown color increased with heating time at 100°C, fluorescence values reached a maximum at an intermediate heating time with subsequent decrease at 11 and 32% RH, and an asympotic approach to a maximum value in dry samples. (Figure 11).

When the heating temperature was lowered to 70°C there were no fluorescence maxima, and fluorescence of eggs appeared to be similar to the pattern of browning in milk (Figure 12). It was observed that the chloroform extract used to remove lipid prior to the KCl extraction

for the fluorescence measurements, had a visual darkness
similar to appearance of the samples prior to extraction.
The possibility of using the chloroform extract as a
measure of browning was thus investigated.

Measurement of absorption of chloroform extracts
of browned egg showed an optimal sensitivity at 390nm.
The browning values of the chloroform extract were observed
to increase rapidly and then level off as time of heating
was increased (Figure 13). Visual comparisons have shown
that the samples could be differentiated, but it was not
possible to conclusively correlate browning values with
visual ranking analysis, because of lack of samples heated
during the early portion of the heating period.

Qualitatively, visual inspection of heated samples
of dry egg shows an appearance which parallels the measured
browning values, in that (1) all the heated samples appear
much darker than the unheated sample and (2) the gradations
of darkness between samples heated for different times
are slight compared with differences between samples heated
at different temperatures.

It has been noted that the visual color of the chloro-
form extracts monotonically increases in darkness with
heating time, which is in constrast to behavior of fluores-
cence (Figure 14). Fluorescence technique is measuring
a browning intermediate which breaks down rapidly at the

temperatures of interest in this study. The intermediate nature of the products contributing to fluorescence is not unexpected and emphasizes the need for additional methods for evaluating the brown color.

The extent and the rate of browning appear to be related to the rate of formation of the intermediates as measured by fluoresence and the onset of their destruction. In Figure 13 are plotted data from two experiments conducted at 90°C on different batches of raw egg. While the curve 90°C(A) appears markedly different from that labelled 90°C(B) it appears that the sources of these differences lies entirely in different "lag" periods for these two samples, and this difference may in turn be related to formation of intermediates. An approximate evaluation of initial rates of browning can be made. While this is only a rough approximation, an activation energy of about 23 Kcal/mole is found, somewhat lower than that found for dried milk though close to the value found for the model system studied in Phase I (19kcal/mole).

Table 1

Browning of freeze-dried non-fat milk

B.V. = mt + b

t = time in minutes
r = correlation coefficient
m = browning rate

Temperature		RH	m	ln m	b	r
°C	$\frac{1}{T}$(°K^{-1}x10^{-3})					
70	2.92	32	0.0099	-4.6135	1.2383	0.9938
80	2.83	11	0.0031	-5.7682	2.55	0.8309
		32	0.0837	-2.4803	-7.6557	0.9966
90	2.75	0	0.0033	-5.7125	2.5714	0.9170
		11	0.0157	-4.1548	2.4971	0.8778
		32	0.4083	-0.8957	-36.7143	0.9997
100	2.68	0	0.0157	-4.1548	1.4140	0.9715
		11	0.2581	-1.3546	-31.1514	0.9704
		32	0.6649	-0.4082	-35.0946	0.9945
110	2.61	0	0.0923	-2.3827	-2.5030	0.9691
		11	0.8271	-0.1897	-69.74	0.9578
		32	1.8804	+0.6316	-104.33	0.9687
		32*	2.1920	+0.7848	-208.62	0.9991

Activation energy

$$\ln m = m'\frac{1}{T} + b'$$

RH	m'	b'	r
0	-23.7843	59.6578	-0.9993
11	-26.7203	69.7455	-0.9875
32	-16.4836	43.9083	-0.9825

Table 2

Browning of Dried Milk at 90°C

Visual and Chemical Evaluation

Ranking by visual test*		Browning value**
32/6	a	109.5
32/4	b	49.6
32/3	c	23.2
11/6	_d_____ ***	8.4
32/2	d,e	10.4
11/4	_e,f_ ****	5.4
11/3	f	4.5
00/4	g	1.39
00/6	g	1.06
00/3	g	0.96
32/0	h	2.4
11/2	h	3.50
11/0	i	1.82
00/2	i	0.27
00/0	i	0.31

* Sample codes (RH/HRS): samples with same lower case letters are not different at 5% level

** Browning value equals $\dfrac{OD_{420} \times 100}{Wt\ sample}$

*** Acceptability limit - independent judgement

**** Acceptability limit - group judgement
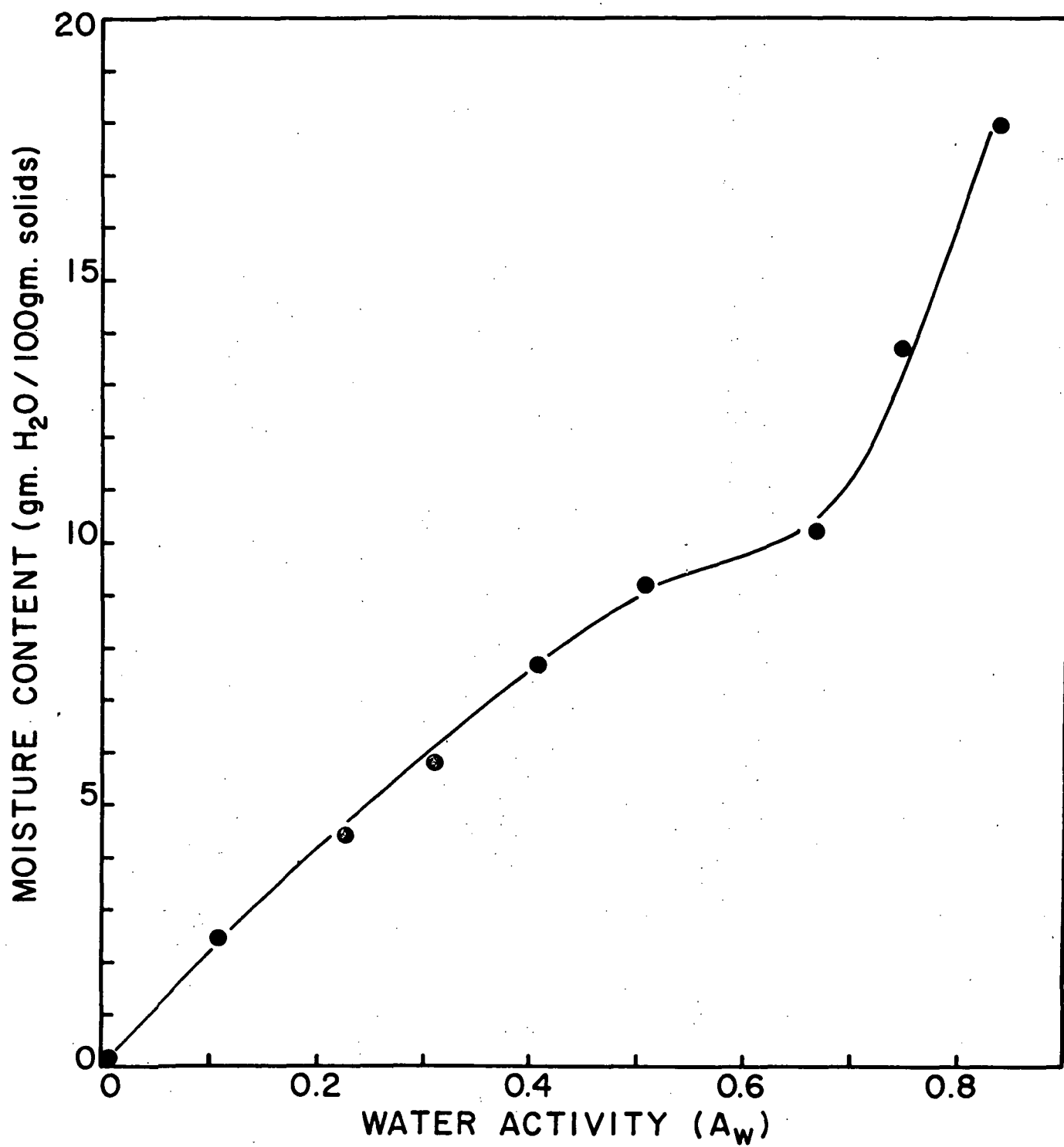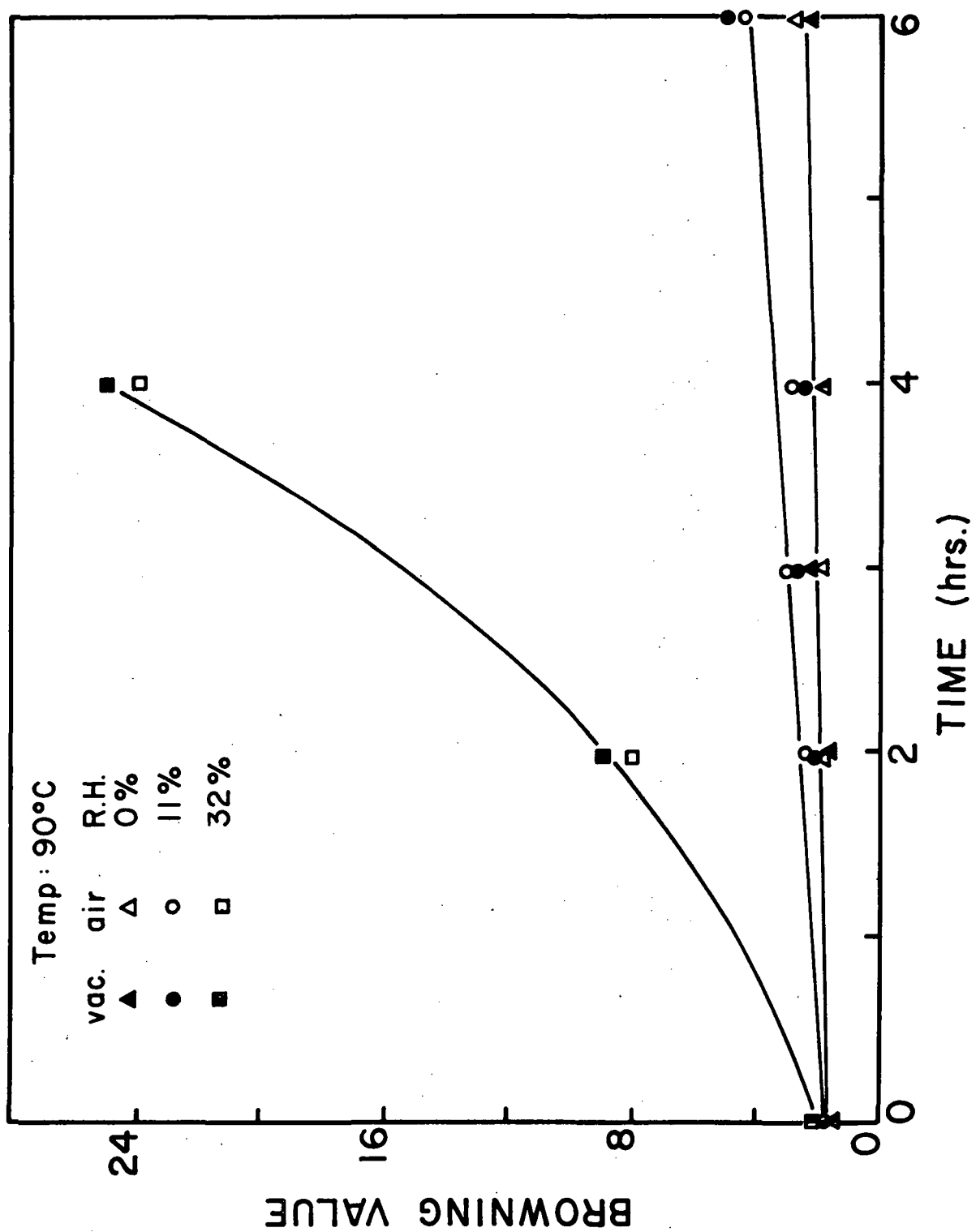
Figure 1
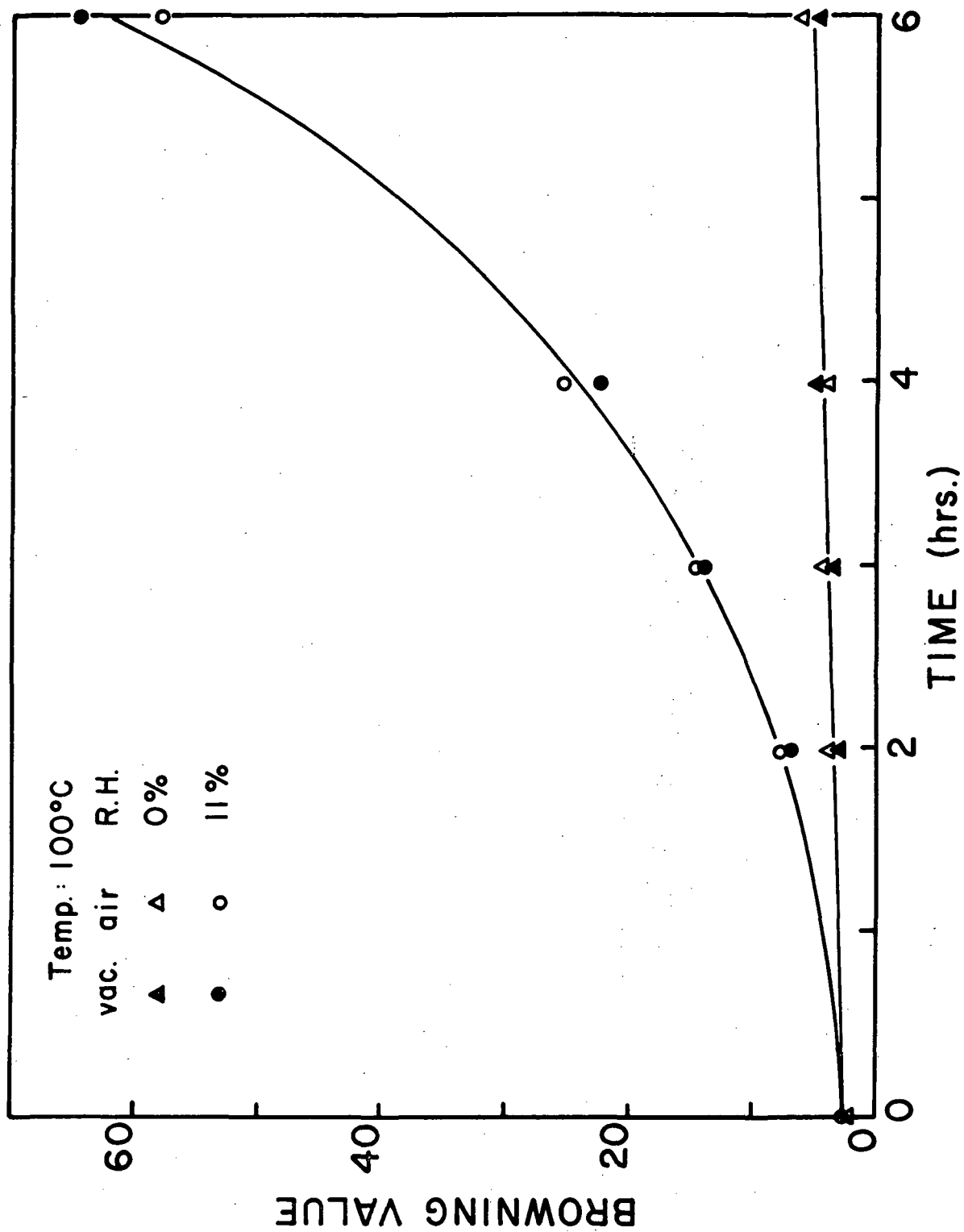
Figure 2
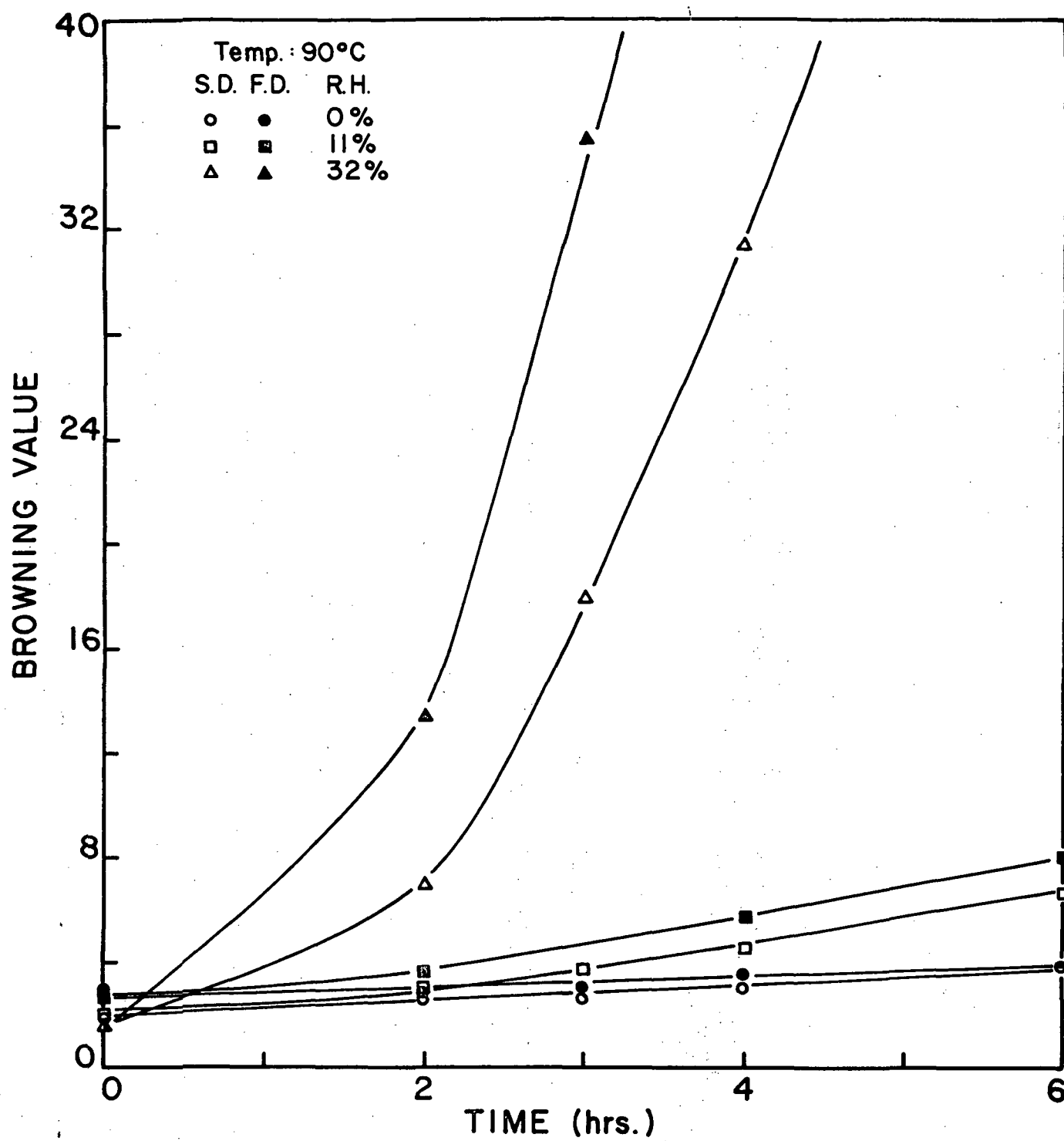
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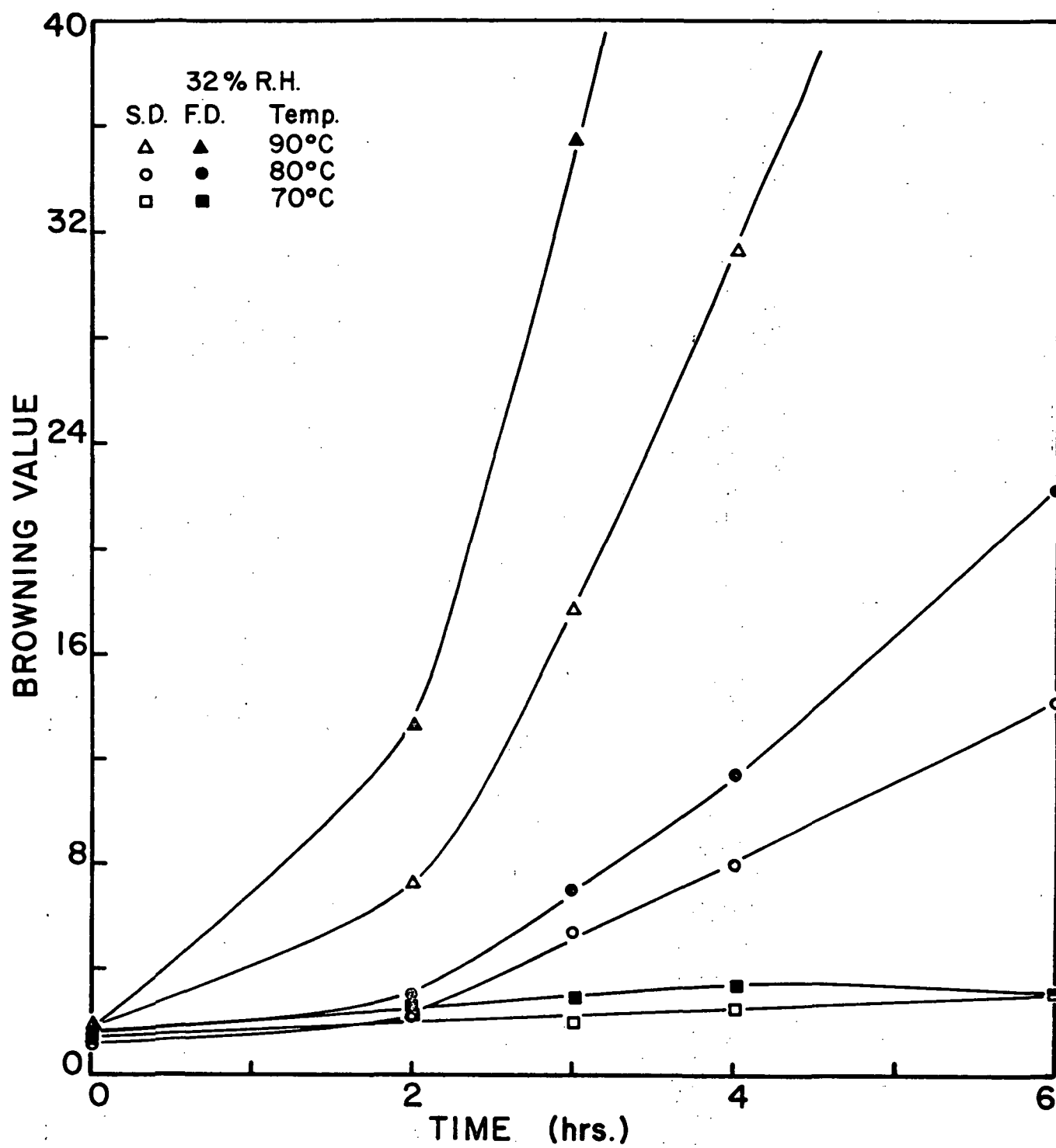
Figure 4

Figure 5

Figure 6
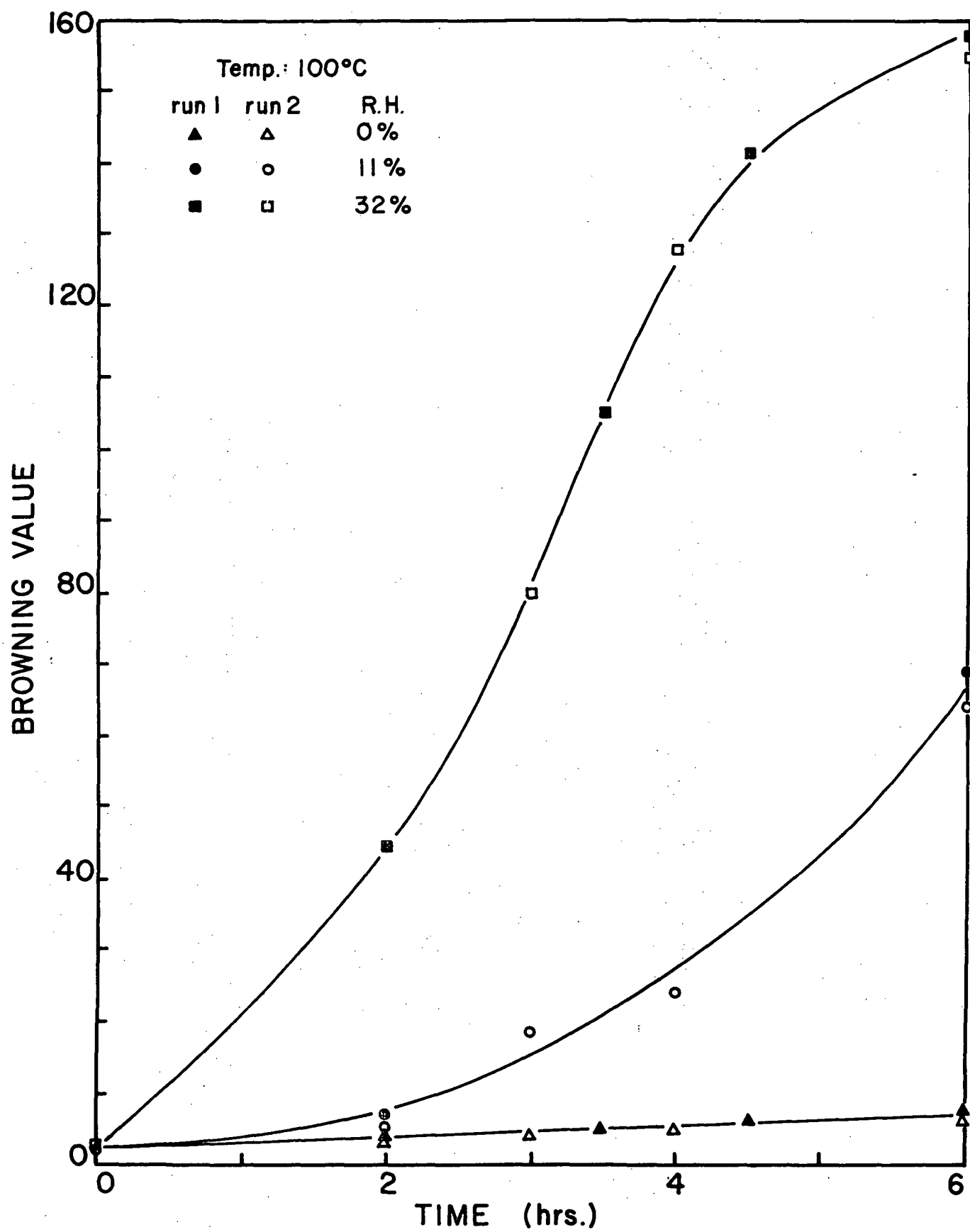
Figure 7
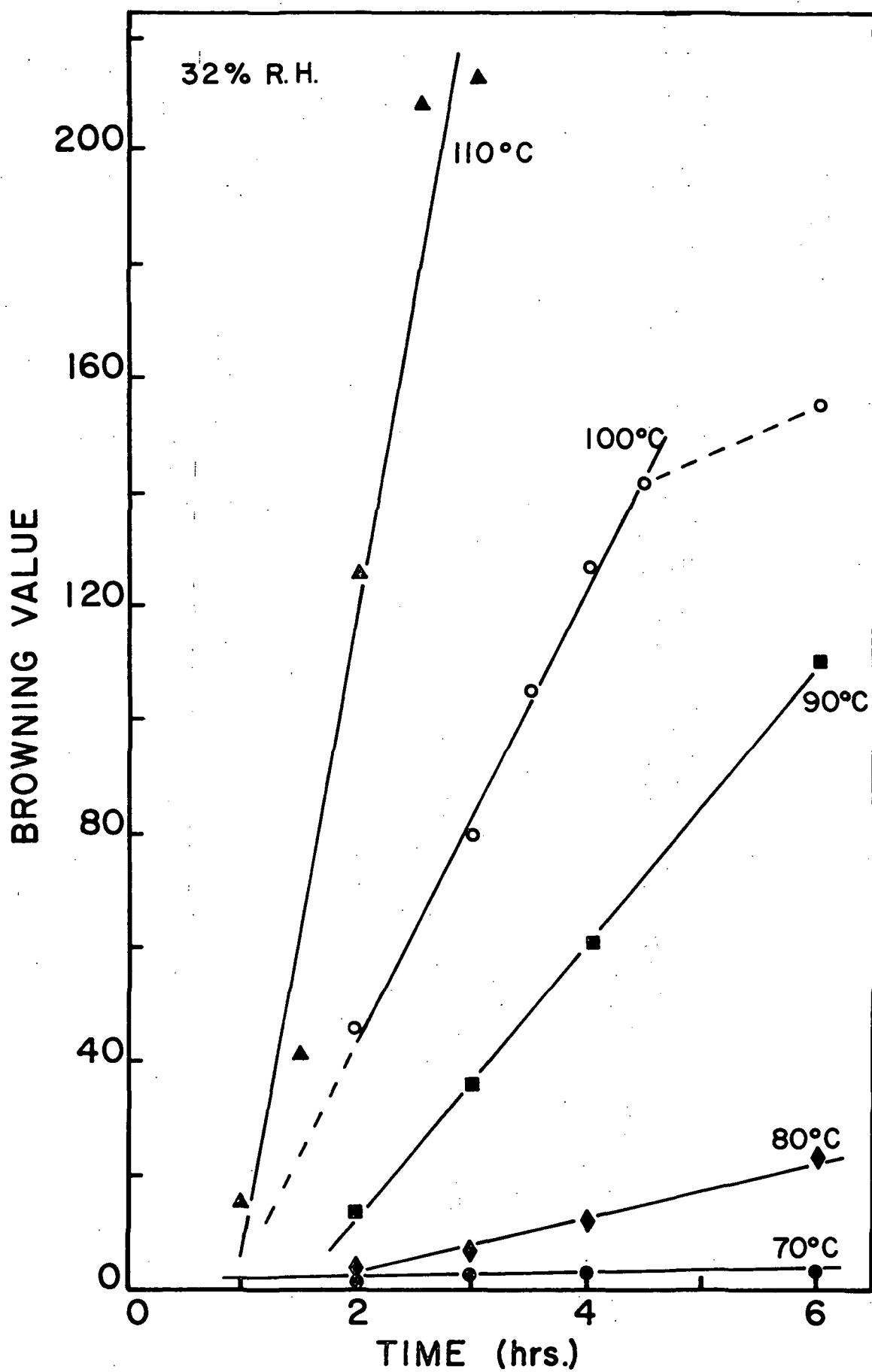
Figure 8
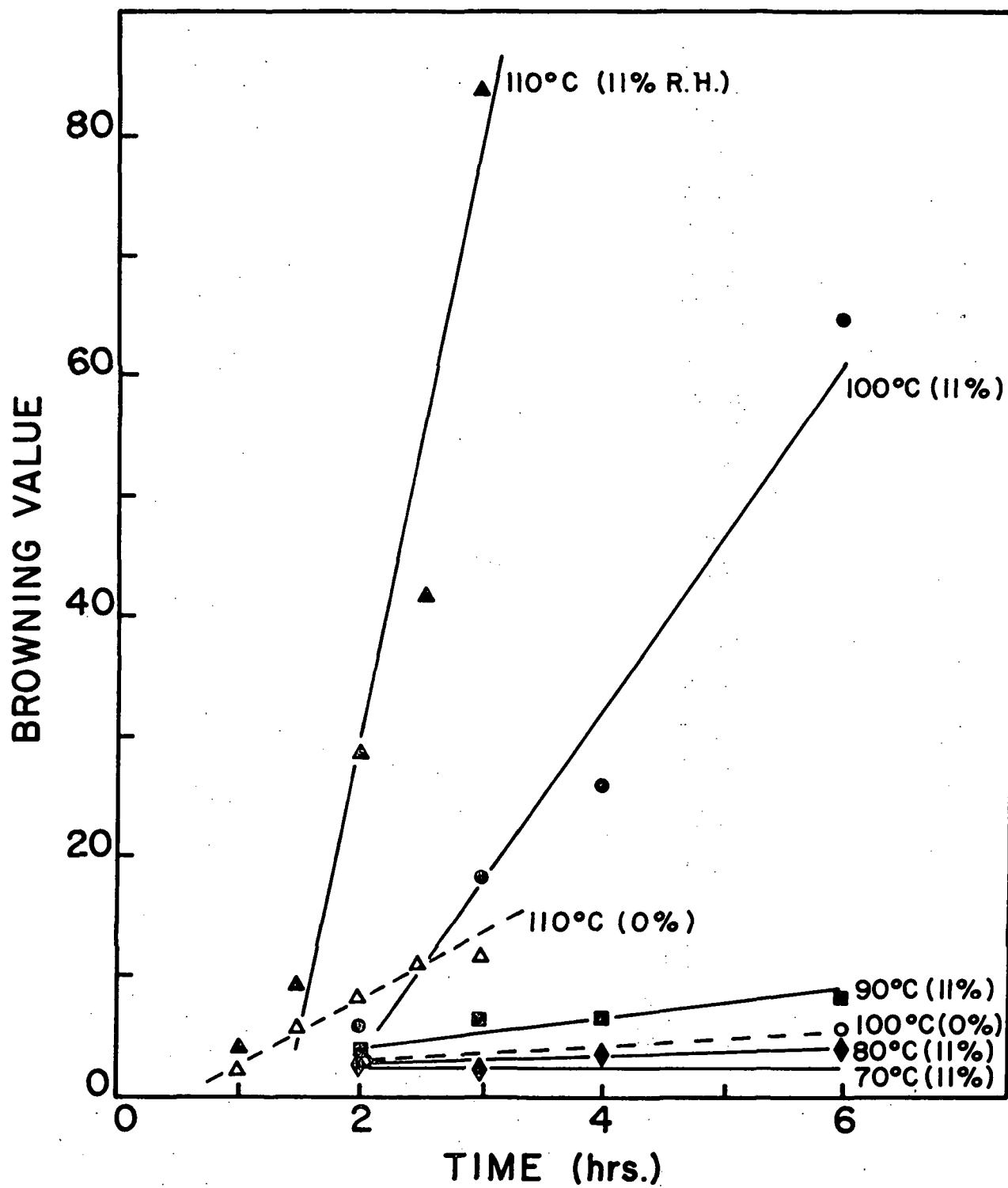
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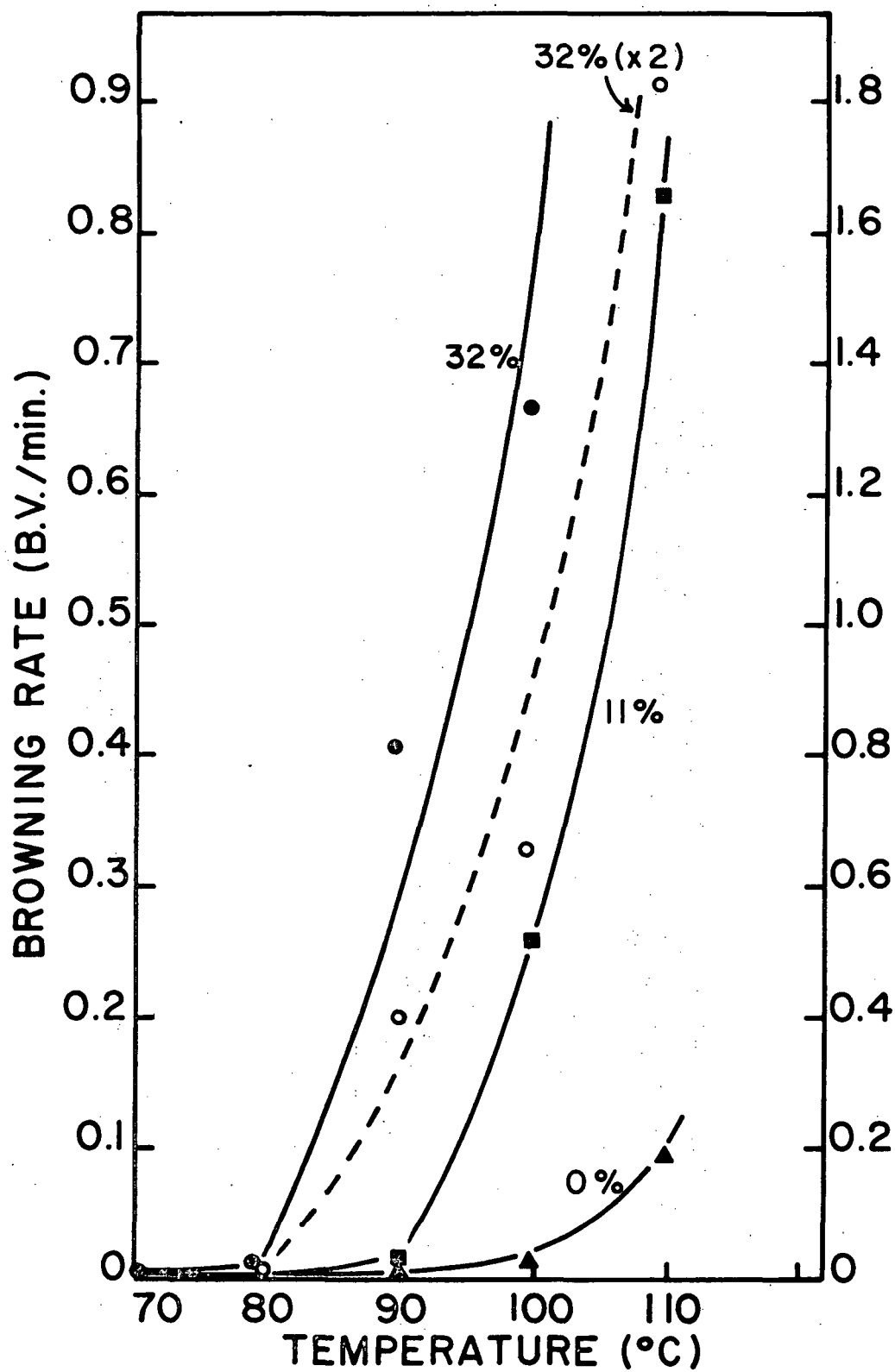
Figure 9

Figure 10

Figure 11

Figure 12

Temp.: 70°C

Y-axis: FLUORESCENCE (%E)

X-axis: TIME (hrs.)

32% R.H.

11% R.H.

0% R.H.

Refrigerator stored, not humidified.
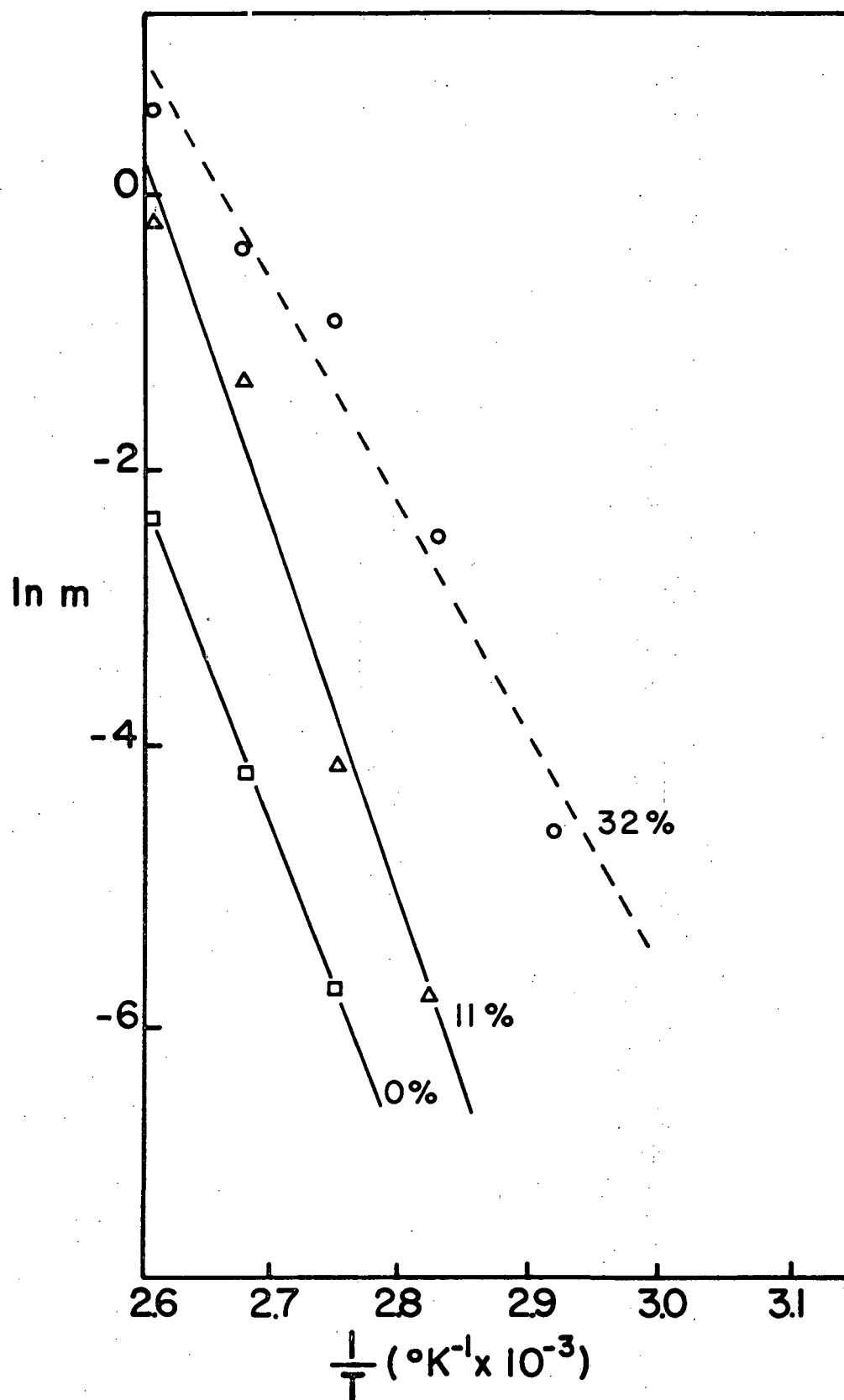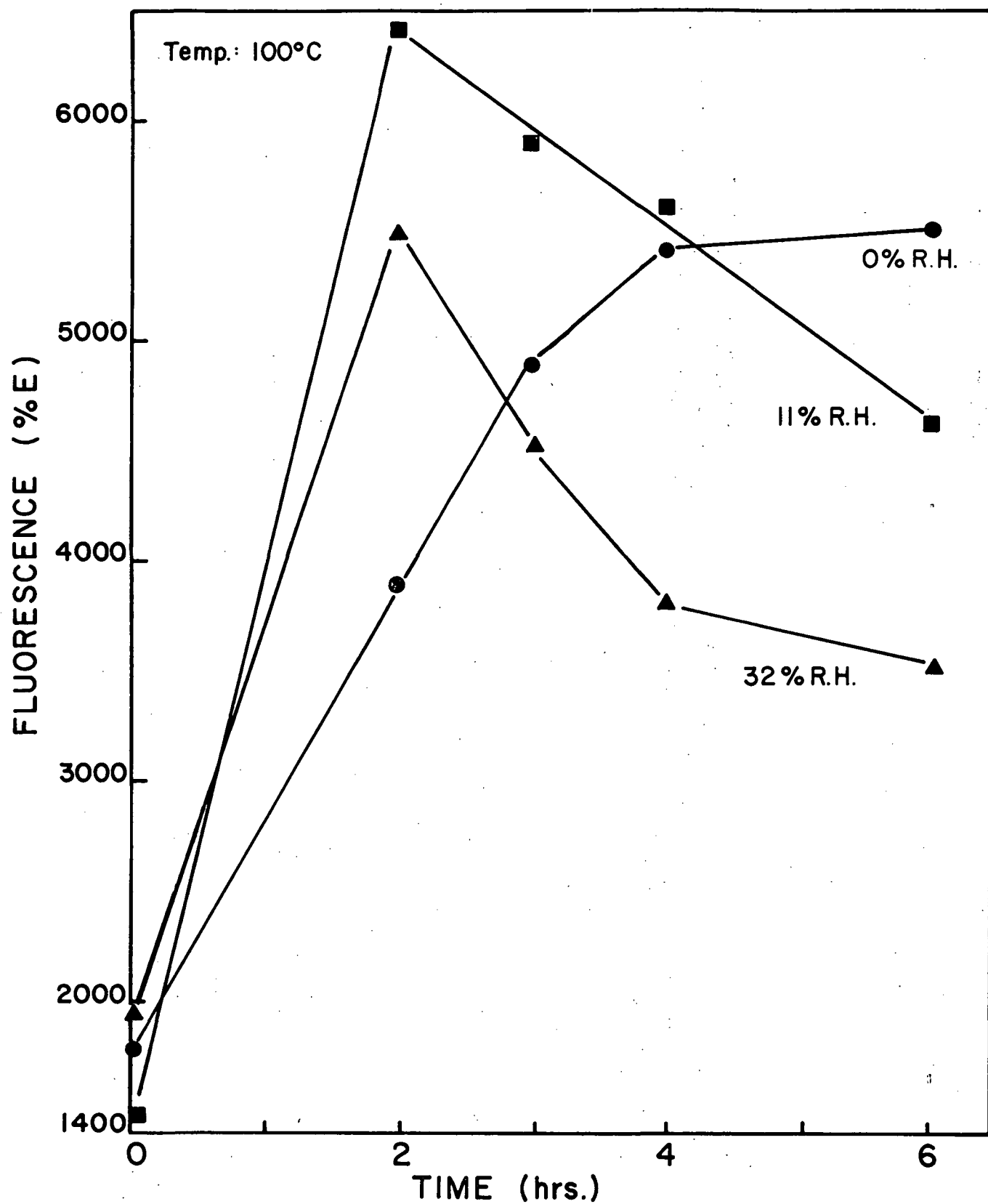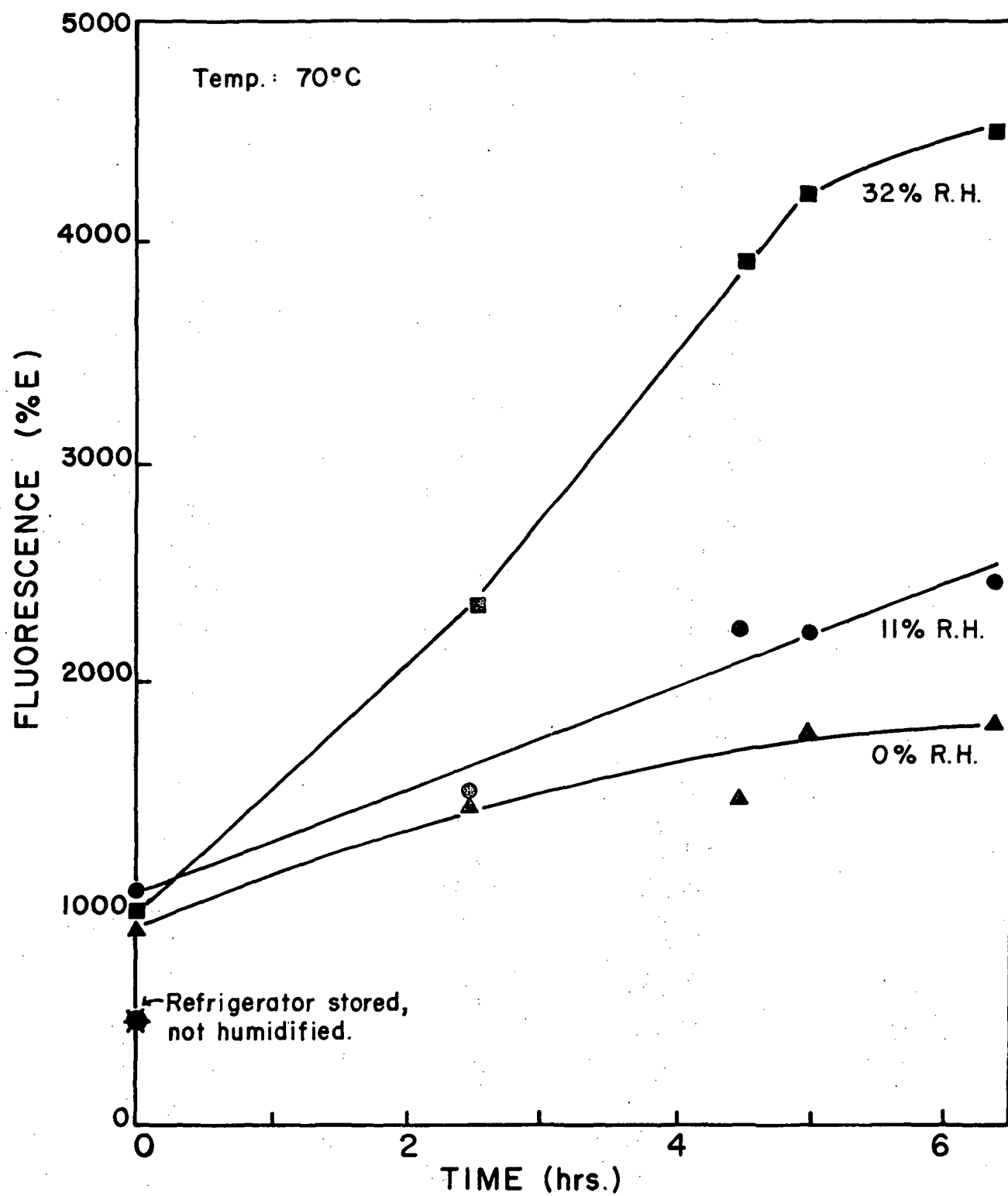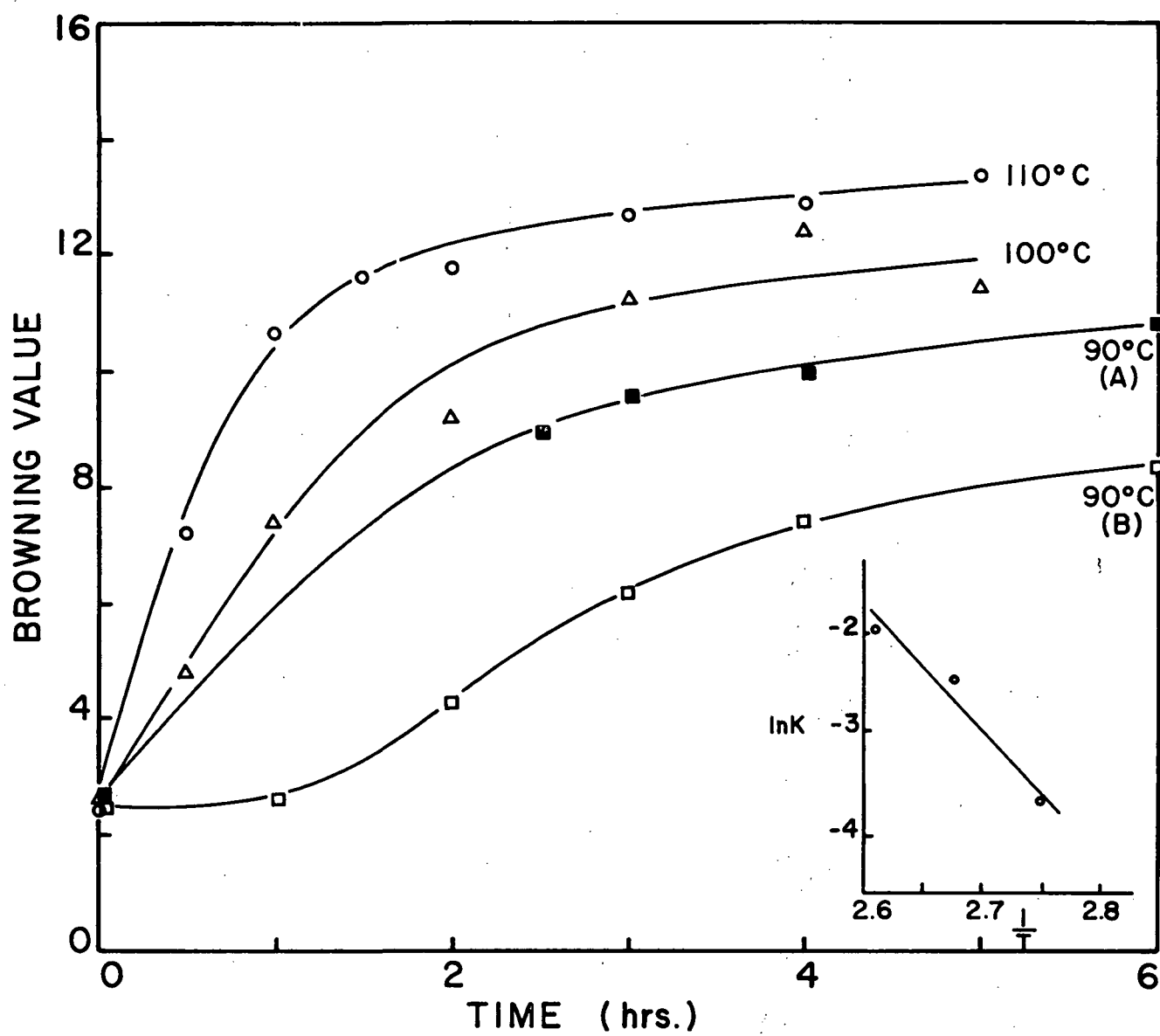
Figure 13

Figure 14
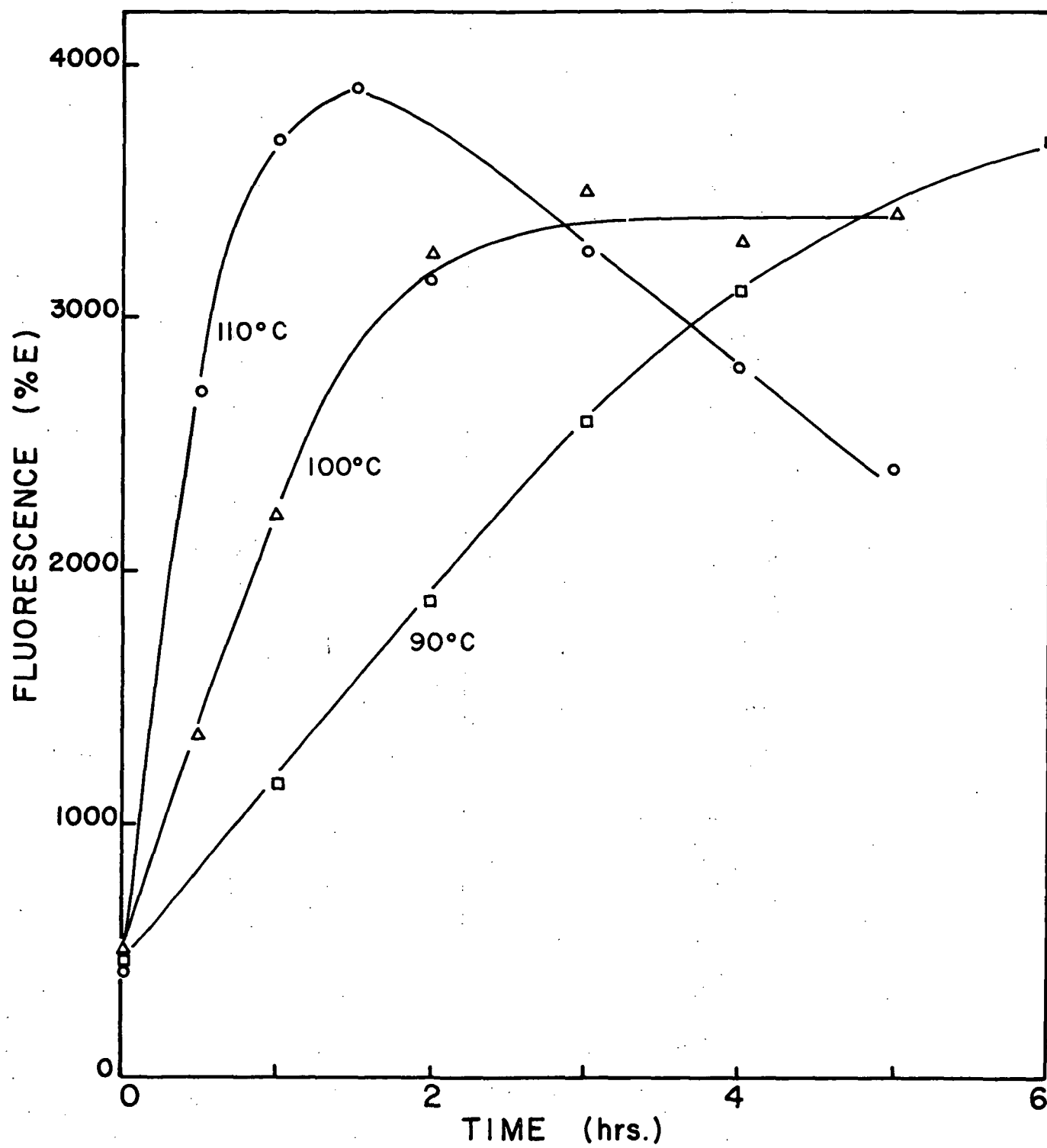
4. Artificial gel structures for engineered space foods

4.1 Introduction

The future supply of space missions requiring a palatable, stable and controlled-nutrient level diet, may rely to an increasing extent on artificially created foods based on a variety of key ingredients. One of the important attributes of such systems is the development of texture simulating natural foods or even exceeding in quality such natural food items. At present the commercial emphasis in this field has been almost entirely on thermo-plastic extrusion, or solution-spinning of oilseed- or bean- derived proteins. The commercial approach has been predicated on creation of a mass market with relatively rapid recovery of investment. These requirements pre-cluded the utilization of expensive technology or of simulated food replacing actual foods with low inherent cost. The commercial approach has therefore concentrated on very inexpensive "extenders" of meats and on somewhat more expensive "analogs" meant to substitute for the increasingly costly foods derived from animal tissues (meats, fish, crustaceans).

In our preliminary approach to the present problem, therefore, we have decided to concentrated on two aspects which are not at present subjects of active commercial activity:

A)   Non-aqueous and complex freeze drying methods designed to produce structured dehydrated systems.

B)   Preliminary experiments on establishing parameters and approaches for creation of gel systems approximating organoleptic characteristics of fruits and vegetables.

4.2   Non-aqueous materials and the freeze drying process

      The following review of this subject was presented
by Professor Flink at the A.I.Ch.E. Meeting in Detroit
in June of 1973, and was subsequently published in
AIChE Symposium Series 69(132),63-69,(1973).

NONAQUEOUS MATERIALS AND THE FREEZE DRYING PROCESS

James M. Flink

Drying processes involving the substantial presence of nonaqueous materials can be divided at present into two major areas:

1. Freeze drying of nonaqueous systems.
2. Freeze dehydration of aqueous systems using nonaqueous solvents.

The first process is conceptually similar to the conventional freeze drying process except that nonaqueous solvents are being sublimated. This allows the preparation of porous dry materials which are not normally or easily soluble in water. The second process is somewhat different, being an organic extraction of frozen aqueous-based materials which results in dried products that have characteristics similar to conventionally freeze dehydrated materials.

The basic concepts of the two processes are discussed, and examples of their potential application to the food industry are presented.

Drying procedures utilized in the processing or production of foods almost exclusively involve the removal of water from the primarily nonvolatile organic solute. Dehydration is generally accomplished by contacting the foods with heated air; for example, by tunnel or spray drying. Separate mediums for heat and mass transport are also utilized, as in drum drying. In describing these traditional drying processes, the term *nonaqueous materials* refers to organic materials generally present in limited concentrations.

During this symposium, however, we are considering situations in which nonaqueous materials comprise a major portion of the items being dried or used in the drying process itself. Most interactions of nonaqueous materials and drying processes (especially freeze drying in our case) can be divided into the following topics:

1. Freeze drying of metal salt systems present in high concentration in aqueous solutions.

2. Freeze drying of nonaqueous systems, or mixtures of nonaqueous and aqueous systems.

3. Drying of water-containing samples by contacting them with nonaqueous materials.

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These three areas are relatively independent since they approach the interaction of nonaqueous materials and drying processes from quite different viewpoints.

Topic 1 employs the rather loose interpretation of inorganic acid and metal salt solutions as nonaqueous even though the fluid medium is water. This liberty is taken so that interesting ideas from the field of metallurgy and material science might be included and considered for possible adaptations to food processing.

The tendency, when considering food drying, is to think in terms of removal of the system solvent, water. In topic 2, nonaqueous materials serve as the system solvent, and freeze drying in this case refers to the removal of volatile organic solvent, with the essentially solvent-free, nonvolatile solutes remaining. Mixed aqueous-nonaqueous systems have also been studied.

In topic 3, conventional food materials (that is, products composed primarily of water) are dried by contacting them with an excess of nonaqueous material. Studies have been conducted with the material to be dried in both the frozen and unfrozen states. Although use of a frozen product is closest conceptually to conventional freeze drying, we will discuss both situations since claims of quality equal to normal freeze drying are made for both frozen and unfrozen materials.

FREEZE DRYING OF AQUEOUS INORGANIC SYSTEMS

In the past few years metallurgists and material scientists have become increasingly interested in the utilization of freeze drying for the production of ceramic (1 to 3) and metal (4 to 6) powders having novel properties. In contrast to the usage of freeze drying in the food industry where the sample composition is generally accepted as that present in the raw material, processes for preparation of freeze-dried metal or ceramic powder start with a controlled formulation of various pure chemical species. These species mix on a molecular level when they are in solution, forming a completely homogeneous dispersion. The importance of freeze drying to the overall process is that, if proper processing conditions are utilized, this homogeneous molecular dispersion can be maintained in the dry product. Proper processing essentially consists of the following:

1. Choosing chemical species which maintain their solubility during the lowered temperatures of the freezing steps.

2. Very rapid freezing so that gross partitioning of solute from the ice cannot occur.

3. Maintenance of freeze drying conditions which allow no melting or other structural changes during the drying steps.

Contrary to freeze drying of foods, the freeze-dried powder resulting from this processing scheme is generally an intermediate in the overall process. Thus, for example, Landsberg and Campbell (6) describe further treatments of tungsten-rhenium powders formed by freeze drying an aqueous solution of the ammonium salts of tungsten and rhenium. (The initial solution was prepared by mixing a H_2WO_4 –NH_4OH solution with a Re_2O_7 solution.) The freeze-dried powder was first heated stepwise from 200° to 900°C. while exposed to hydrogen gas to reduce the salts to the metal. Following this reduction, the metal powders were compressed (with binders) and sintered to form the desired tunsten-rhenium alloy having uniform composition.

Roehrig and Wright (5) report similar treatments on a wide variety of metals and ceramics. They further note that one can selectively transform components of the homogeneous powder so that the resultant product could be either a two-component metal alloy or a ceramic-metal mixture.

Another interesting characteristic of the freeze drying process concerns the very fine particle size of the powder. Roehrig and Wright (3, 5) describe the preparation of tungsten carbide particles 200 to 500 Å in diameter although they had been present as particle chains 0.7 μm in diam. (presumably due to overheating in the conversion to the carbide). These chains could be comminuted to give the individual particles. Landsberg and Campbell (6) also report extremely fine particles for their freeze-dried ammonium tungstate (0.03 to 0.05 μm), although the particles increase in size following reduction to metallic tungsten (0.2 to 0.4 μm).

These fine grain powders provide very large surface areas, as noted by Trambouze (7) in his discussion of the use of freeze drying for the preparation of adsorbents and catalysts. This fact in itself is of interest when considering possible uses for these very fine freeze dried powders. At the same time, fine-particle, freeze-dried alumina powders were treated by calcining, compressing, and sintering to form very dense specimens (99.8% of the theoretical maximum) (1). These very dense alumina materials were shown by electron microscopy to have a very smooth surface essentially free of pores.

These concepts which have been developed for the production of metal and ceramic powders, may be relevant for developing future food processing techniques. Of particular interest are:

1. Preparation of controlled formulations homogeneously distributed at the molecular level in the dry state.

2. Freeze-dried material which serves as an intermediate for further processing treatments.

3. Preparation of powders with fine particle size and subsequent control of surface properties.

FREEZE DRYING OF NONAQUEOUS SOLVENT SYSTEMS

For food or biological systems, the solvent occurring in nature is water. Thus, preservation techniques based on the lowering of the activity of the solvent have been concerned with the removal of water. One of these techniques is freeze drying, or lyophilization. In the freeze drying process, samples of nonvolatile solutes dissolved in water are maintained in the frozen state, and the water, having a higher vapor pressure than the solute, is volatilized by a sublimative transformation. Materials containing solutes that have higher vapor pressures than water are subject to some special considerations.

The same principles that exist for freeze drying aqueous systems apply to all systems where a relatively nonvolatile solute is present in a more volatile solvent. Therefore, materials dissolved in nonaqueous solvents can be dried (desolventized) by freeze drying. The success of this technique has been demonstrated by Rey and his co-workers for a number of purely nonaqueous solvent/solute systems as well as for mixed aqueous/nonaqueous systems (8 to 11). As most of the fundamental aspects of this area of freeze drying have been conducted by Rey et al., the following discussion will draw heavily on their developments.

For the successful freeze drying of nonaqueous systems, it is necessary that the solvent and solutes have widely differing vapor pressures at temperatures for which the system exists in the solid state. Other considerations involve the ability to maintain the sample in the solid state so that structural changes will not occur. As with aqueous samples, the nonaqueous system must be kept at a temperature well below the solvent melting point so that it is maintained in the solid state during sublimation of solvent. To conduct the freeze drying, it is also necessary to have a condensing surface at a temperature lower than that at the frozen-dry interface of the freeze drying sample. Finally, the rate of vapor transport from the sample to the condenser is greatly enhanced by conducting the sublimation at reduced pressure.

Rey (8) described some initial work demonstrating that several organic solvents could be utilized for freeze drying. Glycerol distearate, at a concentration of 7.5%, or polystyrene at concentration of 0.1 to 1% was freeze dried from solutions of benzene, carbon tetrachloride, dioxane, or chloroform. A 5% maltose solution in diethylamine was also freeze dried.

In these processes, the sample temperatures generally ranged from -90°C. at the initiation of the process to -30°C. as the last traces of solid solvent were removed. At the completion of the drying process, the temperature was allowed to reach 20°C. Chamber pressures during sublimation were approximately 10^{-1} torr. To achieve the low temperatures necessary, liquid nitrogen (-196°C.) was used for the initial freezing and for cooling the vapor condenser.

Rey et al. (9) later reported on expansion of their earlier work to systems that utilize two solvents. They have named the freeze drying of multisolvent systems, where some solvents are nonaqueous in nature, as complex freeze drying. Complex freeze drying can be subdivided into two subsets according to the methodology used:

1. Successive complex freeze drying.

2. Joint, simultaneous, or staggered complex freeze drying.

Successive complex freeze drying consists of successive simple freeze dryings in which some of the systems treated were nonaqueous. This type of complex freeze drying is conducted in the following manner:

1. A simple solvent-solute system (either aqueous or nonaqueous solvent) is freeze dried.

2. The freeze-dried cake is impregnated with a second solute-solvent system in which the cake is not soluble.

3. The impregnated solvent-solute system is frozen and then freeze dried.

An example is given in which a mixed low density solid of polystyrene and dextran is obtained (9).

This complex freeze drying process can be repeated any number of times provided that the subsequent solvents used would not dissolve any of the solutes which comprise the freeze-dried cake. Use of such a solvent is conceivable, however, where controlled solubilization might be used to produce desired product characteristics.

The products obtained by successive freeze dryings with different solvents have several interesting characteristics. As with most freeze-dried materials, a highly porous solid is obtained. The process can be devised, however, so that the porous cake is composed of normally incompatible materials which are present in close association on a molecular level. Furthermore, these materials are present in a highly extended state with respect to internal surface area. The order of preparation of the material can be expected to influence the physical and chemical properties of the final material. The solvent-solute system freeze-dried first will determine the primary structure of the final solid since further impregnations and freeze drying will occur in the void spaces remaining. The

second solute phase can exist as islands in a matrix of first solute phase, or the second phase could form an intermingling matrix with the primary phase, such that both could be considered independent backbones of the overall structure.

Current practice for the freeze drying of immiscible systems is to prepare an emulsion of the materials and freeze dry the emulsion. Complex freeze drying can be expected to give dry materials having different properties even though the overall compositions would be the same.

Successive complex freeze drying allows the preparation of dry materials with closely controlled physical and chemical properties. In the fabrication of food materials, for example, texture might be improved by the addition of a second water-insoluble solute which will prevent overall softening of the food following rehydration. The fabrication of structures on the molecular level by complex freeze drying is an area for potential development.

The incorporation of water-soluble materials in a water-insoluble matrix, or vice versa, is another potentially significant area in food processing. Consider the possibility of controlled release materials (water-soluble compounds released from a water-insoluble matrix) where the influence of temperature on diffusion controls the release. The influence of water on release of the water-soluble compounds could be predetermined by properly designing the water-insoluble matrix. It should be possible, for example, to have matrices which do not release when contacted with water vapor, but allows leaching when contacted with liquid water, or matrices which will not release when contacted with water in either the liquid or vapor state. In the later case, release would be temperature controlled.

Successive complex freeze drying seems to offer the best possible control of the properties of the final product since each step can be independently regulated. This level of control is obtained only by utilizing multiple freeze drying steps, which will result in a high total cost. Freeze drying mixed systems in one operation will significantly reduce processing costs but presumably at some loss of quality.

Complex freeze drying in one step has been subdivided by Rey et al. (9) into two mechanisms, simultaneous and staggered, depending on whether the solvent vapors are sublimated simultaneously or if one vapor is preferentially removed. As has been noted, the properties of final materials having the same solids composition can differ depending on the solvents used and on the conditions utilized for freezing the solutions initially.

We will consider a few general cases. Homogeneous systems are defined as those in which the solvents are miscible and the solutes, each being miscible in its particular solvent, are completely mixed when in the liquid state. This homogeneity disappears during freezing, when the solvents with their respective solutes undergo phase separation and crystallize. Freeze drying can now occur either in a simultaneous or staggered manner. An example of simultaneous sublimation is the mixed solvent system, water:dioxane. The dioxane is used to solubilize polystyrene; water is the solvent for dextran. During freezing, dioxane (with the polystyrene) crystallizes

first and the dioxane crystals are eventually surrounded by ice crystals (with the dextran). Sublimation, however, occurs with equal loss of dioxane and water. The solvent system, dioxane:carbon tetrachloride has a different sublimation behavior. This homogeneous system separates upon freezing; dioxane crystallizes first, initially as isolated regions, although it later is surrounded by solidifed carbon tetrachloride. During freeze drying, carbon tetrachloride sublimates much more rapidly than dioxane; only 44% of the dioxane has been lost when the last of the carbon tetrachloride is removed.

Most systems considered for complex freeze drying will be heterogeneous rather than homogeneous. A degree of homogeneity is achieved by forming an emulsion of the solvents. While it is not necessary that water be included, most systems studied have been water:solvent emulsions. The final structure achieved depends on the state of the emulsion and thus primarily on the solvent material. For example: when water is emulsified with carbon tetrachloride, it crystallizes first, as a continuous phase; when emulsified with benzene, it forms a dispersed phase and crystallizes second.

An interesting aspect of complex freeze drying of emulsified systems is that, although the sublimation rate is different in each phase, the nonaqueous solvent is usually eliminated first. Although this characteristic is primarily related to vapor pressure differences, other factors such as solutes and emulsion properties exert an influence. The preferential elimination of the nonaqueous solvent occurs whether the solvent is the continuous or dispersed phase. Thus, when solvents are the dispersed phase, solvent vapor must be able to pass through the continuous phase, ice. Lambert et al. (12) recently demonstrated this phenomenon under more controlled conditions.

Freeze drying of nonaqueous materials has been investigated with liquid ammonia and liquid carbon dioxide as solvents (8). Both of these good solvents have wide-ranging usefulness, especially in cases where water proves inadequate. The particularly reactive environment provided by liquid ammonia is conducive to the formation of new chemical species. Unstable compounds produced in this system can then be freeze dried at low temperature to yield stable dry powders. Dry powders containing stabilized free radicals can be prepared by irradiating frozen solutions of free radical formers in liquid ammonia at -196°C. The frozen liquid ammonia solution freeze dries at such low temperature (approximately -110°C.) that the free radicals remain immobilized and retain their activity.

Liquid carbon dioxide is increasingly recognized as an important solvent for foods due to its special physical and chemical characteristics. A promising area for the expanded use of liquid carbon dioxide is for extraction of food flavors. Following this extraction, several processes can be considered for obtaining the solvent-free extract. Carbon dioxide-soluble, nonvolatile solute can be added to the liquid system, which can then be frozen by contact with a surface chilled by liquid nitrogen and at a vapor phase pressure of about 10 atmospheres. Freeze drying at -78°C. will commence spon-

taneously when the pressure is reduced to atmospheric and heat input is allowed; it will be rapid at low temperature and atmospheric pressure, and the product will be a dry matrix containing the flavor extract. Another use of the liquid carbon dioxide extract, involving impregnation of freeze-dried coffee (not soluble in liquid carbon dioxide) is discussed by Rey (11). This is essentially a successive complex freeze drying.

The influence of complex freeze drying process variables on properties of the final product have been investigated by Blond (13) and Blond et al. (14). The physical property of primary interest was the specific surface of the dry material as measured by krypton gas adsorption and pore size evaluation using sectioned samples under the optical microscope. These studies showed that the speed of freezing, the solute concentration, colloidal state and degree of polymerization (influencing solution viscosity), and the type of solvent all influence the specific surface of the dry material. Results are presented for polystyrene freeze dried in benzene and for corn starch in water. Similar trends for surface area response are noted for both systems for most parameters; however, solute concentration produces differing behaviors. In general, more rapid freezing or increased solute concentration gives an increase in surface area of the polystyrene although the choice of solvent produces quantitative differences. Polystyrene freeze dried from either benzene, carbon tetrachloride, or dioxane vary greatly in surface area as measured by BET analysis of the krypton adsorption (38, 4.2, or 3.5 m.^{2}/g., respectively). It was suggested that these differences were due to variable development of microporosity (pores of submicron size) depending on molecular solvent:solute interactions since the overall surface, as measured in the microscope, was relatively constant for all solvents (between 1.3 and 2.2 m.^{2}/g.). Similar behavior was reported for polyvinyl pyrrolidone where a 3-fold increase in surface area resulted from freeze drying from acetic acid instead of water (13).

Attempts to control the porosity of cellulose acetate membranes by freeze drying from nonaqueous solutions was reported by Rothbaum (15). Dioxane was used to dissolve the cellulose acetate. Following freeze drying, electron microscopy revealed that the cellulose acetate membrane was essentially unidirectionally transversed by large diameter pores (~1000 Å). Increased concentration of cellulose acetate resulted in a more random spatial crystallization of dioxane but little change in the dioxane crystal size (that is, pore diameter). The desired reduction of pore diameter must be achieved by changes in the solvent crystallization steps. Rothbaum found that increased freezing rate will lead to glass formation and poor freeze drying. Thus, if some intermediate freezing rate is not successful, it might be necessary to evaluate other solvent systems for species which crystallize as smaller units.

Thus, the various methods of complex freeze drying offer the possibility of fabricating dried materials with closely regulated properties. A wide variety of solvents is available for selective solubilization of materials to be incorporated into a very complex matrix, for example:

1. A reactive matrix of high internal surface to be used for adsorptive or catalytic purposes.

2. A mixed matrix of catalytically reactive species which will be inert during storage but can react at a high rate due to its intimate molecular mixing when the catalytic agent is applied.

3. Texture improvement of fabricated foods by incorporation of compounds of limited water solubility within a water-soluble matrix.

DRYING BY CONTACTING WITH NONAQUEOUS SOLVENTS

A quite different interrelationship of freeze drying and nonaqueous solvents from that already presented is dehydration process based on contacting of aqueous materials with nonaqueous solvents. This type process has been variously labeled extractive freeze drying, solvent extraction, solvent drying, azeotropic distillation, azeotropic drying, and azeotropic freeze drying. The terms *extractive drying* and *extractive freeze drying* will be used here for processes above and below the freezing point.

Another nonaqueous drying technique is used by biologists for the preparation of structurally unaltered samples for electron microscopy. It is called *critical point drying* (*16* to *18*).

Extractive methods have been developed for situations where the aqueous sample (generally animal or vegetable tissue) is present in either the nonfrozen or frozen state. Extractions have been conducted with both polar and nonpolar solvents. In the case of nonpolar solvents, the term *extraction* is not truly correct as the water obtained from the product will not be dissolved in the nonpolar solvent. However, the term *extractive drying* will be used to describe dehydration by contact with nonaqueous materials. Literature references for these various processing conditions are:

	Polar solvent	Nonpolar solvent
Nonfrozen sample	*19, 20, 24, and 29*	*19, 21, to 24*
Frozen sample	*25, 27, 28, 31 to 33*	*26, 30*

A wide variety of solvents have been evaluated for use in drying of food products. Polar solvents are generally alcohols, predominantly ethanol (*27, 28, 31* to *33*). Acetone (*16, 20, 25*) and acetates (*19, 20, 27*) have also been investigated. The use of a more novel polar solvent, polyethylene glycol 400, has been reported by Thijssen (*29*). In this case, the proper choice of sample composition allows unfrozen liquid materials to be successfully dried by extraction without loss of their initial round shape. Further, flavors present in this liquid can be retained to a high degree.

Though more potential difficulties exist with nonpolar solvents, primarily due to toxicological and organoleptic aspects of the residual solvent, studies have been conducted using a wide variety of nonpolar solvents such as benzene (*24*),

toluene (*19, 30*), xylene (*26*), kerosene (*26*), hexane (*23*), and chlorofluorocarbons (*16, 23*).

Some interesting aspects of extractive drying of nonfrozen material will be considered first. This process is essentially a solvent extraction, but is continued to the point where the material being extracted is finally obtained in the dry state. This requires that the extracting solvent be replenished with fresh make-up as the process continues. On a commercial scale, this would require the recycling of the extracting medium after the extracted water had been separated. Thus, one important consideration is the ease of separation of the solvent and water. Processes can be designed for nonpolar solvents where the phase separation following condensation of vapors gives an automatic separation; as for polar solvents, a distillation step will probably be required.

The rate of drying of shrimp was shown by Hieu and Schwartzberg (*24*) to be more rapid when polar solvents (ethanol or ethyl acetate) are being used rather than benzene. When drying hardwoods, however, Galezewski and Eckelman (*19*) found that toluene generally gave more rapid drying than ethyl acetate when no concern was shown for sample structural changes. Once these considerations were reflected by changes in the drying procedure, the relative effectiveness of the two solvents was closer. Thus, it was shown that interactive effects of solvent and the aqueous material (such as swelling behavior) can greatly influence the allowable conditions for the extractive drying. The investigation by Hieu and Schwartzberg (*24*) gives an extensive mathematical analysis of the transport properties and processing conditions for a number of solvents used for the extractive drying of shrimp. Unfortunately, quality aspects of the final product were only cursorily presented.

Bacterial spores were shown to be viable after extractive drying with acetone or *n*-butylacetate to residual moisture contents of between 1 to 3%. Extractive drying was conducted in two manners: one as a simple extraction with acetone; the other, an extraction and azeotropic distillation under vacuum with *n*-butyl acetate.

A description of a commercial-scale process for extractive drying with ethylene dichloride as the solvent is available with some cost figures (*21*). While the commercial facility has been used for drying various animal and vegetable materials, there is mention of methods by which concentrated liquids might be dried. It is necessary to add liquids as concentrated drops. This generally would be accomplished by spraying the liquid concentrate into the extracting solvent. Forming an emulsion of the feed liquid with cold solvent prior to spraying results in more uniform dry material.

Thijssen (*29*) has recently reported briefly on another system by which liquids might be extractively dried. Viscosity control of the feed material enables drying of droplets without an emulsification step. By proper control of all processing steps, retention of model flavor compounds in the dry particles was greater than 90%.

Extractive freeze drying is essentially identical with extractive drying except that the material being dried is in the frozen state. This offers potential advantages over regular extractive

drying in that the frozen product will undergo water loss with little or no change in volume. Ordinary extractive drying will suffer from some shrinkage effects during water removal although they will be less severe than in normal air drying since the material solvent, water, is replaced by the extraction solvent. This means that there is less surface tension changes in the piece, and consequently, less tendency to try to reduce the interfacial area. As in normal freeze drying, processing conditions must be chosen to balance heat and mass transfers at a level where the product will remain frozen.

Slight differences in concept can be associated with the use of either nonpolar or azeotrope forming polar solvents. With nonpolar solvents, the solvent serves primarily as a heat transfer medium. The ice sublimates due to the heat transfer from the liquid medium. The vapor is insoluble in the solvent and thus is rapidly transported to the vapor space of the vessel due to density and pressure differences between the water vapor bubble and the liquid solvent. A chilled condenser will remove water vapor from the vapor space, while a vacuum pump is used to remove noncondensables. The receiver flask is designed so that solvent vapors which are condensed will be returned to the extraction vessel. Extractive freeze drying with nonpolar solvents is conducted with essentially no interaction of the water vapor with the solvent.

Extractive freeze drying with low boiling azeotropes (28, 30) is more of a true extraction procedure than when nonazeotrope nonpolar solvents are used. Azeotropes of both polar (27) and nonpolar solvents (30) have been described. The solvent medium supplies heat to the frozen material, causing some ice to sublime. The water vapor produced forms an azeotrope with the solvent which boils at a reduced temperature.

By maintaining the vessel pressure at some fixed level, a constant boiling point is obtained for the azeotrope. Heat is supplied to the solvent at a rate such that the solvent temperature is incrementally above the azeotrope boiling point. In this case, all azeotrope which forms at the sample surface is vaporized and condensed elsewhere in the system. Agitation of the solvent insures that the concentration of water at the sample surface cannot accumulate to the point where insufficient azeotrope is formed, resulting in water at the interface and a reduction of interfacial mass transfer; this would lead to melting of ice in the frozen sample.

Extractive freeze drying has been also described for polar solvents where the extraction solvent is present in large excess and the water extracted is not removed from the extraction vessel simultaneous with the drying process (25). Malecki (28) used molecular sieves to dehydrate the extraction solvent. Drying of gelatin capsules at -78°C. was more effective using ethyl ether than absolute ethanol, though in both cases drying at this temperature was incomplete even after 15 days. Tests using cryogenic liquids, such as liquid nitrogen, with desiccants proved to be too slow.

A method of drying which was developed specifically for the preservation of sample structure is critical point drying (16 to 18). Critical point drying involves bringing the solvent phase in the sample material to its critical point (critical

temperature and critical pressure). At this point gas and liquid phase boundaries disappear—all the solvent existing as one phase without surface tension. The vessel is warmed slightly above the critical temperature to prevent condensation during sample removal and the pressure is then slowly lowered by controlled removal of the gas.

As critical pressures are quite high for most solvents, this process must be carried out in a pressure vessel. Water, for example, has a critical pressure of 217.7 atm. and a critical temperature of 374°C. Thus, for aqueous based materials it is desirable to replace the water by a material having a lower critical point. Carbon dioxide (17) and fluorocarbons (16, 18) have been used. The process itself is relatively rapid [tissue samples processed with freon-113 take 45 min. (16)] although preliminary procedures to impregnate the sample with the desired solvent may prove to be time consuming.

CONCLUSIONS

Nonaqueous materials offer many interesting relationships to the freeze drying process. Materials of controlled and novel properties can be prepared using nonaqueous materials as solvents in normal freeze drying processes. Texture, catalytic effect, adsorbent capacity, flavor, etc. can be designed into such materials by careful stepwise or simultaneous complex freeze drying procedures. Systems can be prepared with mixing of components at the molecular level. Such systems can possibly be used for controlled chemical reaction rates.

Nonaqueous materials can also be used for freeze drying of aqueous samples by extractive procedures. A novel drying method which rivals freeze drying in the retention of sample structure is critical point drying. Nonaqueous materials, which offer simpler operating conditions, will be of significance here as well.

ACKNOWLEDGMENT

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4.3 Artificial gel systems

Work on artificial gel systems has concentrated on calcium alginate gels. The major effort has been in evaluating methods by which the desirable textural qualities can be retained. In addition, the influence of process and composition on taste quality has been considered.

The general method for preparation of the calcium alginate gel consists of equilibrating a sodium alginate solution (usually 3-5%) (contained in a plastic sample cell having nylon membranes at both ends) in a calcium lactate bath (usually 4.5%). The calcium ions diffuse into the cell and crosslink the alginate molecules to form the gel. The strength and thus textural properties of the gel will depend on the initial concentration of alginate and lactate solution in the sample cell and bath, other molecular species present in the systems, and to some extent sample cell-bath geometries.

Tests with various concentrations of sodium alginate and calcium lactate showed that an optimum relative to textural quality was achieved with initial solution concentrations of 3.0% and 4.5%, respectively. Approximately 48 hours of equilibration time was required to completely gel samples when in a cell of 30mm diameter and 50mm height. These samples have a crispy texture after equili-bration.

Sucrose was added to the sodium alginate solution, both to give the product sweetness and to modify the texture by improved water binding. Sucrose concentrations of 0 to 25% (in the sodium alginate) were evaluated initially. The textural behavior was not greatly altered by sucrose concentration. It was noted that the gel sweetness was not sufficient, even at 25% sucrose levels. Refractometric measurements allowed a system mass balance to be obtained. This showed that sucrose was diffusing from the sodium alginate solution into the bath. Sucrose loss ranged from 50-75% of that initially present depending on (1) initial sucrose concentration (2) relative amounts of the alginate and lactate solutions and (3) size of the sample holder. One attempt to halt the sucrose diffusion involved using a calcium lactate (4.5%) - sucrose (22%) gelling bath. This was unsuccessful as no gelling occurred after 3 days. One further attempt, somewhat more successful, involved a higher initial sucrose concentration (50%) and a smaller sample holder which reduced equilibration times to 24 hours. While 60% of the sucrose was lost, the 20% remaining in the gel was adequate to give a distinct sweet taste.

Artificial sweetener (saccharine) at a level of 0.32 grams per 400 ml of alginate solution gave no better quality than the sucrose.

Textured modification was attempted using two polymers as additives to the basic alginate solution. Dextran-10 or a pregelatinized starch were used at various concentration combinations.

starch/alginate			dextran/alginate	
(%)	(%)		(%)	(%)
1.5	3.0		0.5	3.0
1.5	4.0		1.0	3.0
1.5	5.0		2.0	3.0

The effects of the added polymer were not significantly beneficial to warrant further investigation.

Three fruits: apple, pear and cherry, were simulated using the calcium alginate gels. The formulations are listed in Table 1. While problems associated with diffusion loss of sucrose and flavor was noted, good texture was achieved for the fresh gels.

In addition to some preliminary organoleptic tests, the rheological behavior of some fruits and of selected gels was compared using a simple load cell in which a load could be applied and the corresponding fruit (or gell) compression measured in terms of fractional decrease in slice thickness. Figure 1 shows the compression loading and relaxation behavior of peach and apple slices.

Figure 2 shows a comparison of peach and gel behavior. In Figure 3 the compression behavior of two gels is compared with that of peach and apple, showing a good similarity in this particular rheological characteristic.

In general the progress in obtaining fresh gels with fruit characteristics was good. Substantial problems, however, were encountered in the attempts to freeze dry the gel structure and this aspect will require much work. Freezing and thawing caused substantial deterioration of texture and attempts to freeze dry the gels resulted in poorly rehydrating and very tough matrix. Preliminary work on counteracting the effects of freezing and drying by modification of freezing rate and by addition of polymers was not successful. Figure 4 shows compression data for two gels each at a 2% algin and 1% starch level, but with either 3% and 6% sucrose both with and without a freezing treatment. The toughening effect of the freeze-thaw cycle is evident.

Figure 5 compares the compression data for an algin gel before dehydration with the same gel after freeze drying and rehydration. The toughening effect of the drying is apparent.

Work is currently in progress on new approaches to exploring the effects of freezing and drying in order to counteract these effects.

Table 1

Sodium Alginate Solutions for Formulations Simulating Fruits

Apple

45 ml	(3% starch, 15% sucrose)
14 ml	5% malic acid
60 ml	3% sodium alginate
0.2ml/100ml	apple flavor

Pear

36 ml	(5% starch, 50% sucrose)
24 ml	2.5% malic acid
60 ml	3% sodium alginate
0.2ml/100ml	pear flavor

Cherry 1

100 ml	20% sucrose, 3% sodium alginate
1.0ml/100ml	cherry flavor

Cherry 2

100 ml	50% sucrose, 3% sodium alginate
1.5ml/100ml	cherry flavor

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

5. Volatile retention during freeze drying

5.1 Introduction

Work conducted prior to this contract primarily with low molecular weight carbohydrates showed that flavor retention during freeze drying could be explained on the basis of the microregion theory of Flink and Karel, and that this theory could serve as a basis for optimization of process parameters. The Annual Report for Phase I presented results of studies on the behavior of the non-carbohydrate polymeric material polyvinylpyrrolidone (PVP), and also introduced our use of a radiochemical technique for studies of volatile retention. This technique has been used subsequently to evaluate the retention behavior of a number of polymeric materials present in foods and of potential significance in formation of food matrices. This work has been presented in a series of research papers which have been published (section 5.3), accepted for publication (sections 5.4, 5.6) or submitted for publication (section 5.5). In addition current results which have not yet been written for publication are presented as section 5.2 These reports are assembled to form section 5 of the Phase 2 Annual Report.

5.2 The influence on volatile retention of dextrose equivalent (D.E.) for a series of dextrins

Dextrins are formed by partial hydrolysis of starch. The degree of starch breakdown, which depends on a number of process parameters, results in a distribution of molecular units ranging in molecular weight from the monosaccharide, dextrose, to the higher polysaccharides. While it is possible to determine the complete molecular weight distribution for any dextrin sample, it is more usual for dextrins to be classified according to the percentage distribution of the lower oligosaccharides. The most widely utilized method of classification, however, is the so-called dextrose equivalent (D.E.) which is a measure of the number of reducing groups per unit weight of sample. Dextrose, which has one reducing group per glucose unit , is defined to have a D.E. of 100. Maltose, with one reducing group for each two glucose residues (i.e. one reducing group for each maltose molecule) has a D.E. value of 50. As the dextrin molecular weight distribution moves toward larger molecular units, the D.E. values decrease with a lower limit generally being about 2-4. Depending on the D.E. values, these products are generally referred to as maltodextrins or dextrins.

Maltodextrin materials have often been included in studies on flavor retention during freeze drying, but

there has been no effort to investigate the influence of molecular weight distribution of maltodextrins on the volatile retention. Since maltodextrins are utilized in a number of formulated foods, and may be incorporated into freeze dried food matricies, we conducted an investigation of the influence of dextrose equivalent on volatile retentions.

[14]C-labelled volatiles (acetone and ethyl acetate) were used to evaluate the retention in several commercial dextrin preparations. The characteristics of the dextrins are listed below (Maltrin was obtained from Grain Processing Corporation, and Stardri from A.E. Staley).

Material	D.E. value	Percentage of the material in fractions with different number of glucose units			
		1 unit	2 units	3 units	4 or more units
Maltrin 100	9-12	0.5	3.5	6.5	89.5
Maltrin 150	13-17	1.0	3.5	7.5	88.0
Maltrin 200	18-22	1.0	6.0	8.0	85.0
Maltrin 250	23-27	2.5	5.0	8.5	84.0
Stardri 24F	24-28	5	14	14	67
Stardri 35F	33-36	14	12	11	63
Stardri 42F	42-45	20	14	12	54
Maltose	50	0	100	0	0

The volatile retention was evaluated using solutions containing 1% of volatile and either 10, 20 or 30% of the dextrin preparations. Two (2) ml samples were frozen at

-40°C in scintillation vials, freeze dried and rehydrated with 2 ml of water. Scintillation liquid (PPO, napthalene, dioxane) was added and normal counting procedures used.

The results, in the form of two graphs, presents the retentions of acetone (Figure 1) and ethyl acetate (Figure 2) for the three concentrations as a function of dextrose equivalent. An increase of the dextrose equivalent is indicative of a change of the polysaccharide composition to units of lower molecular weight. It can be seen that for both volatiles, as the solids concentration increases, the influence of dextrose equivalent is reduced. Also, as is expected, it can generally be stated that a higher solids concentration results in higher retentions, though at high D.E. with acetone, a slight reversal is observed. An example of the complex interactions of solids and volatile can be seen by the fact that retention shows a slight decline with increasing D.E. for ethyl acetate, while a sizable rise is observed for acetone over the range DE 10 to 25, followed by a leveling off. It cannot be certain but the leveling of the acetone curves above 25 D.E. may be related to the change from Maltrin samples to the Stardri samples, where there is a large increase in the saccharides of 1 to 3 glucose units. In Figure 2, two different polysaccharide distributions at a DE value of 24 (Maltrin 250 and Stardri 24F) were

evaluated. It can be seen that the sample which had the <u>higher</u> percentage of low MW species (Stardri 24F) had a sizably higher retention.

These studies have shown that a variety of dextrin materials show the same general volatile retention behavior as observed for a number of simpler carbohydrate systems. At the higher solids concentration investigated (30%) the level of retention was above 60% for all D.E. values investigated. While differing shapes of the retention-D.E. curves were observed for acetone and ethyl acetate, at a 30% solids concentration these variations in retention are not very large when compared to the overall level of retention.
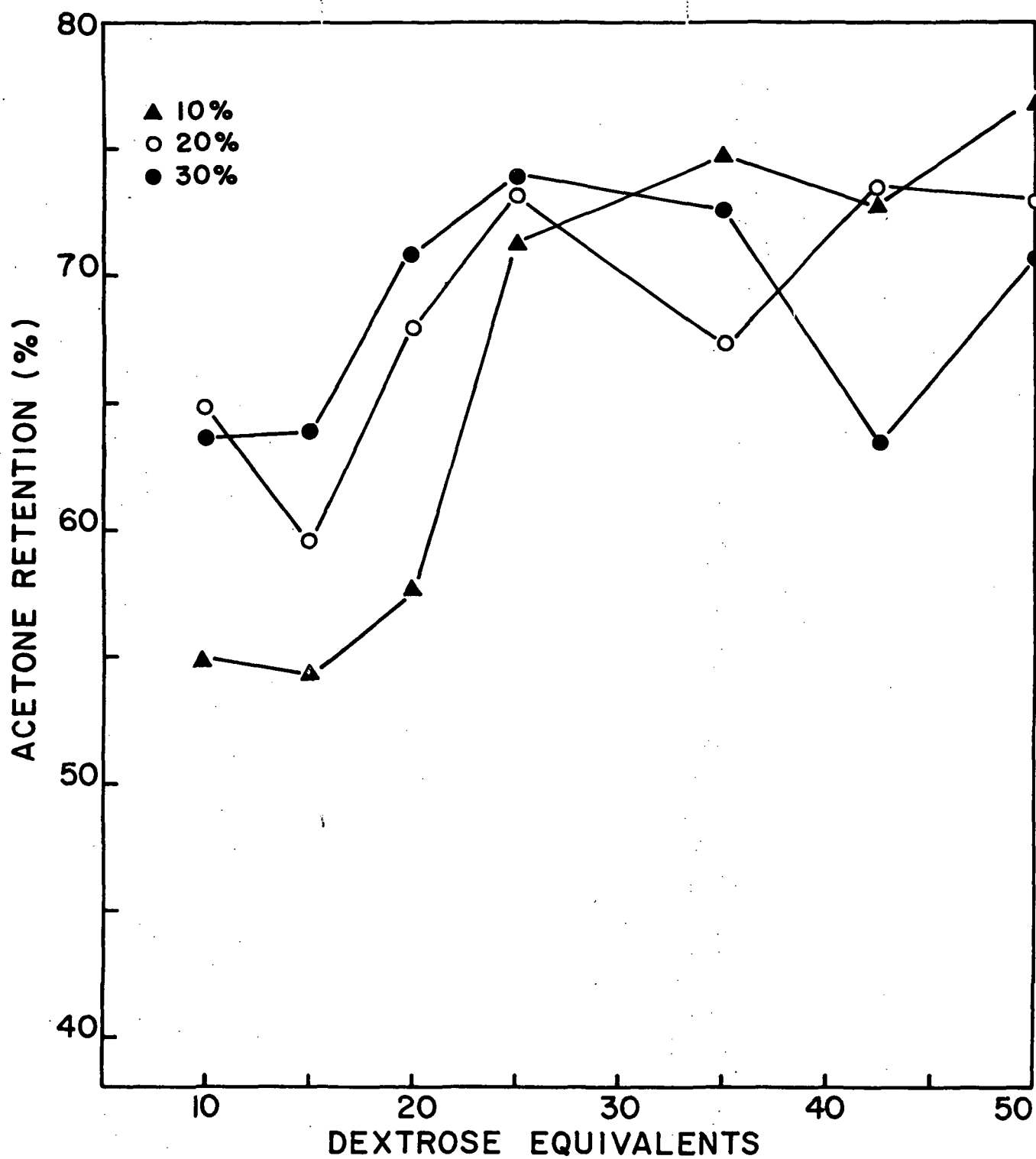
Figure 1

Figure 2

5.3 Volatile retention during freeze drying of aqueous suspensions of cellulose and starch

Volatile Retention during Freeze Drying of Aqueous Suspensions of Cellulose and Starch

Jorge Chirife[1] and Marcus Karel*

This paper studies the retention of ^{14}C-labeled 2-propanol in freeze-dried starch or cellulose suspensions. Among the variables affecting the retention level are concentration of solids and initial concentration of the alcohol in the suspension. The observed retentions can be explained by inclusion within the polymer chains, the predominant mechanism of retention, and adsorption. Cellulose gave a much lower retention than starch, probably because the low mobility of the chains in the highly crystalline cellulose reduces the capacity for retention of the alcohol through inclusion.

In the past few years, significant progress has been made in studies on the mechanism of volatile retention in freeze-dried foods. Most of these studies have been based on model systems, mainly carbohydrate solutions (Flink and Karel, 1970a,b; King, 1970; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and water-soluble polymers (Chirife and Karel, 1973b; Chirife et al., 1973). It is to be expected that studies on model systems, based on individual food components, could eventually lead to a better understanding of volatile retention in more complex food systems.

In this study we present results which characterize the retention of 2-propanol in model systems based on cellulose and starch, polysaccharides widely found in fruits and vegetables. The observed retentions are analyzed in terms of possible interactions between the polymeric substrates with volatile.

EXPERIMENTAL SECTION

Model Systems Preparation. The model systems consisted of either cellulose powder (Whatman CC 41, mean particle size passing 200 B.S.S.) or starch (Merck, Soluble Starch), ^{14}C-labeled 2-propanol, and water. They were prepared by suspending the desired amount of cellulose or starch in water and adding 2-propanol; 0.1% (w/w) of carboxymethylcellulose (CMC) was added to facilitate the handling of the suspensions.

Five-milliliter aliquots of the suspensions were pipetted into 50-ml Erlenmeyer flasks and frozen immediately in liquid nitrogen to maintain the solids in the suspended state. The resultant sample thickness was about 4 mm. The samples were then freeze-dried for 48 hr at ambient temperature and at a chamber pressure of less than 100 μm in a Virtis freeze drier (model 10-MRTR).

Reagent grade 2-propanol was mixed with ^{14}C-labeled 2-propanol to give the desired specific radioactivity. The

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radioactive propanol was obtained from International Chemical and Nuclear Corporation, Irving, Calif.

Humidification Experiments. In several experiments, freeze-dried samples were humidified by placing tared and weighed flasks in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

2-Propanol Analysis. The 2-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

Reproducible measurements with a constant counting efficiency were obtained by dispersing the dried samples of cellulose or starch in water (to 10% w/w) and adding 1 ml of this suspension to 10 ml of the scintillator solution [2,5-diphenyloxazole (1 g), naphthalene (100 g), dioxane to 1000-ml volume]. The resulting dispersion was counted with a liquid scintillator counter (Beckman LSD series).

RESULTS

Figure 1 shows the effect of initial solids concentration on 2-propanol retention by freeze-dried cellulose and starch suspensions. In both cases the alcohol retention increases linearly with solids content in the range examined. For these experiments the initial concentration of 2-propanol was fixed at 0.1% (w/w) for the cellulose system and 0.05% (w/w) for the starch-based model.

For both cellulose and starch, when solids content is kept constant, relative retention increases as initial alcohol concentration decreases. The use of ^{14}C-labeled 2-propanol of relatively high specific radioactivity allowed for a wide range of concentration from 2–3 ppm to 5000 ppm for the starch system.

Results obtained during freeze drying of 20% (w/w) suspensions are shown in Figure 2. The curve which characterizes the behavior of starch suspensions is particularly interesting because the low volatile concentrations investigated are similar to those encountered in natural food systems. The 2-propanol retention in freeze-dried cellulose suspensions appears to level off at 7.3% retention as the initial volatile concentration is decreased.

Table I summarizes the alcohol retention (1-propanol or 2-propanol) observed during freeze drying of several model systems (carbohydrates and polymers). All the experiments were performed in very similar processing conditions (plate temperature, drying time, chamber pressure, frozen layer thickness) and system composition (solids concentration, volatile content), so the observed retentions give a direct indication of the particular ability of each solid substrate to retain the volatile. For high initial alcohol concentrations (0.5 to 1.0% w/w), the low molecular weight carbohydrates are much more effective than the polymeric systems (PVP, Dextran, Starch, Cellulose). However, at low volatile concentrations, the polymers are also able to produce significant alcohol retentions. The exception is cellulose, which even at low initial volatile concentration gives low retention values.

Readsorption of 2-propanol in the dry layer of cellulose during freeze drying was investigated in the following experiment. Samples were prepared by freezing alternate layers of a cellulose suspension containing no volatile and layers of a solution containing the volatile. Each layer was completely frozen before the next layer was added. The composition of the systems for these experiments was fixed: cellulose 20% (w/w) and 2-propanol 0.5% (w/w). During freezing and drying, the layers were separated by thin brass mesh to avoid any "contamination" between them. After the standard cycle of freeze drying (48 hr), the layers were separated for individual analysis. Excellent agreement was found among all the samples. It was observed that the amount of 2-propanol adsorbed in the layers originally containing no volatile was 31.5% of the retention found in the layers originally containing the vol-
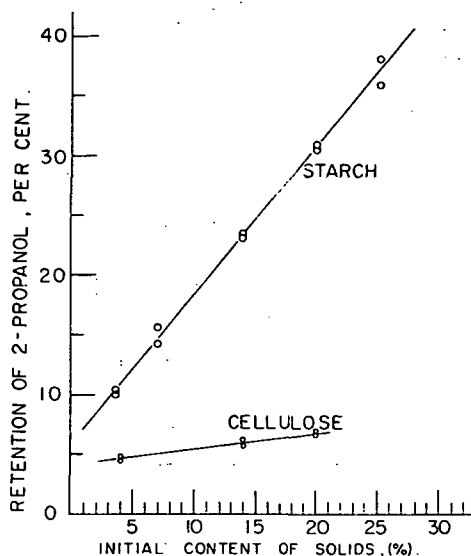
Figure 1. Effects of initial solids content on retention of 2-propanol in freeze-dried aqueous suspensions containing starch or cellulose. Initial 2-propanol content: 0.05% (w/w) in starch suspensions, and 0.1% (w/w) in cellulose suspension.
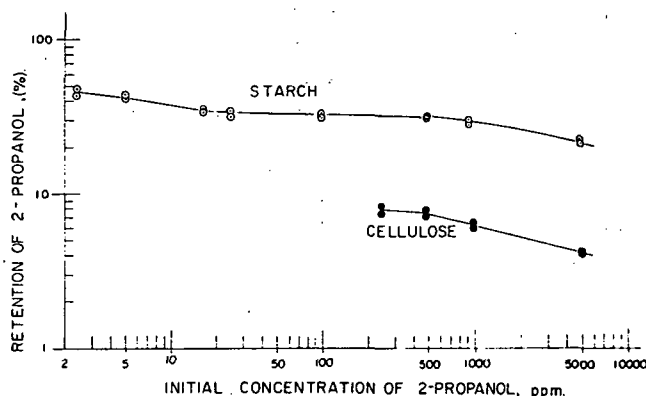
Figure 2. Effects of initial concentration of 2-propanol on retention in freeze-dried suspensions containing 20% of starch or of cellulose.

atile, the absolute amount being between 0.0244–0.0269 g of 2-propanol/100 g of cellulose.

In another experiment, we studied alcohol release due to adsorption of water. Twenty percent suspensions of starch containing 0.05% (w/w) were freeze dried under standard conditions. These conditions resulted in the retention of 0.0775 g of 2-propanol/100 g of starch. The freeze-dried systems were then equilibrated to different relative humidities and the loss of alcohol was determined. Figure 3 shows the 2-propanol retention in freeze-dried starch humidified to 11, 52, and 75% relative humidity.

DISCUSSION

Two types of interactions between the solid substrates and the volatile will be discussed in connection with the mechanism of 2-propanol retention during freeze drying of cellulose and starch suspensions; they are inclusion and adsorption in the dry layer. The first one refers to an entrapment of the volatile between the polymeric units of cellulose and starch, and the second to the binding of the alcohol to specific sites of these polymers. Russell et al. (1937) studied the sorption isotherm of alcohols in cellulose. They found that after sorption of alcohol, evacuation at room temperature did not completely remove the alcohol. They postulated that as a result of the process of removal of alcohol, cellulose chains interact, forming internally stressed structures that hold the residual "solvent."

Table I. Alcohol Retention during Freeze Drying of Carbohydrate and Polymer Solutions

Solid	Concentration of initial solids, % (w/w)	Volatile	Initial concentration of volatile, % (w/w)	Retention, % Rapidly frozen samples	Retention, % Slowly frozen samples	Reference
			Initial alcohol concentration between 0.5–1.0%			
Maltose	20	1-Propanol	1.0	69.5		Chirife and Karel (1973b)
Maltose	18.8	2-Propanol	0.75	67.6		Flink and Karel (1970a)
Malto-dextrin	20	1-Propanol	0.75		80	Flink and Gejl-Hansen (1972)
Glucose	18.8	1-Propanol	0.75	47.8		Flink and Karel (1970a)
Glucose	18.8	2-Propanol	0.75	52.8		Flink and Karel (1970a)
Starch	20	2-Propanol	0.5	21.0		Present work
PVP	20	1-Propanol	0.5		25.5	Chirife et al. (1973)
PVP	20	1-Propanol	1.0	9.8	24	Chirife et al. (1973)
Dextran 10	20	2-Propanol	0.75	7.5		Flink and Karel (1970a)
Dextran 10	20	1-Propanol	0.75	4.2		Flink and Karel (1970a)
Cellulose	20	2-Propanol	0.5	4.0		Present work
			Initial alcohol concentration between 100–250 ppm			
Dextran 10	20	2-Propanol	100	56	97	Flink and Labuza (1972)
Maltose	20	2-Propanol	100	16	88	Flink and Labuza (1972)
PVP	20	1-Propanol	100		58	Chirife et al. (1973)
Starch	20	2-Propanol	100	38		Present work
Cellulose	20	2-Propanol	250	8.4		Present work

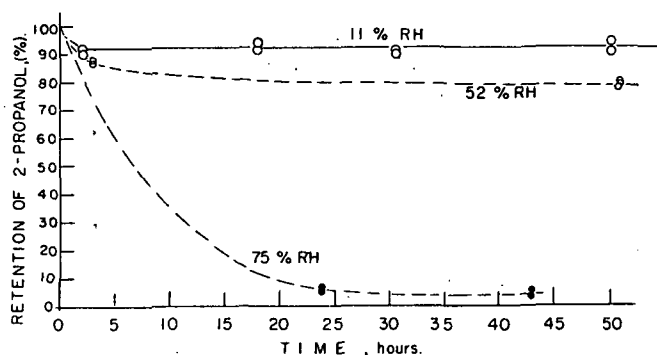**Figure 3.** Retention of 2-propanol during exposure of freeze-dried starch suspensions to specified levels of relative humidity at 25°.

Staudinger et al. (1953) also showed that nonpolar liquids which do not react with cellulose cannot be completely removed by drying in vacuum. They also postulated an inclusion of the organic liquids produced when hydroxyl groups of the cellulose chains hydrogen-bond together.

We have demonstrated in previous studies that most of the retention of volatiles in water-soluble polymers, in particular dextran and polyvinylpyrrolidone, is due primarily to entrapment in microregions similar in their retention properties to those formed by low molecular weight carbohydrates (Chirife et al., 1973; Flink and Karel, 1970a). However, in polyvinylpyrrolidone, adsorption also plays a role and, in fact, a small but significant readsorption of propanol occurs in dry layers formed during freeze-drying (Chirife and Karel, 1973). The "layered system" experiments reported in this paper show that readsorption of 2-propanol in the dry layer can contribute to retention in freeze-dried cellulose suspensions.

Sorption of alcohols in dry cellulose has been measured by several workers, including Lauer and Ayer (1957), Colombo and Immergut (1970), and Le Maguer (1972). Through measurements of heats of sorption, Colombo and Immergut (1970) found that the interaction cellulose-methanol involves hydrogen-bond formation which extended over a wide range of vapor coverage. However, sorption isotherms measured at high volatile partial pressures may not be valid at the low levels expected in freeze drying of model systems. A small amount of very active sites may be available for strong adsorption of small quantities of the volatile, leading to retention. Sorption of

volatile compounds in starch was studied by a number of authors; Maier and Bauer (1972) suggest that most aroma compounds may be bound by hydrogen bonds or inclusion. Starch has the capacity to complex many ligand molecules such as aliphatic alcohols, fatty acids, and aliphatic hydrocarbons (Bear, 1944; Kuge and Takeo, 1967; Osman-Ismail and Solms, 1972; Takashi and Takeo, 1968). It was considered that complexing occurs mainly within the helical regions of the amylose fraction. Several possibilities were taken into account for the forces that stabilize the inclusion complex, namely hydrophobic bonding, hydrogen bonding, dipolar interactions, etc. (Kuge and Takeo, 1967). Based on this property of amylose, Solms et al. (1973) suggest that the formation of starch-inclusion complexes is an important mechanism of volatile retention during food processing. However, some characteristics which regulate the formation of such complexes strongly indicate that this is not the case for the 2-propanol retention during freeze drying of starch suspensions. To begin with, a certain minimum concentration of ligand was necessary to initiate the formation of insoluble complexes (Solms et al., 1973). Also, reaction mixtures did not give inclusion compounds under constant temperature conditions but only if a temperature gradient was applied ranging from 90° to room temperature (Solms et al., 1973). This suggests that the reaction requires some sort of activation. Furthermore, the humidification experiments shown by Figure 3 are another indication that no irreversible complex was formed, resulting in 2-propanol retention. Humidification to 75% RH produces almost total loss of retained 2-propanol. This effect can be attributed to the swelling effect of water molecules which permits the loss of the entrapped alcohol.

We may conclude that inclusion of the volatile between the polymer chains and readsorption in the dry layer are the main mechanism of retention. The low levels of retention observed with cellulose can be explained on the basis of the low mobility of the cellulose chains, as compared with starch or other polymeric materials.

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5.4 Volatile retention during freeze drying of protein solutions

Volatile Retention During Freeze Drying of Protein Solutions

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INTRODUCTION

Retention of organic volatiles in freeze-dried systems is determined by properties of non-volatile solutes which often form an amorphous matrix of the freeze-dried solid. Retention presumably results from an entrapment mechanism which immobilizes the volatile compounds within that amorphous solute matrix (7, 13, 33, 36). At present two approaches appear to give mechanistic descriptions for observed volatile retention phenomena. These are "selective diffusion" (19, 20, 29, 34, 36) and "microregions entrapment" theories (13, 15). While these are based on different approaches, they may represent macro and micro views of the same basic phenomenon (8).

Most of the research on volatile retention has been concerned with interactions between carbohydrates and various volatiles. Recently, Chirife *et al.* (8). were able to extend the applicability of the microregions theory to a system other than carbohydrates. Model systems of a water soluble polymer, polyvinylpyrrolidone (PVP) virtually duplicate the behavior of carbohydrates with the exception that a small fraction of volatile may be retained by adsorption phenomena (9). In that work, PVP was chosen as a model system because it is a water-soluble polymer containing a polar group different from

those of polysaccharides but also present in proteins.
PVP does not, however, manifest the complexity of
interactions that occurs in proteins.

We are now presenting results which describe
retention of 2-propanol in a model system based on a
water soluble protein, bovine serum albumin (BSA). Some
experiments were also performed in a model system based
on the enzyme, pepsin.

EXPERIMENTAL

Model system preparation

The model systems consisted of either bovine serum
albumin (BSA) or pepsine, ^{14}C labeled 2-propanol and
water. The model systems were prepared by dissolving
the desired amount of protein in distilled water and
adding 2-propanol. In preparing the pepsin model
system, the pH was increased to 8.5-9.0 using NaOH.
Five ml aliquots of the solution were pipetted into
50 ml Erlenmeyer flasks which were frozen as specified
below and then freeze dried for 48 hr in a Virtis
freeze drier (model 10-MRTR). The drying took place
at room temperature and low chamber pressures (< 100 μm).
Temperatures were not measured during freeze drying.
No partial melting or collapse was observed in any of
the samples during freeze drying. The conditions of
freeze drying were identical to those that we previously

found gave high volatile retentions in low molecular weight carbohydrate-volatile systems.

The sample thickness was varied in some experiments as noted under RESULTS & DISCUSSION. In most experiments the composition of the systems was fixed as (initial concentration expressed in weight percent): protein 20%, 2-propanol 0.05%, water 79.95%. In several experiments the effect of changing concentrations was studied; the changed compositions are noted under RESULTS & DISCUSSION.

Samples were frozen by one of two methods: fast freezing was accomplished by immersion of flasks in liquid nitrogen; slow freezing refers to placing the stoppered flasks in still air at -40°C.

Some freeze-dried samples of BSA containing no 2-propanol were analyzed gravimetrically for residual moisture content by drying in a vacuum oven at 87°C for 32 hr. The moisture content was found to be < 0.4%.

BSA

Bovine serum albumin, Cohn Fraction V, powder of purity 95% was obtained from Nutritional Biochemical Corporation.

Pepsin

Pepsin, N.F. powder was obtained from Fisher Scientific Co. The proteins were not further purified.

2-Propanol

Reagent grade 2-propanol was mixed with ^{14}C labeled

2-propanol to give the desired specific radioactivity.
The radioactive propanol was obtained from International
Chemical and Nuclear Corporation in Irving, California.

2-Propanol analysis

The 2-propanol content was determined by measuring
the radioactivity of the samples with a liquid scintilla-
tion counter.   Reproducible measurements with a constant
counting efficiency were obtained by dissolving the dried
samples in water (to 10% w/w) and adding 1 ml of this
solution to 13 ml of the scintillator solution (2,
5-diphenyloxazole 1 g, naphtalene 100 g, dioxane to 1000
ml volume). The resulting mixture was counted with a
liquid scintillation counter (Beckman LSD Series).   The
results were corrected for background counting, although
this correction was significant only at the lowest volatile
concentrations. It was demonstrated that the sedimentation
of insoluble protein did not affect the reproducibility of
the measurements.

RESULTS & DISCUSSION

*Effects of pre-treatments and processing conditions on
freeze-drying.*

As discussed previously (8, 15), we conceive the
retention of volatiles in freeze-dried solutions to be
caused by the formation of microregions during freezing.
During dehydration the microregions are stabilized by

formation of a matrix which becomes completely impermeable
to organic volatiles but not to water when the local
moisture content drops to a critical level. The matrix
is stabilized by noncovalent bonds between suitable
polar groups of the solute units and the critical moisture
content tends to occur at the B.E.T. monolayer value
(8, 15).

Process conditions affect the formation of micro-regions
and, consequently the extent of retention. Freezing rate
is of considerable importance as it influences volatile
incorporation in the solute matrix (13); slow rates promote
volatile retention in microregions.

Table 1 presents a comparison between retention of
propanol in freeze-dried BSA and pepsin systems and
retentions previously observed in freeze-dried PVP and
maltose. In all systems slow freezing resulted in
higher retention than rapid freezing; this effect was
particularly notable with the high molecular weight
($\%$ 70,000) BSA. These findings are compatible with the
microregion theory: slow freezing, which allows diffusion
of solute from the freezing front, results in fewer,
larger, more concentrated microregions, which are less
permeable than those created by rapid freezing (14).

Figure 1 shows the effect of initial alcohol
concentration on retention of 2-propanol by BSA (rapidly

frozen) and pepsin (slowly frozen). The relative retention
by BSA can be seen to decrease with increasing alcohol
concentration. Similar results were found by Chirife
et al. (8) in the PVP-n-propanol system and are also
consistent with the microregions theory (14). It is
expected that as the volatile concentration decreases the
volatile loss would approach a minimum, which would depend
on volatile amount transiently entrapped within ice,
(and therefore outside the non-volatile solute matrix)
and other factors. Relative volatile retention will
therefore approach a maximum. In the case of BSA-propanol
system this maximum has not yet been reached down to an
initial concentration of 50 ppm, at which concentration
propanol retention was only about 20%. With pepsin,
however, retention is high (over 80%) at all initial
concentrations studied (up to 1000 ppm). Apparently
maximum retention here is attained already at relatively
high initial concentrations of propanol.

The microregions theory predicts that increasing
the solid concentration increases volatile retention
up to a limiting concentration which depends on the
type of solid and type and amount of volatile. Figure 2
shows the results obtained with the BSA-2-propanol
system (slowly frozen). The shape of the curve is in
good agreement with those obtained by other workers in

several model systems (8).

Thickness of sample is also a factor in retention. Figure 3 presents the retention of 2-propanol (initial concentration 0.05%) in BSA (slowly and rapidly frozen) and pepsin (rapidly frozen). The retention decreases with increasing thickness primarily because the more rapid drying and steep moisture gradients in thin samples decrease the period during which the moisture content is high enough to permit volatile escape (14).

We also investigated the possibility that changes in the proteins induced by heat or by pH changes could influence the 2-propanol retention during freeze drying. Samples of BSA and pepsin were subjected to pH changes and/or heat treatment prior to addition of the volatile. The samples were then freeze dried under standard conditions and the observed volatile retentions were compared with control samples. Results are shown in Table 2.

BSA was partially denatured by shaking a 20% solution in a bath at 65.5°C during 120 min (33). An increase in viscosity after heating was easily observable indicating unfolding of the molecule. Table 2 shows that heat denatured BSA is as effective in 2-propanol retention during freeze drying as the native protein. This result indicates that preservation of native structure of the

native protein is not necessary for entrapment of the volatile. Table 2 also shows that an increase to pH 10 has only a minor influence in the observed retention, in spite of the known conformational changes occurring in BSA at this pH (6, 27).

Pepsin shows a similar behavior; samples heated at 82°C did not show any difference in 2-propanol retention as compared with the unheated samples. However, this experiment is not as conclusive as the one with BSA because it is known that at pH 7 or higher, pepsin is already "alkali denatured" (4).

A combination of heat and low pH was able to produce a small reduction in volatile retention. However, it is likely that this effect was due to a change in the substrate composition, because some hydrolysis of peptide bonds occurs in these conditions due to autolysis (4). The physical appearance of the freeze-dried cake also confirmed that a change in composition of the system occurred.

The possibility of binding of 2-propanol on specific sites of BSA was also considered. BSA binds a great variety of small uncharged molecules (12, 28, 32). Very little information is available, however, about identification of binding sites. In the presence of any specific molecule, a particular configuration of BSA is stabilized which permits the various portions of the

small molecule to interact with the appropriate groups in the protein (30).

Also, the folded structure of globular proteins is partially stabilized by hydrophobic interactions, and these interactions are important in binding (28). However, we believe that the role of hydrophobic interactions, if any, is not significant in retention of propanol during freeze drying. In discussing the interaction of volatiles with food components, Solms et al. (31) have suggested that hydrophobic interactions between proteins and ligands are of importance in the mechanism of flavor retention during food processing. They based this suggestion in experimental results obtained by Arai et al. (2) who found that some n-hexanal and n-hexanol were retained by soy protein after vacuum distillation at 30°C, and the amount of bound ligand increased with heat denaturation of the soy protein. According to Solms et al. (31) proteins retain volatiles interacting with them by unfolding and destruction of tertiary structures with the binding mechanism being a hydrophobic one. Binding, in this view, depends partially on the capacity of the ligand to cause unfolding of the protein. Heat denaturation increases the amount of binding through the unfolding of the molecule.

We believe that the role of hydrophobic interactions

as a mechanism of volatile retention by protein systems have been over-emphasized by Solms et al. (31); the amount of n-hexanol in soy protein after vacuum distillation of 9.1% (w/w) soy protein solution represents only a retention of 0.13% for an initial volatile concentration of 1000 ppm, whereas in our study retention was in the range of 10 to 90%. In addition, an examination of the experimental procedure of Arai et al. (2) concludes that heat denaturation of the protein was not responsible for the observed increase in volatile retention. Arai et al. (2) mixed the protein solution with the volatile and then heated the mixture to produce the denaturation at a temperature of 90°C during 1 hr. Heating in presence of the organic compounds may have resulted in chemical reactions between the protein and the volatiles.

Release of entrapped volatile by humidification

BSA solutions (20% solids, 0.05% 2-propanol) were freeze dried under standard conditions (slowly frozen, freeze dried at room temperature). These conditions resulted in the retention of 0.16 g 2-propanol/ 100 g BSA. The freeze-dried systems were then exposed to different relative humidities and water uptake and volatile loss were measured as a function of time. From results

in Figure 4 it can be seen that humidification leads
to loss of propanol until a new level of retention is
reached, which is again stable unless the humidity is
increased further.

These results are of striking similarity with those
observed by Flink and Karel (15) during humidification
of freeze-dried carbohydrate-volatile systems. They
found that at low relative humidities (in equilibrium
with moisture contents below the B.E.T. monolayer value)
there was no volatile loss; at higher humidities a rapid
volatile loss occurred until a new level of volatile
content was reached.

The adsorption of water by BSA was studied by
several authors. Using the B.E.T. equation and the
vapor pressure data of Bull (5) on horse serum albumin,
Pauling (25) estimated that the first layer of sorbed
water contained 6.7 g water/100 g BSA. Amberg (1) and
Eley and Leslie (11) reported values of 6.2 g water/
100 g BSA for the B.E.T. monolayer.

Fuller and Brey (17) determined the nuclear magnetic
resonance line widths of water sorbed on solid BSA. Their
results indicated that the sorbed water exists in different
states, and some 6.5 to 8.5 g water/100 g BSA was strongly
bonded to the protein. Figure 4 shows that there is little
volatile loss during humidification to 20% and 32% RH;
the water adsorbed in these conditions being respectively,

5.5 and 8.5 g water/100 g BSA. This appears to indicate that water corresponding to that "strongly bonded" by BSA does not significantly disrupt the microregions entrapping the volatile. However, at 52% and 75% RH the adsorbed water is well above that range and results in significant volatile release due to microregion disruption. These results can be considered surprisingly consistent with those obtained with carbohydrates, considering the diversity of polar groups and complexity of interactions that can occur in a protein as compared to carbohydrates.

Role of adsorption

Adsorption has to be also considered in explaining the 2-propanol retention by BSA. Proteins are able to adsorb polar vapors in the "dry" state, in contrast to carbohydrate-volatile systems (13, 21). The sorption of aliphatic alcohols in BSA and related proteins has been measured by a number of authors. Among them, Benson and Richardson (3) measured the sorption isotherm of ethanol, methanol and butanol vapors on egg albumin and of ethanol on BSA. Eley and Leslie (11) studied the sorption of methanol on BSA; Fogiel and Heller (16) reported on the sorption of ethanol by egg albumin, and Puri and Malik (26) on the sorption of methanol and ethanol vapors on casein. Most of these studies have

been concerned with the B.E.T. analysis of the measured

isotherms. However, this kind of analysis is of little

utility from the point of view of the adsorbate which

is irreversibly adsorbed (under the experimental

conditions) and does not take part in the equilibrium

relations of the B.E.T. theory. Unfortunately, it is

not always possible to decide whether chemisorption is

involved in addition to non-covalent forces.

Some indication of the ability of the non-volatile

solids comprising the freeze-dried matrix to interact

strongly with the volatile is given by "layering"

experiments such as those reported by Flink and Karel

(13), and Chirife and Karel (9). The same experiments

were done on the system BSA-2-propanol. Samples were

prepared by rapidly freezing alternate layers of a

solution containing the volatile and layers of a

solution containing no volatile. Each layer was

completely frozen before the next layer was added. The

initial 2-propanol content was fixed for this experiment

at 1% (w/w) for purposes of comparison with the

experiments reported on the PVP-n-propanol system (9).

After the standard cycle of freeze drying (48 hr), the

layers were separated for individual analysis. The

absolute amount of 2-propanol adsorbed in the dry

layers was about 0.051 g 2-propanol/100 g BSA. This

value is equal to those reported by Chirife and Karel

(9) for PVP-n-propanol, and Flink and Karel (13) for

maltose-2-propanol, and shows that a small amount of

very active sites may be available for strong adsorption

of small quantities of alcohol. In this connection, it

is noteworthy that Fogiel and Heller (16) in studying

the sorption isotherm of ethanol on freeze-dried egg

albumin found a strong deviation of B.E.T. theory at very

low relative pressures (below 0.05), which were tentatively

interpreted in terms of chemisorption or a small fraction

of active sites having a very high affinity for the

vapor.

Attempts at thermal desorption of entrapped volatile

Freeze-dried samples of BSA-2-propanol containing

about 0.16 g 2-propanol/100 g BSA were placed in a

vacuum oven over anydrous calcium sulfate and activated

charcoal and evacuated at 50°C, 72°C, 87°C and 106°C.

The samples were placed on a bed of purified sand to

insure good thermal contact. The 2-propanol content was

measured as a function of time.

The results are shown in Figure 5; it can be seen

that there is no significant loss of volatile in the

range of temperatures examined. In addition, we did not

observe any change in the external structure of the

freeze-dried BSA by effect of temperature. These results

are in good agreement with those obtained by Chirife and Karel (18) who studied the effect of temperature on the release of entrapped n-propanol from freeze-dried maltose. They noted especially the coincidence of structural changes observable visually at the onset of volatile release. We believe that the observed results support the microregion theory of volatile retention. The loss of volatile appears associated with disruption of structure present in the freeze-dried cake. Thermal energy from heating will cause disruption only when it is above some energy level (24). Therefore, as shown in Figure 5, 2-propanol retention in freeze-dried BSA will remain constant when the sample is heated at temperatures at which no major structural changes occur.

The lack of apparent change in the external structure of BSA does not preclude the possibility of denaturation in the "dry" state. Mirsky (22) heated crystals of horse serum albumin (HSA) at various temperatures from 60°C to 115°C. At no temperature were the crystals destroyed and even after heating at 115°C the crystals seemed as perfectly formed as before heating. However, Mirsky confirmed the occurrence of denaturation in the solid state for the highest temperatures, without apparent change in the external crystal form.

SUMMARY

Retention of ^{14}C labeled 2-propanol was studied in freeze-dried systems containing either bovine serum albumin (BSA) or pepsin. Retention during freeze drying varied with: initial concentration of solids and 2-propanol, rate of freezing and sample thickness. Heat denaturation of the proteins did not significantly affect the volatile retention. When freeze-dried BSA was rehumidified, volatile retention dropped to a new level which depended on the final water content. Heating the freeze-dried BSA systems in vacuum had no effect on the 2-propanol retention. The results are indicative of structurally dependent retention and can be mainly interpreted by the "microregions" theory of volatile retention.

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FIGURE LEGENDS

Figure 1   Effect of initial 2-propanol content on the
            retention during freeze drying of BSA and
            pepsin solutions (20% solids).

Figure 2   Effect of concentration of solids on 2-propanol
            retention during freeze drying of BSA solutions
            (initial 2-propanol content: 0.05% w/w).

Figure 3   Effect of sample thickness on 2-propanol
            retention during freeze drying of BSA and
            pepsin solutions (20% solids content, initial
            2-propanol content: 0.05% w/w).

Figure 4   Loss of 2-propanol from freeze dried BSA
            humidified to specified relative humidities
            at 25°C.

                Initial 2-propanol content: 0.16 g/100 g BSA
                Equilibrium water content at 20% RH: 5.7 g/100 g BSA
                                          at 32% RH: 8.5   "
                                          at 52% RH:12.6   "
                                          at 75% RH:20.2   "

Figure 5   Retention of 2-propanol in freeze dried BSA
            heated at several temperatures.

                Initial 2-propanol content: 0.16 g/100 g BSA

TABLE 1

Effect of Freezing Rate on Retention of Propanol
by Model Systems

Solid	Solid conc. %	Volatile	Initial volatile conc. %	Retention(%)	
				Slow Freezing	Fast Freezing
Maltose	18.8	2-propanol	0.75	87.5	67.5
PVP	20	n-propanol	1	24	10
			0.05	33.5	-
			0.005	65	-
BSA	20	2-propanol	0.05	62.5	11.5
			0.005	-	20
Pepsin	20	2-propanol	0.05	82	59

TABLE 2

Effects of Heat and of pH on Retention of 2-propanol
in Freeze-dried Protein Systems

Solids conc. 20%; Volatile: 2-propanol; Slowly frozen samples

Protein	Initial volatile Conc. %	pH	Heat	Retention %	No. of samples studied .
Pepsin	0.1	8.5-9.0	no	81	3
Pepsin	0.1	8.5-9.0	2 hr at 82°C	80.5	3
Pepsin	0.05	8.5-9.0	no	82	4
Pepsin	0.05	2	2 hr at 70°C	75.5	4
BSA	0.05	6.7	no	62.5	8
BSA	0.05	10	no	67.5	4
BSA	0.05	6.7	2 hr at 65.5°C	61.5	4
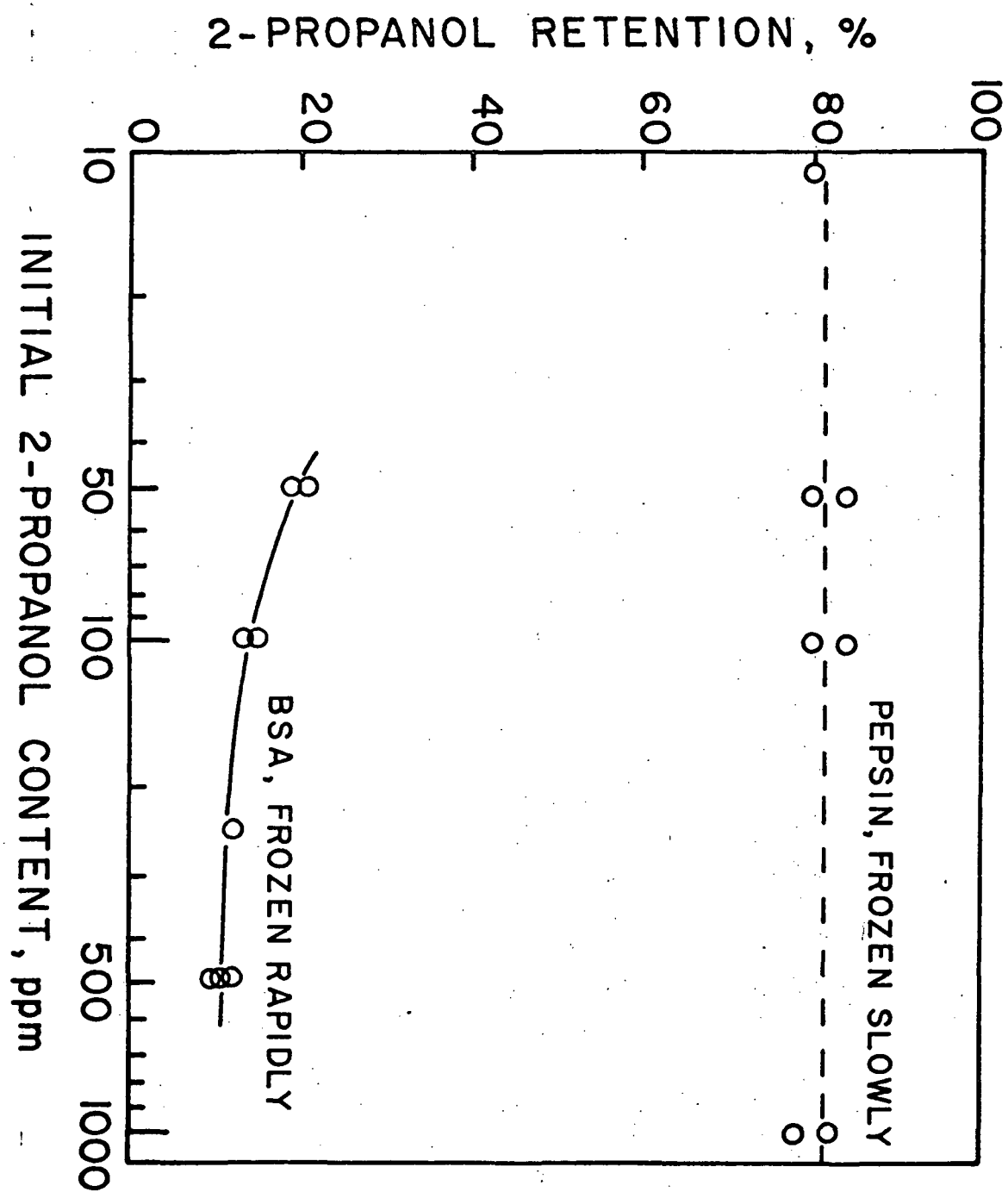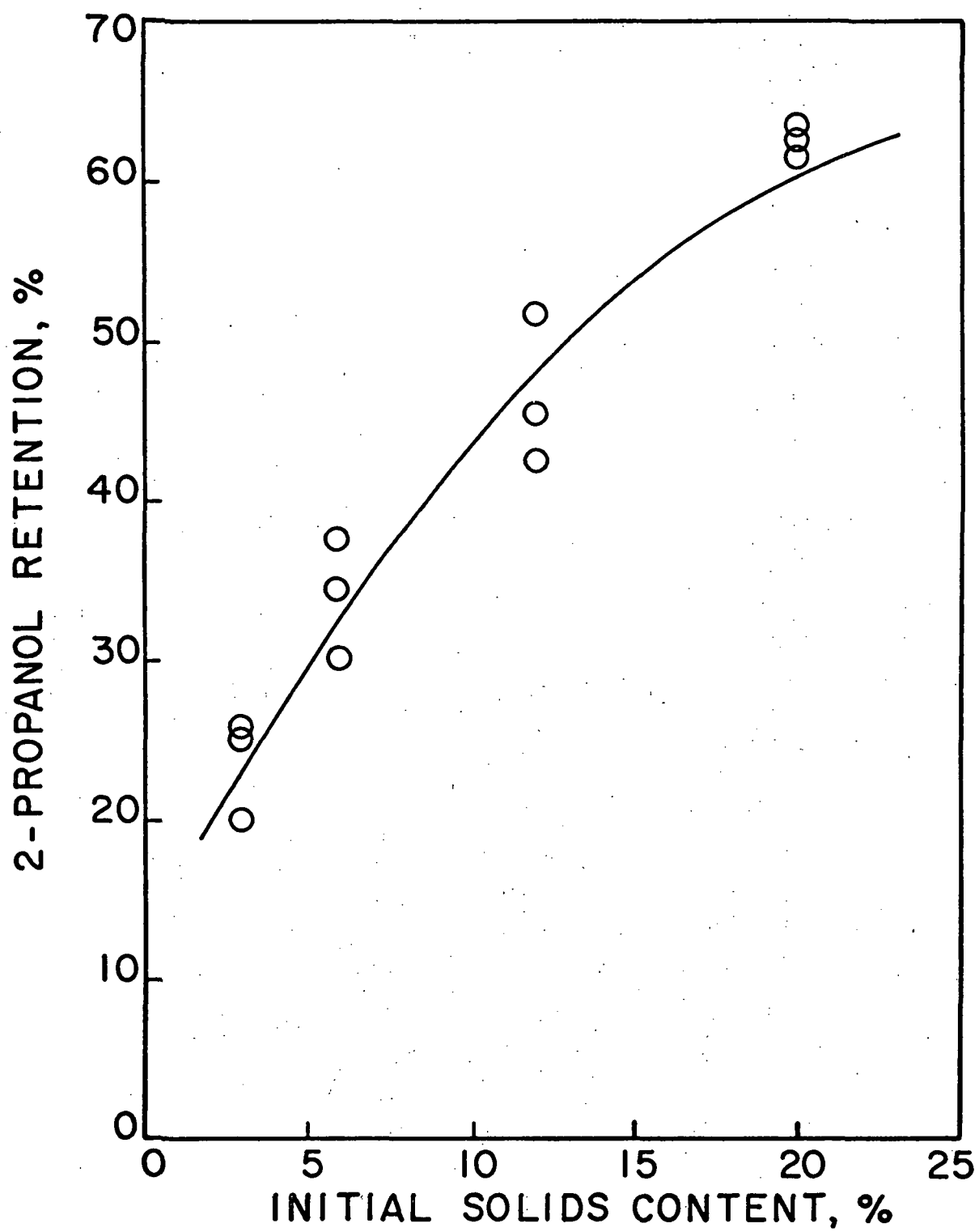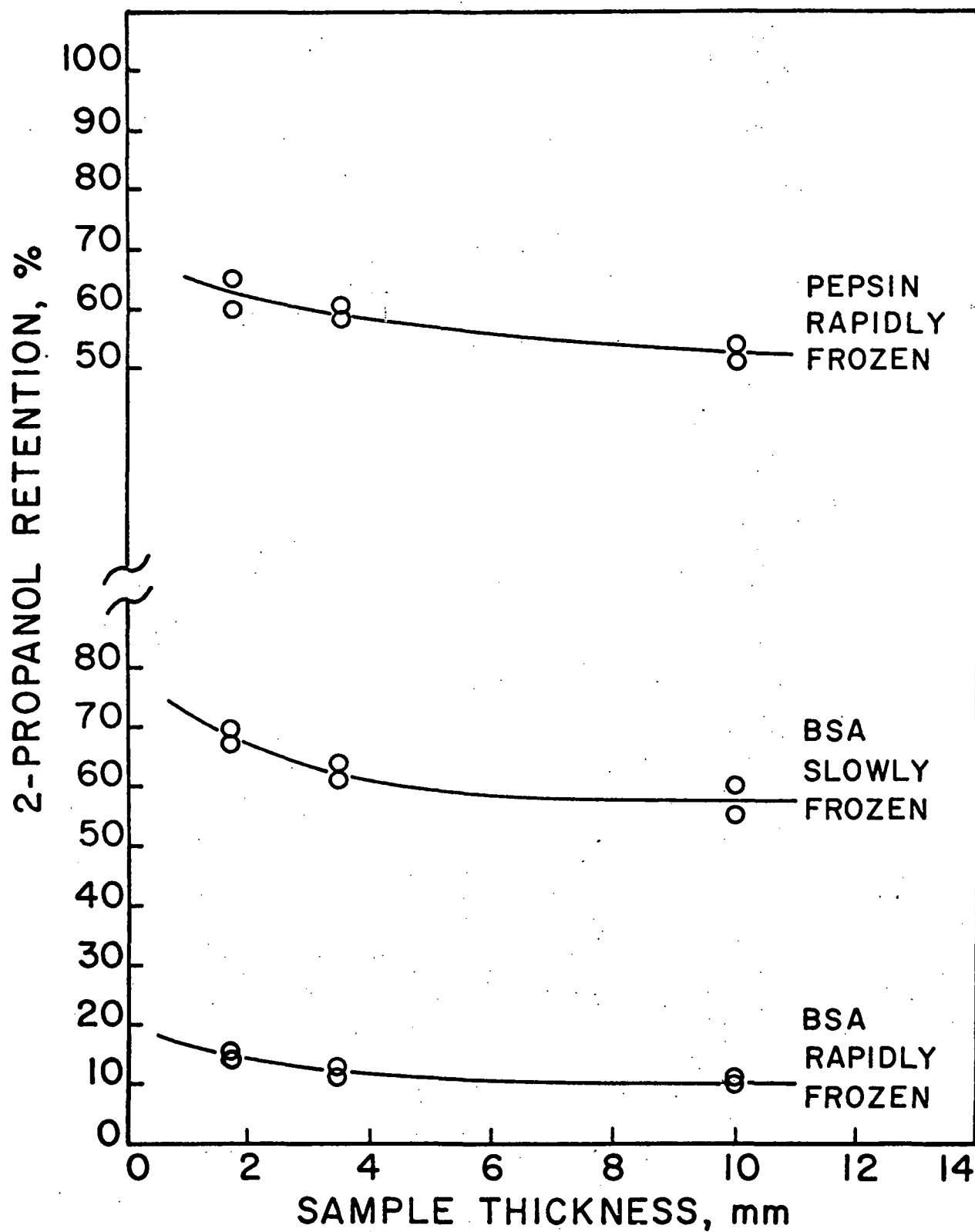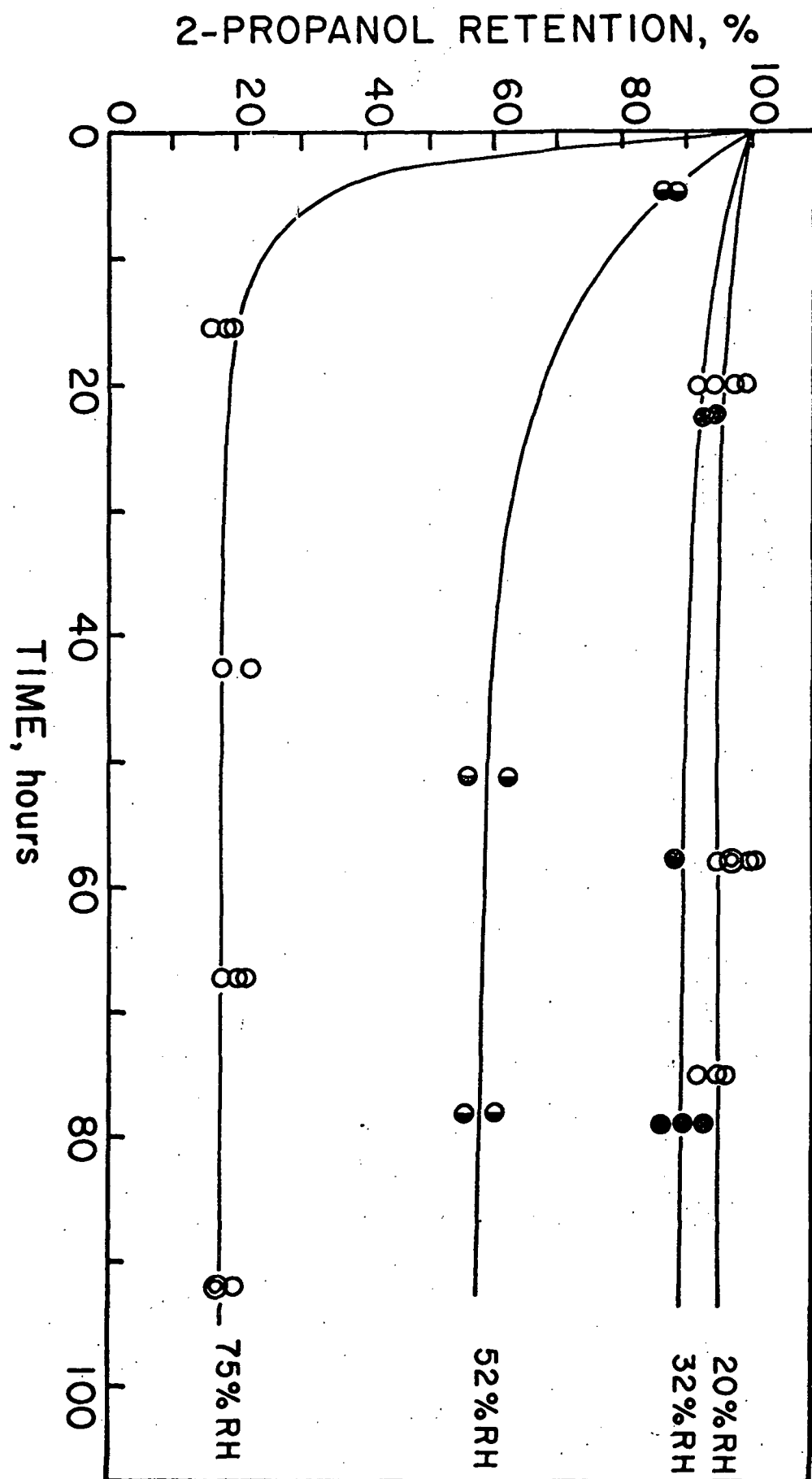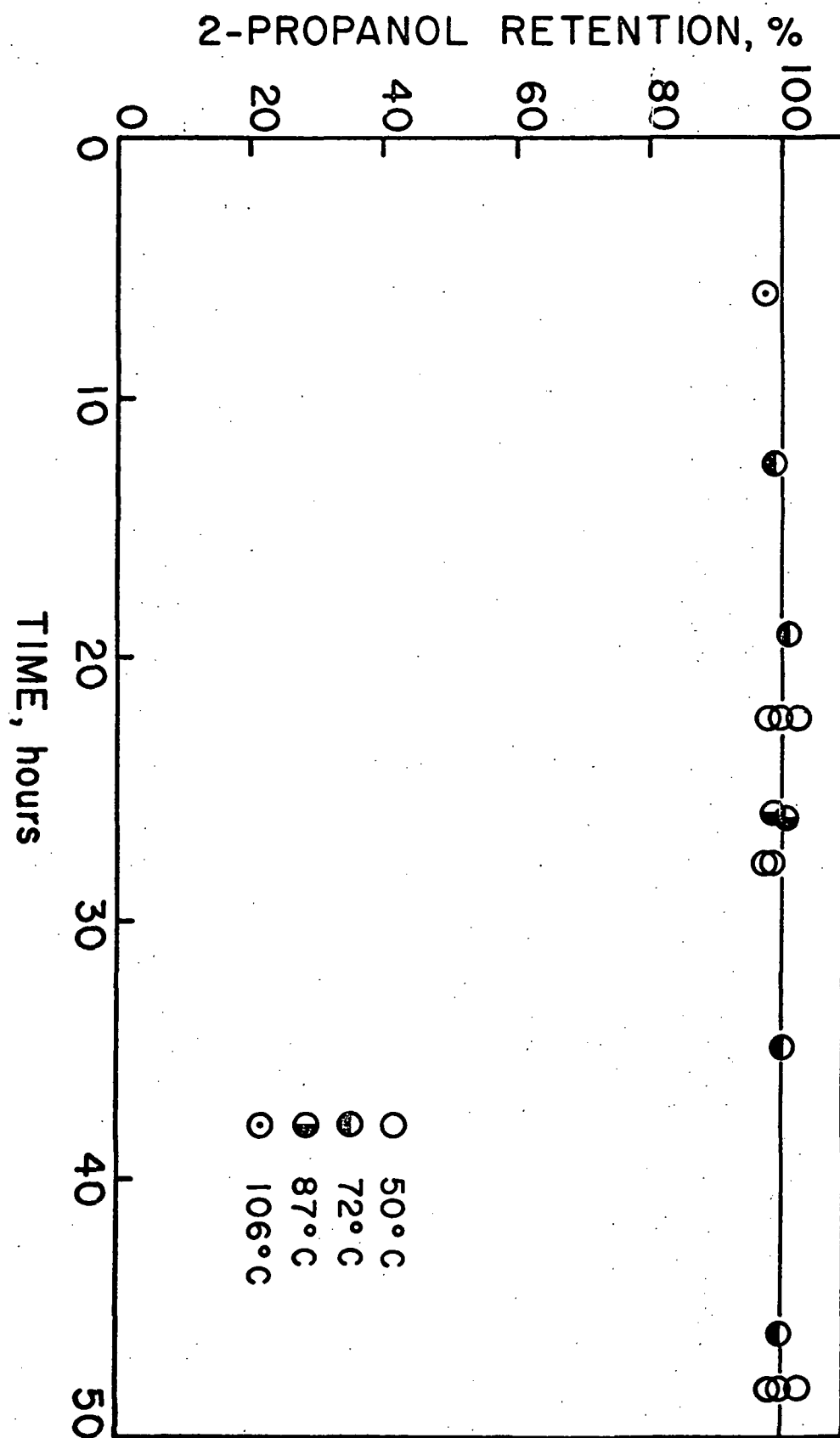
5.5 Retention of volatile organic compounds in a complex freeze dried food gel

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Retention of volatile organic compounds in a complex freeze-dried food gel

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Running title: Volatile retention in freeze-dried food gels

Summary

The retention of ^{14}C-labelled ethyl acetate, n-propanol and acetone in freeze-dried food gels is studied. Retention of volatiles increases with increasing solids concentration and decreases with increasing initial volatile content. Retention also decreases with increasing sample thickness in fast-frozen samples, and is lower in rapidly-frozen than in slowly-frozen samples.

Humidification causes release of retained volatiles, the new level of retention depending upon relative humidity. The results indicate that the predominant retention mechanism is entrapment in microregions, with a small contribution due to adsorption.

Introduction

The quality of a food product depends in part on its flavor constituents. The retention of these compounds during dehydration and other food processing operations is therefore of considerable significance.

Retention of organic volatiles in freeze-dried systems is determined by properties of non-volatile solutes which often form an amorphous matrix of the freeze-dried solid. Retention presumably results from an entrapment mechanism which immobilizes the volatile compounds within that amorphous solute matrix (Flink and Karel, 1970a; Thijssen, 1971).

At present there are two satisfactory mechanistic descriptions for observed volatile retention phenomena. These are "selective diffusion" (Thijssen, 1971) and "microregion entrapment" theories (Flink and Karel, 1972). While these are based on different approaches, they may represent macro and micro views of the same basic phenomenon (Chirife, Karel and Flink, 1973).

Most of the research on volatile retention has been concerned with interactions between carbohydrates and various volatiles. Recently, Chirife, Karel and Flink (1973) were able to extend the applicability of the microregion theory to a system other than carbohydrates. Model

systems of a water-soluble polymer, polyvinylpyrrolidone
(PVP) virtually duplicate the behavior of carbohydrates,
except that a small fraction of volatile may be retained
by adsorption phenomena (Chirife and Karel, 1973a). Other
polymer systems studied by Chirife and Karel included
cellulose and starch (Chirife and Karel, 1973b; 1973c).

Data on retention of flavors in gel systems are
limited. Saravacos and Moyer (1968) studied retention
of volatiles in different freeze-dried food gels. Sauvageot
et al. (1969) and Voiley, Sauvageot and Simatos (1973)
studied retention of alcohols in a mixed system containing
pectin and sugars, as well as in fruit juices. Kayaert
(1973) studied the effects of different experimental
parameters on the retention of alcohols, aldehydes, 2-
ketones and 3-ketones in a complex gel system used as a
replacement for pectin. All of the above investigators
observed substantial retentions of organic volatiles.
The present study was undertaken in an attempt to establish
the mechanism by which organic volatiles are retained
in a freeze-dried multicomponent food gel and in particular
to determine if it is the same mechanism as in several
polymeric single-component systems studied to date (proteins,
PVP, starch, cellulose).

Materials and methods

Model system preparation

The model systems consisted of the gel solids described below, ^{14}C-labelled volatile organic compounds, and water. The systems were prepared by dissolving the desired amount of solids in a mixture of water and the organic volatile. Concentrations of volatile are expressed in ppm on a volumetric basis. The gel concentration is given on a weight basis. Aliquots of 1.5 ml of the solution were weighed into standard glass screw-capped scintillation vials, frozen in a cold room at -40°C and then freeze-dried for 48 hr in a Virtis freeze drier. The drying took place at room temperature and low chamber pressures (< 100 μm). Temperatures were not measured during freeze drying. These conditions of freeze drying were identical to those that we previously found to give high volatile retentions in low molecular weight carbohydrate-volatile systems.

Non-volatile solute

The non-volatile solute (comprising the solids fraction of the gel) consisted of a mixture of four carbohydrate gums. This mixture is used commercially as a substitute for pectin in the production of fruit jellies in Europe. The composition of this mixture is:

35% locust bean gum

20% guar gum

15% carageenan gum

30% agar gum

The mixture was provided by Pectinfabrik Herman Herbstreib, Germany.

Volatile compounds

^{14}C-labelled acetone, ethyl acetate, and n-propanol were used (International Chemical and Nuclear Corporation, Irving, California). The radioactive materials were diluted with reagent grade nonradioactive volatiles to give the desired specific radioactivity.

Volatile analysis

Volatile content was determined by measuring the radioactivity of the samples with a liquid scintillation counter. Since the counting was conducted in the same vials in which the samples were freeze-dried, no transfer of aliquots was necessary. The following procedure was found satisfactory, as it avoided potential difficulties in complete dissolution of the freeze-dried gel: Samples were rehydrated in the vials by adding water to restore the original sample weight. Fifteen ml of a water-miscible scintillator solution were added (2,5-diphenyloxazole 1 g, naphthalene 100 g, dioxane to 1000 ml volume), and the resulting mixture was counted with a liquid scintillation counter (Beckman CPM 100).

The volatile retention was calculated by multiplying the original volatile content by the ratio of the counts after freeze drying to those in the control solution.

Humidification

Freeze-dried systems were humidified to the desired water activity by placing them in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

Layer experiments

In some experiments, layers of different composition were prepared as follows: 15 ml of a solution containing 1% solids and 500 ppm of acetone were frozen in a 50-ml beaker by immersion in liquid nitrogen. On top of this layer were added and frozen two additional 15-ml layers of 1% gel solution with no acetone. After freeze drying, the layers were separated and analyzed individually for their volatile content.

Results and discussion

In several experiments, the effects of composition and of process variables on volatile retention were studied. Fig. 1 shows the effect of initial gel concentration on retention of n-propanol and acetone. The retention of both volatiles increased linearly with solids concentration. This is very similar to results obtained with cellulose and starch (Chirife and Karel, 1973b). In almost all systems studied, the retention increases in this manner up to a concentration level at which it levels off. In

many systems this level is approximately 20% solids
(Chirife, Karel and Flink, 1973). However, in this study,
the gel concentration was limited to 3% because higher
concentrations of this poorly soluble material showed
erratic behavior (Kayaert, 1973).

Fig. 2 shows the effect of initial volatile concentration
on volatile retention in a slowly frozen, 1.5% gel.
Previous studies on the effect of initial volatile concentration
tended to show that the fractional retention decreases
with increasing concentration, but not linearly (Flink
and Karel, 1970b; Chirife, Karel and Flink, 1973; Chirife
and Karel, 1973a; 1973b). In freeze-dried 20% PVP solutions,
for instance, the retention of n-propanol decreased from
65% when initial concentration was 50 ppm to about 25%
at the 1000 ppm level, and remained constant thereafter.
In freeze-dried cellulose and in starch (each initially
at a 20% level) initial volatile concentration did not
change the fractional retention greatly. The behavior
of this complex mixture as shown in Fig. 2 is qualitatively
similar to that observed in PVP and n-propanol, except
for the apparent decrease of retention at levels below
100 ppm for acetone and ethyl acetate.

The "microregion" theory and "selective diffusion"
theory both predict an effect of thickness, with increasing
retentions expected in thinner samples. Fig. 3 shows
the effect of thickness on retention of volatiles in

freeze-dried 1.5% gels. The shapes of the curves are similar to those observed by Flink and Karel (1970b) for carbohydrates, and by Chirife, Karel and Flink (1973) for PVP, and the behavior conforms to expectations.

Low molecular weight carbohydrates retain more volatile than polysaccharides, probably because they have a higher mobility prior to freeze drying and are able to form a more retentive matrix (Flink and Karel, 1972; Chirife, Karel and Flink, 1973). Saravacos and Moyer (1968) observed that addition of sugar to pectin increased retention of volatiles after freeze drying, but not adsorption of volatiles on an already dry system. Table 1 shows the results obtained in the present study in mixtures of 2% gel and 10% sucrose. These results are in agreement with the prior work cited above. Another process variable expected to affect retention is the rate of freezing. Slow freezing was expected to give higher volatile retention (Karel and Flink, 1973). We observed similar effects with a slowly-frozen 1% gel (containing 500 ppm of acetone), which gave a retention of 12.4%, while only 1.5% was retained in an identical sample frozen rapidly.

The results cited above are in agreement with results for other systems, which indicate that most of the retained volatile content is entrapped in microregions. Previous work has shown, however, that in PVP, cellulose, starch, and other systems, a relatively small amount of

volatile may be retained by adsorption. We performed freeze drying experiments on layered systems described under "Materials and methods," and obtained the results shown in Table 2. These results indicate that adsorption may contribute to the total retention. The concentrations of acetone present after freeze drying in layers originally containing no volatile were a significant fraction of the amount retained in the bottom layer. This behaviour is comparable to that observed in some other systems (Chirife and Karel, 1973a; 1973b). However, most of the retention is apparently due to entrapment.

Previous studies have shown that the microregions entrapping volatiles in freeze-dried systems are sensitive to water and that volatiles are released when water in excess of the B.E.T. monolayer value is adsorbed in humidification experiments (Flink and Karel, 1972; Chirife, Karel and Flink, 1973). Freeze-dried solutions of 1.5% gel and of either acetone, ethyl acetate, or n-propanol in concentrations of 50, 500, and 5000 ppm, were rehumidified over constant humidity solutions and analyzed for retention of volatiles. Typical results are shown in Fig. 4, indicating that at each relative humidity there is a new level of retention. Humidification to 11% relative humidity, which is below the monolayer value for water on the dry gel, resulted in a loss of about 20% of the acetone retained after freeze drying. Similar small losses

of propanol from PVP humidified to levels below the monolayer value were explained as being caused by release of adsorbed volatile rather than disruption of microregions (Chirife and Karel, 1973a).

The results presented in this study lead us to the conclusion that entrapment of organic volatiles in the gel mixture is the primary mechanism of retention, with adsorption contributing to a much smaller extent. This complex mixture shows behaviour similar to that observed with model systems, each containing one type of polymer (Chirife, Karel and Flink, 1973; Chirife and Karel, 1973b; 1973c). Retention levels are comparable with other polymers at the same solids content. The slight lowering of fractional retention at very low initial volatile concentrations also remains unexplained.

Acknowledgment

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Table 1.   Retention of acetone in freeze-dried solutions
of sucrose and of the gel mixture

| Initial concentration of acetone (ppm) | Retention of acetone (%) in specified solution | | |
	2% gel	10% sucrose	2% gel + 10% sucrose
50	19.8	84.8	60.1
100	23.5	77.2	55.8
500	20.3	79.8	57.5

Table 2. Retention of acetone in specified layers of
the freeze-dried gel

| | Acetone content (g/100 g solids) | |
	Before freeze drying	After freeze drying
Top layer	0	.006
Middle layer	0	.009
Bottom layer	4	.031

Figure legends

Fig. 1. Effect of gel concentration on retention of acetone and n-propanol (initial volatile concentration = 2000 ppm).

Fig. 2. Effect of initial volatile concentration on volatile retention in freeze-dried 1.5% gels.

Fig. 3. Effect of sample thickness on the retention of volatiles in freeze-dried 1.5% gels (numbers in parentheses refer to initial volatile concentrations).

Fig. 4. Loss of acetone from a freeze-dried 1.5% gel humidified to specified relative humidities (initial acetone content prior to freeze drying = 50 ppm).
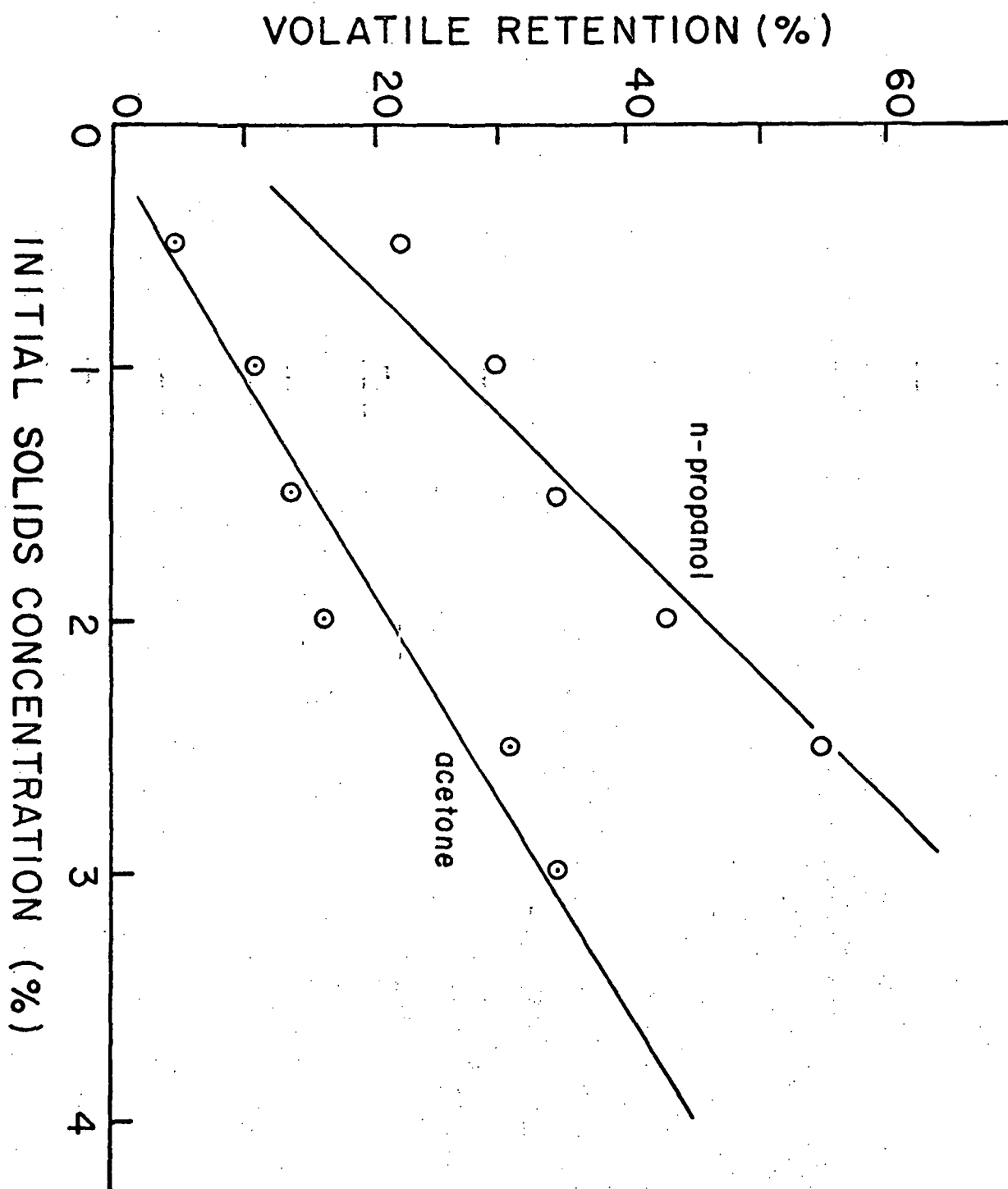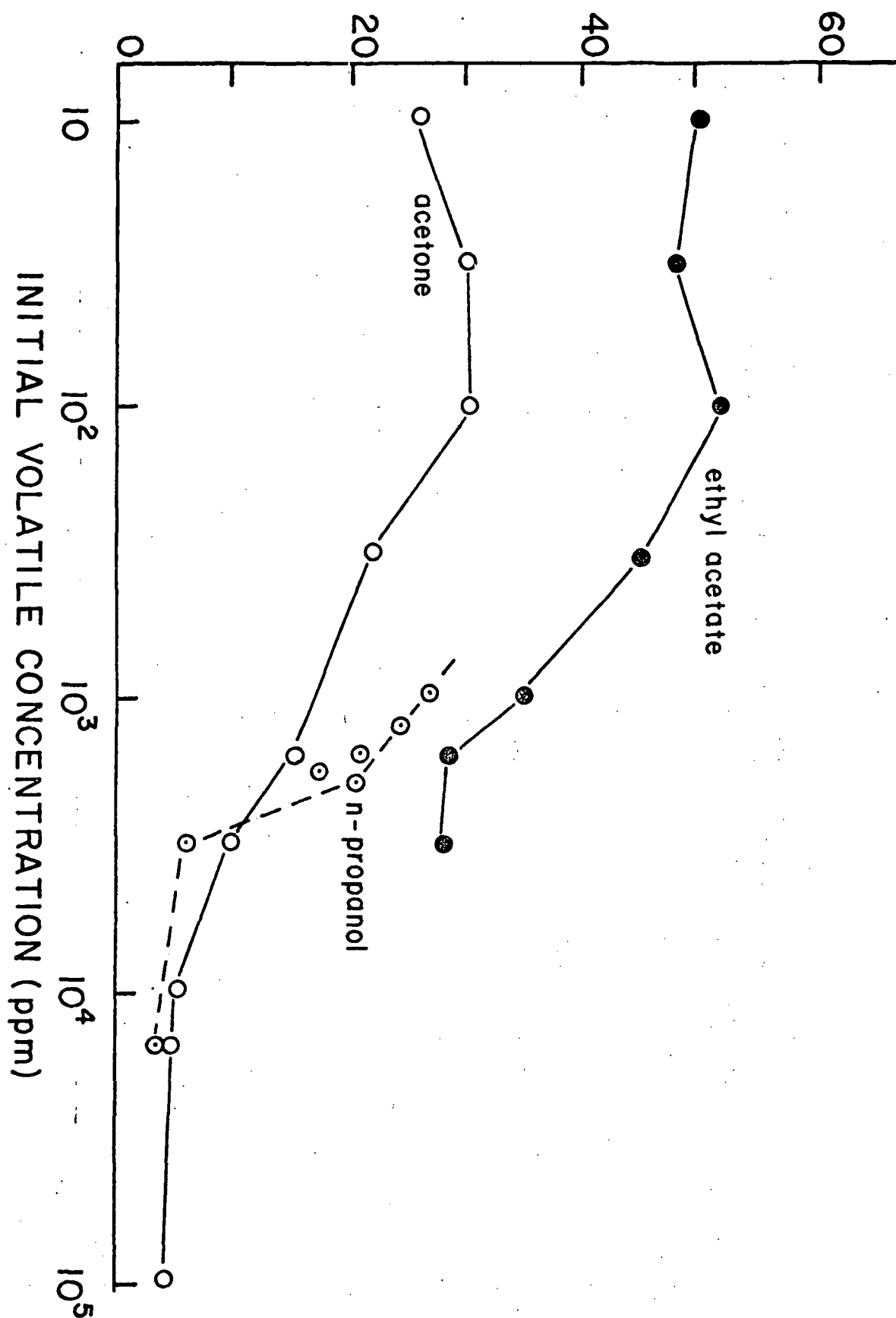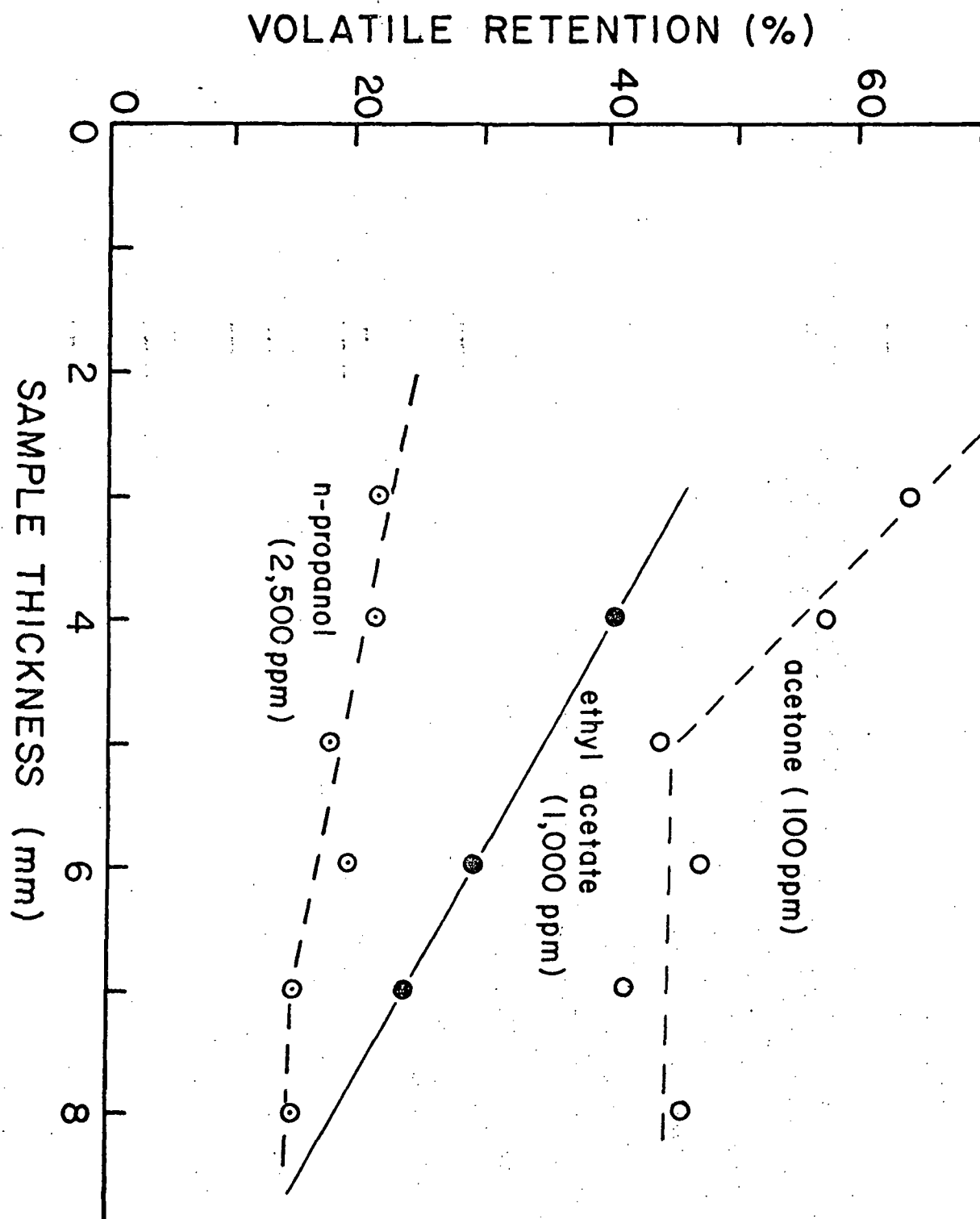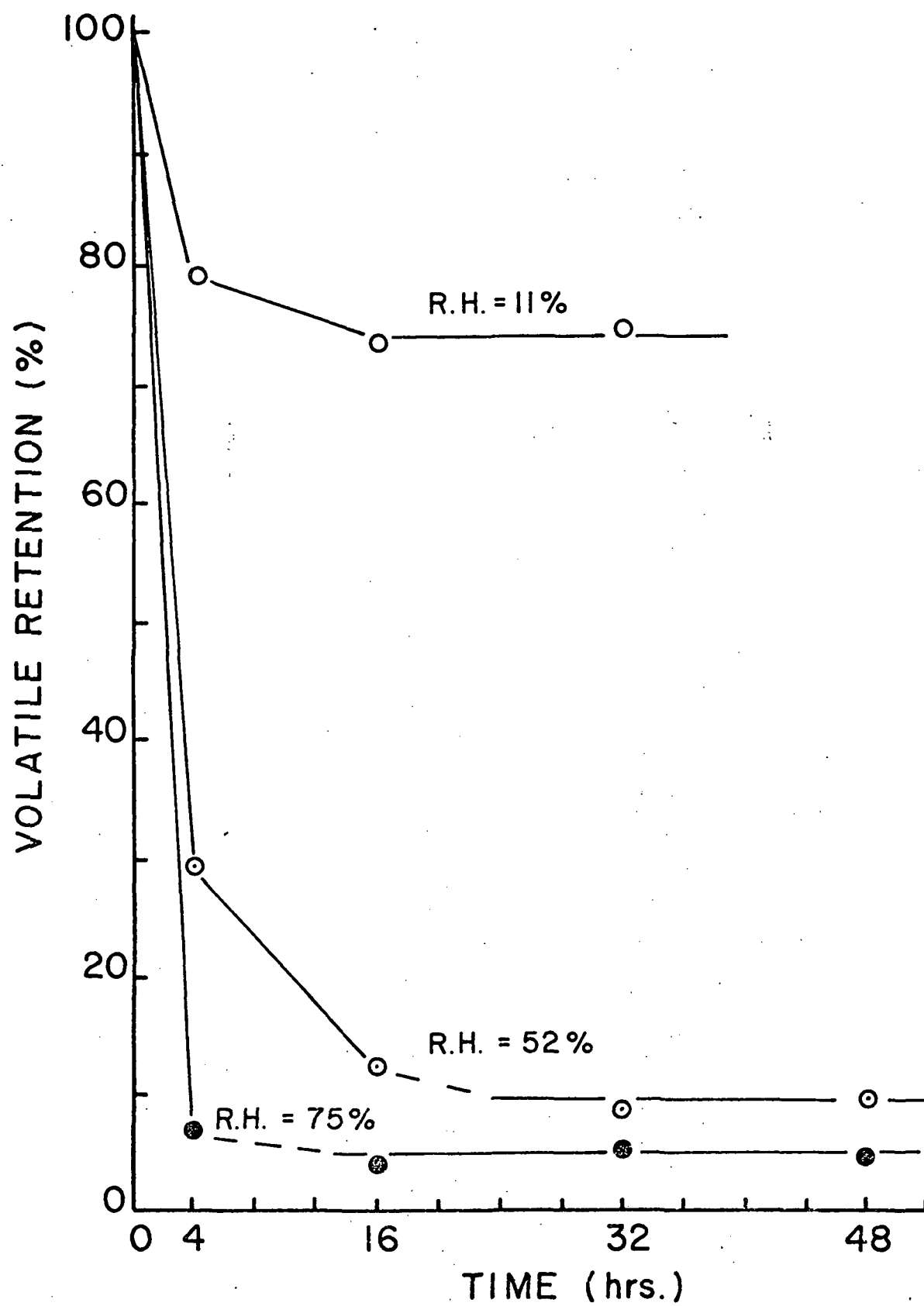
5.6 Effect of structure disrupting treatments on volatile release from freeze dried maltose

Effect of structure disrupting treatments on volatile

release from freeze-dried maltose

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Running title: Structure and volatile release from maltose

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Summary

Solutions of 1% 1-propanol and 20% maltose in water were freeze dried under conditions resulting in retention of 69.5% of the propanol. The freeze-dried solutions were exposed to vapors of several organic compounds which varied in size and polarity. Those vapors which were adsorbed in significant amounts caused a partial release of the entrapped propanol.

Heating the freeze-dried solutions in vacuum at temperatures of 37°C, 50°C, 65°C, and 82°C had no effect, but at 100°C a partial release of the propanol was achieved.

The results support the previously postulated microregion theory of volatile retention in freeze-dried carbohydrate solutions.

Introduction

Retention of volatiles in freeze-dried foods is controlled
by interactions of the volatiles with non-volatile solids
and water during freezing, drying, and storage. Flink
& Karel (1970a) studied volatile retention in freeze-dried
carbohydrate solutions, and postulated that crystallization
of water during freezing results in formation of microregions
containing highly concentrated solutions of carbohydrates
and volatiles. As the local moisture content within these
regions decreases, first due to freezing and then to
sublimation, there occur associations between the
molecules of solute. In the case of carbohydrates these
associations are caused by hydrogen bonds formed between
hydroxyl groups of carbohydrate molecules (Flink &
Karel, 1972; Karel & Flink, 1973). We have recently
observed (Chirife, Karel & Flink, 1973) that molecular
associations entrapping volatiles within microregions
seem to occur also in polar polymers containing no
hydroxyl group.

The structure of the microregions and the permeability
of these regions to water and to organic vapors depends
strongly on local water content. As this content decreases,
the ease of loss of organic volatiles decreases until
at some critical moisture level there is no further loss

(Flink & Karel, 1970 a, 1970b). Exposure of freeze-dried carbohydrate solutions containing entrapped volatiles to water vapor shows the following pattern: at low humidities there is no volatile loss even after evacuation for prolonged periods; at higher humidities a rapid volatile loss occurs, until a new level of retention is reached, which is again stable unless the humidity is increased further.

It was determined that the critical point for initiation of volatile loss corresponds to sorption of water to levels above the calculated B.E.T. monolayer value (Flink & Karel, 1972). Below this level water is sorbed on those hydroxyl groups of the carbohydrates which do not participate in the structure-forming hydrogen bonds. Adsorption of water in amounts below the monolayer value does not therefore disrupt microregion structure, and the volatile retention is not diminished. At moisture contents above this level, however, the sorbed water competes for hydroxyl groups involved in structure forming, the microregion structure is disrupted, and a volatile loss occurs. Humidification resulted in a new level of retention, as long as the original structure of the freeze-dried materials was not destroyed by sufficient water to cause either dissolution or crystallization.

These observations confirmed the existence of a

microstructure which undergoes partial collapse upon
humidification to level above the monolayer value, this
partial collapse becoming complete only upon dissolution
or crystallization (Flink & Karel, 1972).

The microstructure developed in freeze-dried
carbohydrates and responsible for volatile retention
can be disrupted by influences other than sorption of
water. Polar molecules capable of structure disruption
should release entrapped volatiles, and high temperatures
may cause thermal disruption of structure and volatile
release.

Flink (1969) studied the extraction of freeze-dried
carbohydrates containing entrapped volatiles with different
solvents, including water, methanol, ethanol, propanol,
butanol, and acetone. He observed trends which indicated
that size and polarity of the solvent molecules influenced
the removal of retained volatiles from the dry solid,
and that these differences were related to the ability
of the solvent to disrupt microregion structure.

The present paper is devoted to study of microregion
disruption and volatile release by sorption of polar vapors,
and by heating to elevated temperatures.

Materials and methods

Retention of 1-propanol in freeze-dried carbohydrates was studied in a model system consisting of maltose, ^{14}C-labelled 1-propanol, and water. The model system was prepared by dissolving maltose in water and adding propanol. Five ml of the solution were pipetted into 50-ml Erlenmeyer flasks frozen in liquid nitrogen, and then freeze dried for 48 hr at ambient temperature and at a pressure of less than 100 μm in a Virtis model 10 MRTR freeze drier. The system composition was 20% maltose, 1% propanol, and 79% water (by weight). 1-Propanol was mixed with ^{14}C-labelled 1-propanol to give the desired specific radioactivity. The radioactive propanol was obtained from International Chemical and Nuclear Corporation, Irving, California.

In several experiments the freeze-dried solutions were exposed to vapors of various liquids maintained at constant temperatures and vapor activities. The liquids included water, methanol, ethanol, formic acid, acetic anhydride, ethyl ether, benzene, and aniline. All materials used in this study were of reagent grade and were not further purified prior to use.

The control of vapor pressures at saturation levels required only temperature control; where the activities

were to be maintained at levels below saturation, we used binary mixtures. Saturated salt solutions were used in the case of water, since activities of these solutions were readily available from literature. Vapor pressures below saturation were obtained for methanol and ethanol by preparing mixtures with the relatively non-volatile diethyl phtalate. Activity of alcohol vapors over such mixture was known from literature (Dornte, 1929); in the case of formic acid, a mixture of 69 g of 90.9% formic acid with 46 g of glycerol was used (molar fraction of formic acid 0.615). The deviation of the activity from the ideal value was not determined in this case. The activity of water in this mixture was estimated to be well below the levels producing volatile release (Flink & Karel, 1972).

Sorption of the various vapors was accomplished by placing flasks of the freeze-dried model system in vacuum desiccators over the appropriate vapor-generating liquid, or mixture. The amount of vapor sorbed at each vapor activity was determined gravimetrically.

Analysis of 1-propanol

The 1-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

The dried samples of maltose were dissolved in water

(to 10% solution); 1 ml of this solution was added to
10 ml of water-miscible scintillator (2,5-diphenyloxazole
1 g, naphtalene 100 g, dioxane to 1,000 ml) in the counting
vial, and the resulting solution was counted with a
Beckman LS-230 liquid scintillation counter.

Results and discussion

Maltose was chosen as the carbohydrate system to be
investigated, and ^{14}C-labelled 1-propanol was the volatile.

Like other sugars, maltose upon freeze drying gives
an amorphous cake which recrystallizes upon humidification
(Guilbot and Drapron, 1969). The results of humidification
of maltose at 75% relative humidity are presented in
Fig. 1. Humidification causes the moisture content to
increase to approximately 25% (dry basis) and then to
fall, indicating recrystallization. The humidification
had a pronounced effect on retention of entrapped 1-propanol.
After freeze drying and before humidification the
amorphous cake of maltose contained 3.47 g 1-propanol/
100 g maltose. As the water content of the maltose
increased towards 25%, the propanol content was decreasing
slowly towards a new value of about 50% of the original.
Then, as recrystallization began, propanol was lost
rapidly; the final retention was very low. The rate of
propanol loss during this period paralleled the progress

of crystallization. The high rate of propanol loss is a consequence of the extensive disruption of the carbohydrate-carbohydrate bonds present in the amorphous cake. The results obtained with maltose thus are very similar to those found by Flink & Karel (1972) in crystallization of lactose after exposure to 61% relative humidity.

In Fig. 2 we present results obtained when the propanol-containing freeze-dried maltose was exposed to vapors of several organic compounds. Saturated vapor of ethyl ether caused no release of propanol at either 25°C or 37°C. Ethanol at an activity of 0.75 also failed to produce any loss of propanol at 25°C. With both ethanol and ether the lack of propanol release was associated with negligible sorption of the two vapors in the amorphous maltose. Sorption of ether was below detectability limits, and the sorption of ethanol was about 0.55% on dry basis.

Acetic anhydride was adsorbed to a slightly greater extent than ethanol (1.2% dry basis): this sorption resulted in a small but significant amount of released 1-propanol.

Fig. 2 also shows results obtained by exposing the freeze-dried maltose to formic acid over a ternary mixture of formic acid, water, and glycerol (mole fraction of formic acid = 0.615). The total sorption amounted

to approximately 32 g/100 g maltose, and resulted in disruption of maltose cake structure, as evidenced by substantial loss of the entrapped propanol. A rapid initial loss of the entrapped volatile occurred during the first day of exposure to formic acid vapor, followed by an approach to a new retention level.

Similar behavior was observed with methanol vapor at an activity of 0.75, but methanol at an activity of 0.17 had no effect.

The sorption of methanol at an activity of 0.17 was 1.42% (dry basis), and 14.4% (dry basis) at an activity of 0.75.

The ability of methanol to strongly interact with carbohydrates is well-documented in literature: it is significant that methanol can act as a solvent for a number of carbohydrates (Moyle, 1972). The structure-disrupting effect of methanol is similar to that observed with water. In Fig. 3 we compare the effects of water vapor and of methanol vapor at selected activities. At an activity of 0.61 water sorption was 15.0%; at an activity of 0.75 it reached 25.5% before decreasing again due to crystallization. At an activity of 0.75, methanol sorbed was 14.4%, and at an activity of 0.98 it reached 26.4%, then decreased to 18.2%.

As expected from the considerations of both size and

polarity, water is a more effective disrupter of maltose structure than methanol. Methanol at an activity of 0.98 releases less propanol than water at an activity of 0.75; and at an activity of 0.61 water releases slightly more propanol than methanol at an activity of 0.75. The data for release by water at activity 0.61 refer to 2-propanol and were obtained by Flink & Karel (1972).

In a recent review of non-aqueous solvent for carbohydrates, Moyle (1972) noted that the ability of various compounds to act as solvents depends not only on polarity, but also on the ability of the compound to disrupt the crystal lattice of the carbohydrates. Size as well as polarity are involved in such disruptive capability, which is similar to the requirements for the disruption of the structure in microregions in which the volatile was entrapped in freeze-dried maltose.

To demonstrate further the separate effects of polarity and size we exposed freeze-dried maltose containing entrapped propanol to saturated vapors of benzene and aniline. Benzene was not sorbed and produced no release of propanol; aniline, however, was sorbed to the extent of approximately 10%, producing a substantial release of the entrapped propanol (Fig. 4). The shape of the release curve is again consistent with a partial disruption of the retaining structure in the maltose.

In a series of studies on release of entrapped volatiles from freeze-dried carbohydrates, we especially noted the coincidence of structural changes observable visually at the onset of volatile release. Such structure changes are also capable of being produced by exposure to elevated temperatures: often phase transitions occur over a narrow temperature range, below which there is no effect. These transitions occur in crystalline forms (Hodge, Rendleman & Nelson, 1972), but can also be produced in amorphous structures (Mackenzie, 1966).

Freeze-dried samples of maltose-1-propanol were placed in desiccators over anhydrous calcium sulfate and activated charcoal and evacuated at 37°C, 50°C, 65°C, 82°C, and 100°C. The experiments at 37°C and 50°C were conducted in constant temperature rooms. For the higher temperatures a desiccator was partially filled with purified sand as a heat transmission medium, and was placed inside an oven with temperature control. When the steady state at the desired temperature was reached (± 1.5°C), the samples were buried in the sand and the desiccator was evacuated.

In Fig. 5 we show that between 37°C and 82°C there is no significant loss of volatile. However, when the temperature is raised to 100°C a rapid volatile loss occurs, and the 1-propanol content asymptotically approaches

a new level of retention. This loss was associated with observable changes in the structure of the freeze-dried maltose.

These results offer positive support to the postulated mechanism of retention (Flink & Karel 1970 a,b). The microregion structure consists of H-bonded carbohydrate molecules; thermal energy from heating will cause disruption only when it is above some energy level. Therefore, as experimentally found, volatile retention will remain constant when the sample is heated at temperatures at which no structural changes occur.

Acknowledgment

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Figure Legends

Fig. 1. Adsorption of water (dashed lines) and retention of 1-propanol (solid lines) in freeze-dried maltose humidified to 75% relative humidity at 25°C.

Fig. 2. Loss of 1-propanol from freeze-dried maltose exposed to several organic vapors:

     A. Ethyl ether, saturated vapor, 25°C

     B. Ethyl ether, saturated vapor, 37°C

     C. Methanol, vapor activity 0.17, 25°C

     D. Ethanol, vapor activity 0.75, 25°C

     E. Acetic anhydride, saturated vapor, 25°C

     F. Methanol, vapor activity 0.75, 25°C

     G. Formic acid, vapor over solution with a mole fraction of 0.615, 25°C

Fig. 3. Effect of water and of methanol on retention of propanol in freeze-dried maltose at 25°C.

     A. Water, vapor activity 0.61 (2-propanol; Flink & Karel, 1972)

     B. Methanol, vapor activity 0.75 (1-propanol; present study)

     C. Methanol, vapor activity 0.98 (1-propanol, present study)

D. Water, vapor activity 0.75 (1-propanol; present
study)

Fig. 4. Retention of 1-propanol in freeze-dried maltose
exposed to saturated vapors of benzene and aniline. Sorption
of aniline is shown by a dashed line; benzene was not
sorbed in measurable amounts.

A. Benzene, 25°C

B. Benzene, 37°C

C. Aniline, 25°C

D. Sorption of aniline, 25°C

Fig. 5. Retention of 1-propanol in freeze-dried maltose
heated at several temperatures.
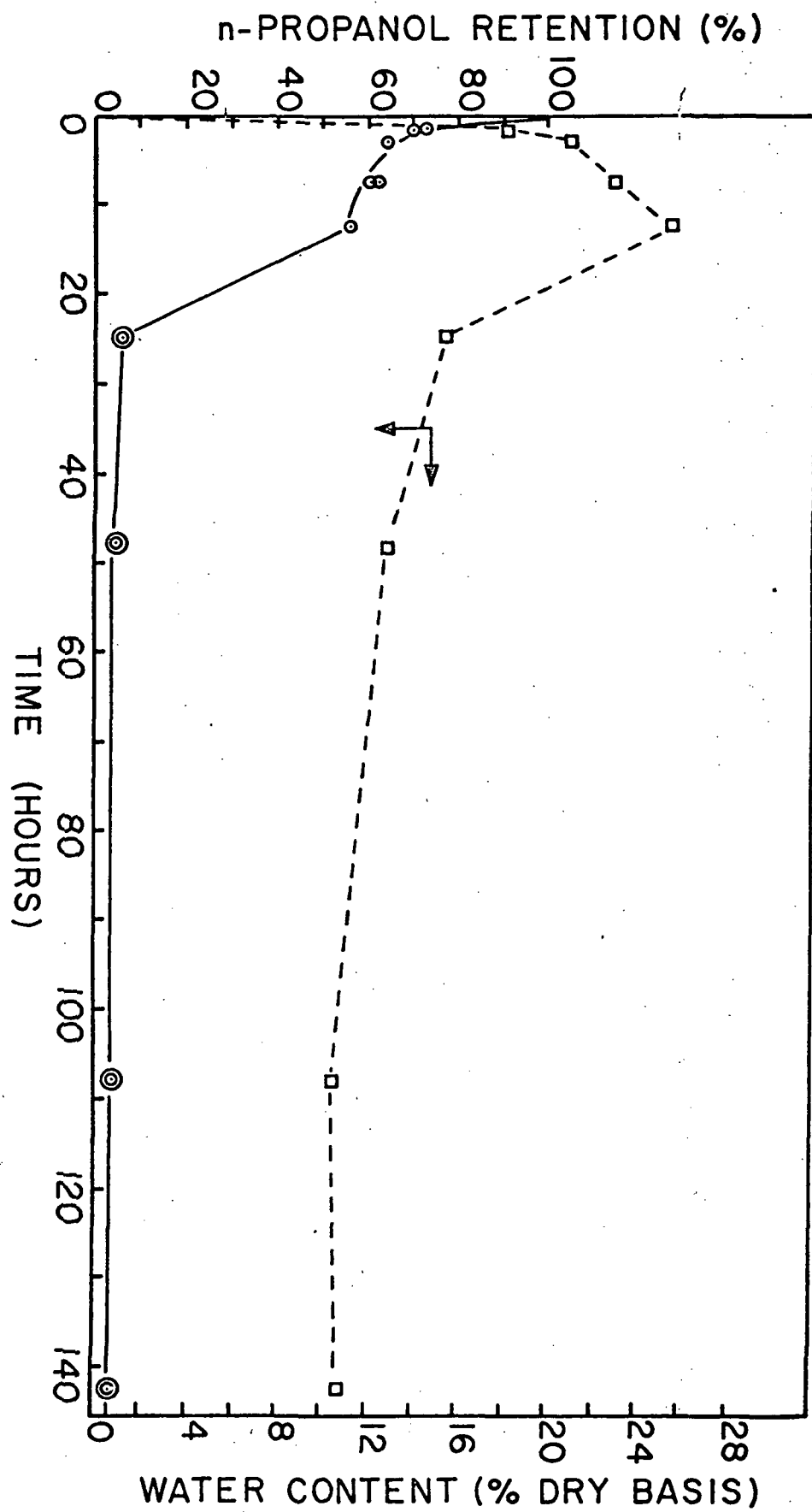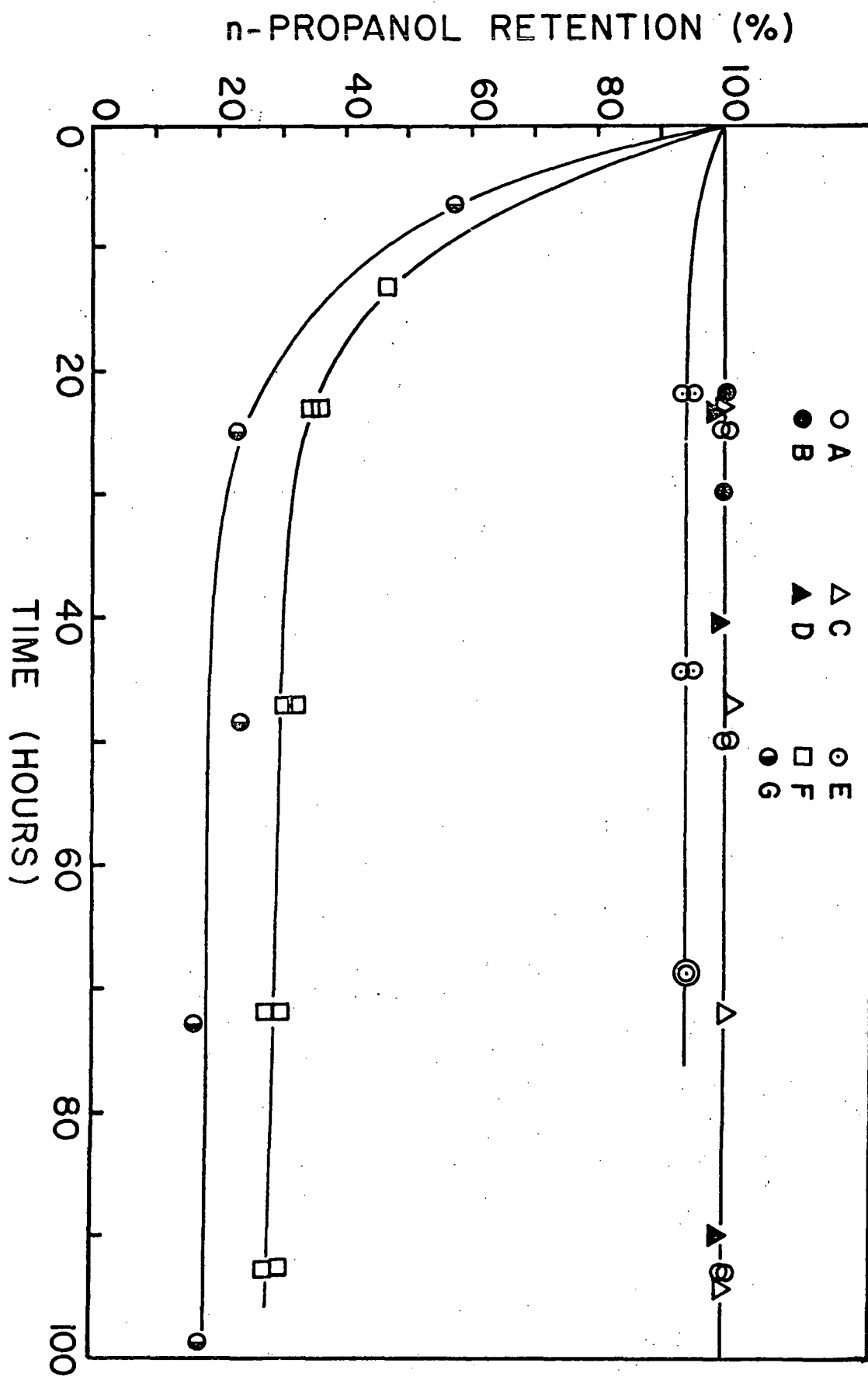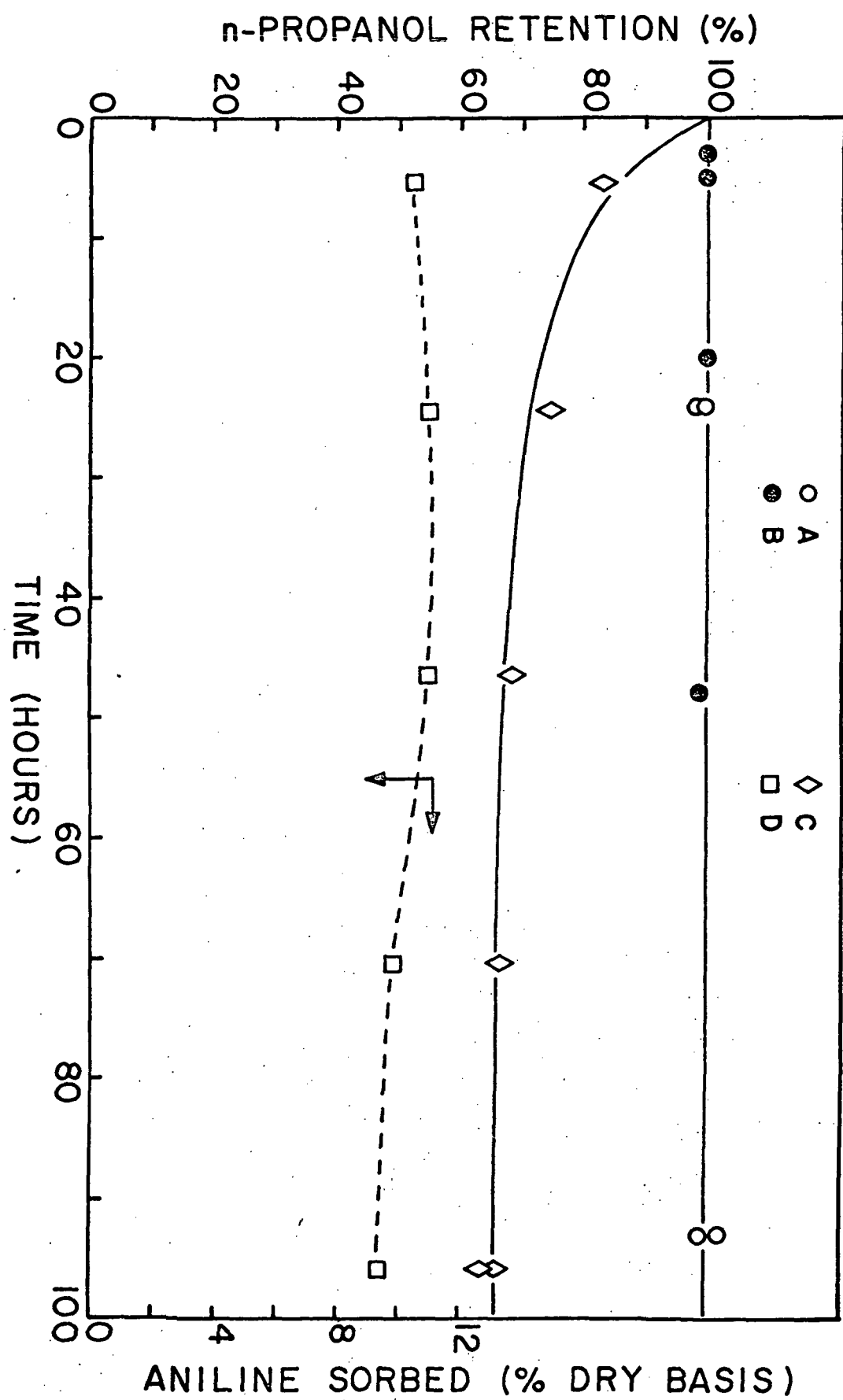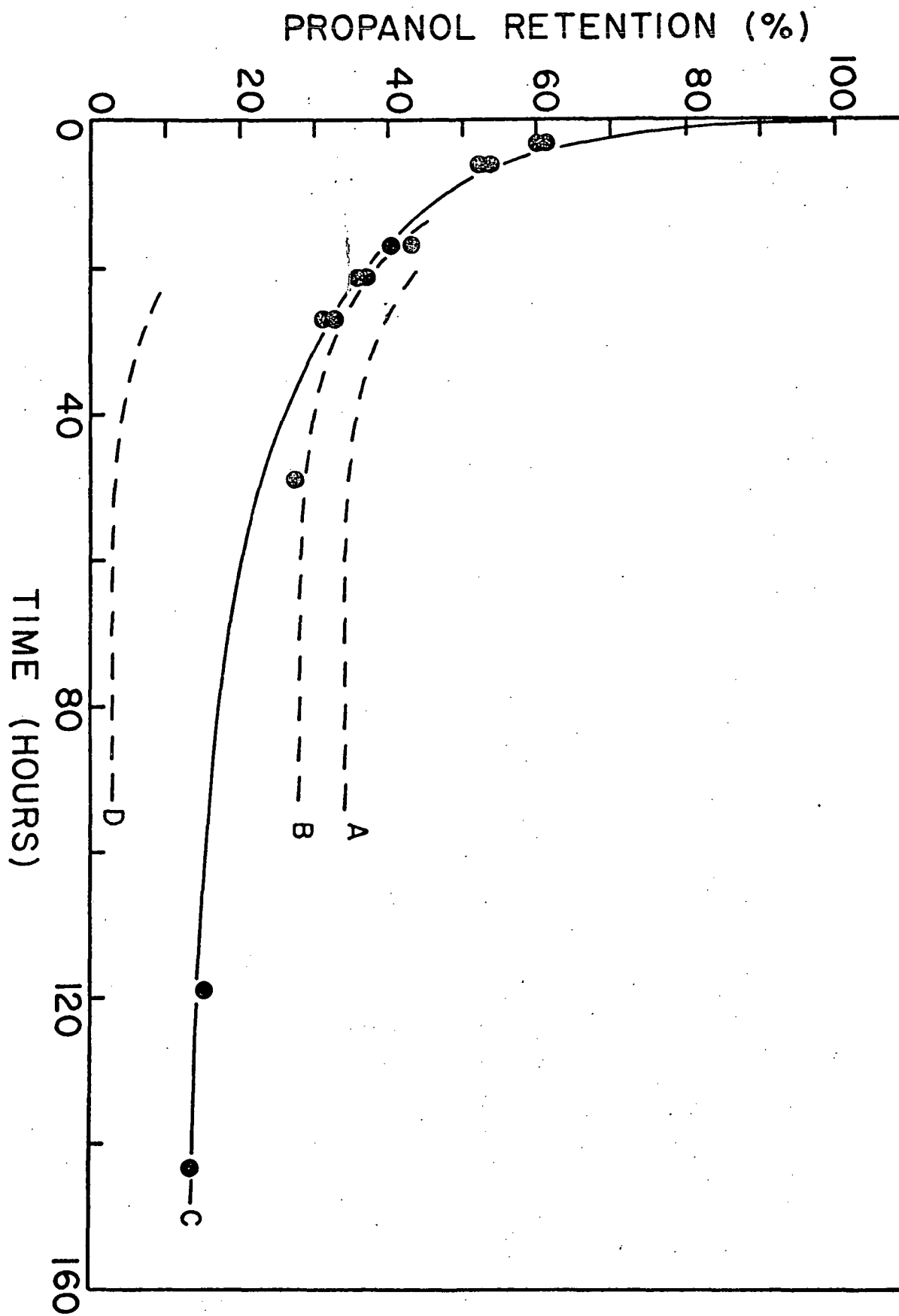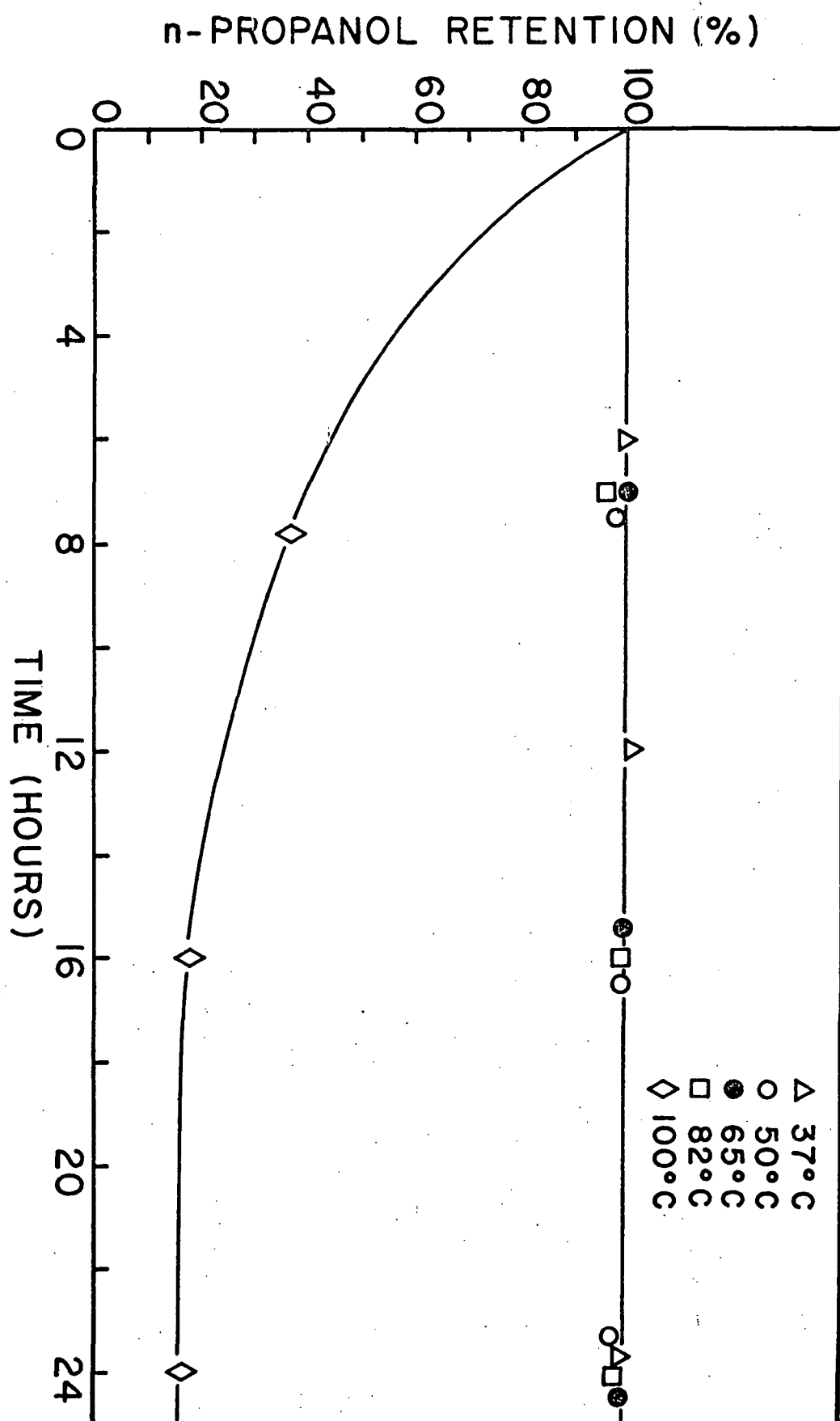
6. <u>Freeze dried food products of improved quality</u>

6.1 <u>Introduction</u>

Utilization of improvements in freeze dehydration
processing suggested by research conducted in this contract
has been directed to the development of dehydrated fruit
products. Process variables having greatest influence
on product quality are freezing rate and initial solids
content.

Phase I studies evaluated methods and processes
for preparing a freeze dried fruit product of improved
organoleptic quality. The influence of a number of
variables, but most notably freezing rate and initial solids
content, on the quality of apple slices was evaluated.
Five pounds of freeze dried apple slices, processed to
give improved quality characteristics, were delivered
to NASA for evaluation in accordance with the pertinent
Phase I end item.

Phase II studies have investigated the applicability
of the processes developed in Phase I to a number of addi-
tional fruit products. These studies resulted in the
selection of freeze dried cantaloupe melon as the most
preferred fruit product and 5 pounds of this product
was delivered to NASA in accordance with the Phase II
end item.

6.2 Methods

6.2.1 Methods of preparation

The methods of sample preparation were described in the Phase I Annual Report (pages VI-1 and VI-2). To summarize that description, the fruits were washed and trimmed (peeled, pitted, etc). The fruit was then cut into uniform pieces and subjected to the following treatments.

1) The sliced fruit is soaked in an aqueous solution of 60% sucrose, 0.52% ascorbic acid and 0.14% malic acid.

2) The fruit is then rinsed for 30 seconds to remove the sugar solution adhering to the surface of the fruit.

This prevents a stickiness after dehydration.

3) For slow freezing the fruit is spread on aluminum foil which is covering the freeze dryer sample trays and these are then placed in a -20°C room for at least 24 hours. For rapid freezing, the rinsed fruit is immersed in a bath of liquid nitrogen and the frozen pieces then placed on the freeze dryer sample trays.

4) Freeze drying is conducted at a low chamber pressure and ambient temperature heating plates.

5) Following freeze drying, organoleptic evaluations were conducted on the dry product and in one case on a

rehydrated paste produced by grinding the dry product and mixing in the desired amount of water.

Solids contents of fresh fruits and treated fruits were determined by measurement of the water loss in the freeze drying process. A water sorption isotherm was measured for the freeze dried cantaloupe of increased solids content by equilibration of samples over saturated salt solutions (Figure 1).

6.2.2 Organoleptic tests

Three methods of organoleptic testing were utilized in evaluating the relative quality of the different processing conditions for a number of fruit products. The tests are completely described in Larmond (Methods for Sensory Evaluation of Foods, Publication 1284, Canadian Department of Agriculture) and will be summarized here.

Products were scored in a difference test for taste and texture using the following scale (together with numerical equivalents): very poor (1), poor (2), fair (3), good (4), very good (5) and excellent (6). By analysis of variance, the difference between samples can be evaluated for significance. In addition, the average value of the scores can be used as a measure of the absolute product acceptability, though some particular psychological and numerical factors must be considered.

As a numerical factor, the values given to the various scores must be taken as mid-range values, to account for the fact that there are no scores granted above and below the end points. Even so while "good" will then range from 3.5 to 4.5, "excellent" will still only have half the range, from 5.5 to 6.0. As psychological factors, there is a reluctance to grant a score of "very poor" or "excellent", as these represent to many judges an ideal. Thus, the scale in reality becomes somewhat compressed with quite good quality product having numerical values of 3-4.

A second test was a paired comparison preference test in which samples were presented in groups of two. In this case, the judge merely has to express a preference for one sample or the other. There was a provision for expressing the degree of preference, but analysis of this information tended to follow the determined significance of the preference test. By consideration of the various combinations of paired comparisons, an overall preference can be determined.

In the third organoleptic test, all samples were presented for ranking according to overall quality. An analysis of variance on the conversion of ranks to scores results in an evaluation of ranking significance. For most tests, four samples were presented and the

numerical conversion of ranks were first (+1.03), second (+0.30), third (-0.30) and fourth (-1.03). The degree to which the sample approaches +1.03 is a measure of its overall acceptance and the difference between values is a measure of the degree of preference.

6.3 Organoleptic evaluations

6.3.1 Evaluation of processing variables

Four types of samples were evaluated for organoleptic quality for all fruits. They are (with code in parentheses):

normal solids, slow frozen   (NS)

normal solids, fast frozen   (NF)

increased solids, slow frozen   (IS)

increased solids, fast frozen   (IF)

The change in solids content from the normal solids and increased solids versions of most of the fruit samples are presented in Table 1. It can be seen that a 2-3 fold increase in solids was achieved.

The scores of the difference tests are presented in Table 2, and numerical evaluations of ranking preference tests in Table 3. The highest scores for taste and texture are given in almost all cases to the increased solids slow frozen (IS) fruits. The notable exception is with cherries where all the samples have a "fair" rating. In most cases, the IS fruits have rated above 4.0 for taste, with a number of samples in the "very good" range (above 4.5). From the ranking preference tests (Table 3) the clear superiority of the IS fruits is observed.

The evaluation of statistical significance of the various organoleptic tests is shown in Table 4 and the

summarized evaluation is given in Table 5. These data demonstrate the superiority of the IS fruits.

6.3.2 Short term storage evaluation

Short term storage stability of Freeze Dried High Carbohydrate Cantaloupe was evaluated for the following conditions:

1) Samples held in vacuum sealed can

2) Storage temperature of 4,20,37 and 55°C

3) Storage times of 1,2,4 and 8 weeks.

At the end of each storage period, samples were evaluated organoleptically using the difference and ranking tests described above. The results of these tests (Tables 6,7, Figure 2) show that all samples undergo some loss of quality, with the rate of loss depending on the temperature of storage. After 8 weeks of storage, there is no significant difference in samples held at 4°C and 20°C.

Table 1

Solids content of fruit slices prior to freeze drying

Fruit	Increased solids (I) %	Normal solids (N) %
Strawberries	23.0	9.4
Honeydew	33.6	9.6
Cantaloupe	28.0	9.6
Peaches	29.4	10.7
Pears	28.0	14.3
Pineapple	27.9	12.1

Table 2

Sample scores for organoleptic tests

6 = excellent; 1 = very poor
solids content: N:normal, I:increased
freezing rate: S:slow, F:fast

Sample	Fruit	Taste	Texture
1	Cherries		
	NS	3.36	3.54
	NF	3.29	2.79
	IS	3.18	3.32
	IF	3.00	2.71
2	Honeydew		
	NS	3.63	3.70
	NF	3.13	3.33
	IS	3.63	3.37
	IF	3.27	2.67
3	Cantaloupe		
	NS	3.92	3.88
	NF	4.00	4.00
	IS	4.77	3.92
	IF	4.08	3.23
4	Strawberries		
	NS	4.21	3.61
	NF	3.57	3.86
	IS	3.93	4.50
	IF	3.79	3.50
5	Cantaloupe		
	NS	3.84	4.05
	NF		
	IS	4.50	3.97
	IF	3.95	3.11
6	Strawberries		
	NS	3.79	3.58
	NF	3.42	3.07
	IS	4.42	4.21
	IF	4.12	3.46

Table 2 (continued)

6 = excellent; 1 = very poor
Solids content: N:normal, I:increased
freezing rate: S:slow, F:fast

Sample	Fruit	Taste	Texture
7	Cantaloupe (rehydrated)		
	NS	3.29	
	NF	2.50	
	IS	3.42	
	IF	2.92	
8	Pears		
	NS	3.90	3.35
	NF	3.90	3.90
	IS	4.65	4.35
	IF	3.60	3.80
9	Peaches		
	NS	2.83	3.00
	NF	2.42	2.83
	IS	4.25	4.42
	IF	3.50	3.17
10	Pineapple		
	NS	3.50	4.05
	NF	2.42	3.36
	IS	4.37	4.05
	IF	3.75	3.45
11	Pears		
	NS	3.55	3.55
	NF	4.20	4.30
	IS	3.75	3.80
	IF	3.10	2.30

Table 3

Sample scores from ranking tests

the extreme values of ranking ± 1.03
solids content: N:normal, I:increased
freezing rate: S:slow, F:fast

Sample #	Fruit	Rank			
		First	Second	Third	Fourth
1	Cherries	NS .190	IS .180	IF .130	NF −.140
2	Honeydew	NS .300	IS .260	NF −.037	IF −.530
3	Cantaloupe I	IS .675	NS .023	NF −.274	IF −.406
4	Strawberries I	IS .380	NS .095	NF −.095	IF −.380
5	Cantaloupe	IS .492	NS −.224	IF −.268	−
6	Strawberries	IS .737	NS .161	IF −.211	NF −.687
7	Cantaloupe (rehydrated)*	IS .333	NS .122	IF 0	NF −.454
8	Pears	IS .678	NF −.060	NS −.206	IF −.412
9	Peaches	IS .969	IF −.001	NS −.233	NF −.726
10	Pineapple	IS .687	IF .172	NS .111	NF −.926
11	Pears	NF .618	IS .326	NS −.266	IF −.678

* Only three samples giving maximum
  range of +.85↔0↔(−.85)

Table 4

Summarized significant results for organoleptic tests of freeze dried fruits

Normal Solids/Slow Freezing NS
Normal Solids/Fast Freezing NF
Increased Solids/Slow Freezing IS
Increased Solids/Fast Freezing IF

Sample #		Difference Test		Preference Test			Ranking Test	
		Taste	Texture	Preference*	Significance		(preferred is underlined)	Ranking Test
1	Cherries	NSD	IF/NS 5% NF/NS 5%	SN/NF 8/14 NS/IS 8/14 IS/IF 10/14	NSD NSD NSD		NSD	
2	Honeydew	NSD	IF/NS 1% IF/IS 5% IF/NF 5%	IS/IF 13/15 NS/NF 11/15 IS/NS 8/15	1% NSD NSD		IF/IS IF/NS	1% 1%
3	Cantaloupe	NS/IS 1% IF/NF 5% IS/IF 5%	IF/NF 5%	IS/IF 11/13 IS/NS 11/13 NS/NF 7/13	5% 5% NSD		IS/IF NF/IS IS/NS	1% 1% 5%
4	Strawberries	NSD	IS/IF 5% IF/NF 5% NS/IS 5% IF/NF 5%	IS/IF 10/14 NS/NF 8/14 NS/IF 8/14	NSD NSD NSD		IF/IS	5%
5	Cantaloupe	IS/NS 5%	NS/IF 1%	IS/NS 14/19 IS/IF 15/19	NSD 5%		IS/IF IS/NS	1% 1%

* Number of judges preferring a given treatment/total number of judges

Table 4 (continued)

Sample #		Difference Test		Preference Test		Ranking Test
		Taste	Texture	Preference*	Significance	(preferred is underlined)
6	Strawberries	NF/IS 1%	NF/IS 1%	IS/NS 10/12	5%	IS/NF 1%
		NF/IF 1%	IS/IF 5%	IS/IF 10/12	5%	IS/IF 1%
		NS/IS 5%	NS/IS 5%	NS/NF 9/12	NSD	IS/NF 1%
						IS/NS 5%
						IF/NF 5%
7	Cantaloupe (rehydrated)	NSD	–	NS/NF 9/12	NSD	IS/NF 5%
				IS/NS 8/12	NSD	
				IS/IF 8/12	NSD	
8	Pears	IS/IF 5%	NS/IS 1%	IS/NS 7/10	NSD	IS/IF 1%
				NS/NF 5/10	NSD	IS/NS 1%
				IS/IF 8/10	NSD	IS/NF 5%
9	Peaches	IS/NS 1%	IS/NF 1%	IS/IF 11/12	1%	IS/IF 1%
		IS/NF 1%	IS/NS 1%	NS/NF 9/12	NSD	IS/NS 1%
		IF/NF 1%	IS/IF 1%	IS/NS 12/12	0.1%	IS/NF 1%
		IF/NS 5%				IF/NF 1%
		IS/IF 5%				NS/NF 5%
10	Pineapple	NF/NS 1%	NSD	IS/IF 8/12	NSD	IS/NS 1%
		NF/IF 1%		IS/NS 7/12	NSD	IS/NF 1%
		NF/IS 1%		NS/NF 11/12	1%	IF/NF 1%
		IS/NS 5%				NS/NF 1%
						IS/IF 5%
11	Pears	NF/IF 1%	NF/IF 1%	NF/NS 8/10	NSD	NF/IF 1%
			NF/IS 1%	IS/NS 8/10	NSD	NF/NS 1%
			NF/NS 1%	IS/IF 9/10	5%	IS/IF 1%
						IS/NS 5%

* Number of judges preferring a given treatment/total number of judges

Table 5

Summarized relative evaluation of quality

Sample #	Fruit	Preference tests	Ranking
1	Cherries	NS > NF,IS > IF	NSD
2	Honeydew	IS > NS [?] > IF > NF	NS,IS
3	Cantaloupe	IS > IF,NF,NS	IS
4	Strawberries	IS > NS > NF,IF	IS
5	Cantaloupe	IS > NS > IF	IS
6	Strawberries	IS > NS,IF > NF	IS
7	Cantaloupe (rehydrated)	IS > IF,NS > NF	IS
8	Pears	IS > NS,IF,NF	IS
9	Peaches	IS > NF > IF,NS	IS
10	Pineapple	IS,NS,IF > NF	IS
11	Pears	IS,NF > NS,IF	IS,NF

For samples 2 and 4, "?" marks appear above some of the ">" symbols in the preference tests column.

Table 6

Storage study on high carbohydrate freeze-dried cantaloupe: Difference analysis

	Week 1	Week 2	Week 4	Week 8
Organoleptic scores (6 is maximum)				
Taste				
4°C	–	4.30	4.30	3.77
20°C	4.58	4.25	4.00	3.45
37°C	3.67	3.25	2.00	1.73
55°C	2.17	2.00	1.00	–
Texture				
4°C	–	4.10	3.95	3.91
20°C	4.18	4.30	3.80	3.64
37°C	4.00	4.10	2.80	1.73
55°C	3.36	3.70	2.30	–
Differences noted				
Taste	55/20**	55/4 **	55/4 **	37/4 **
	55/37**	55/37**	55/20**	37/20**
	37/30*	55/20**	55/37**	
		37/20**	37/20**	
		37/4 *	37/4 *	
Texture	55/37*	NSD	55/4 **	37/4 **
	55/20*		55/20**	37/20**
			55/37*	
			37/4 **	
			37/2 **	

* significant on 5% level
** significant on 1% level

Table 7

Storage studies on high carbohydrate freeze dried cantaloupe:

Ranking analysis

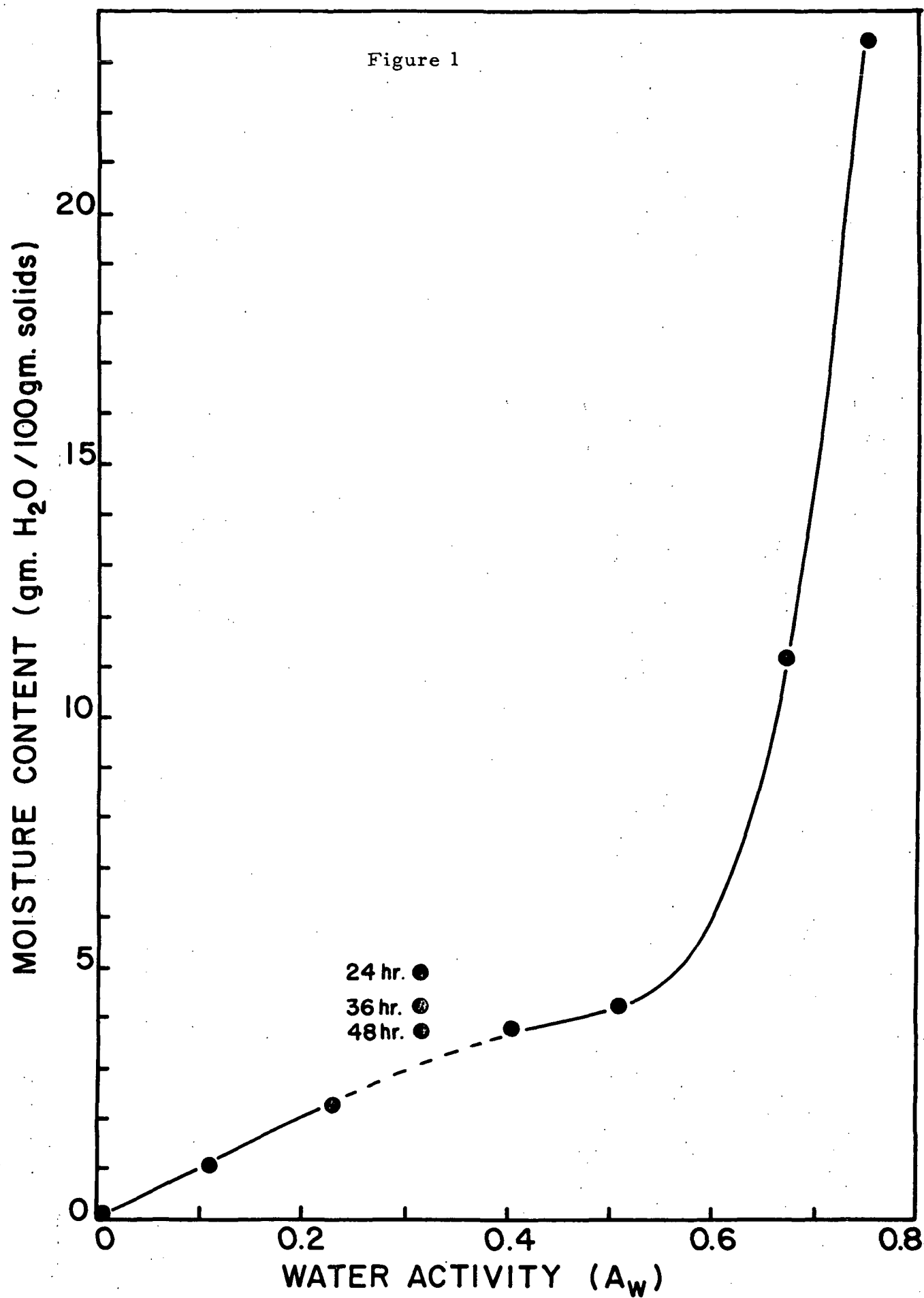|	Week 1	Week 2	Week 4	Week 8
Preference	20>37>55	4,20>37>55	4,20>37>55	4,20>37
Sample				
4° sample	-	first .605	first .738	first .708
RT sample	first .708	second .472	second .592	second .142
37° sample	second .142	third -.120	third -.300	third -.850
55° sample	third -.850	fourth -.957	fourth -1.03	-

Figure 1

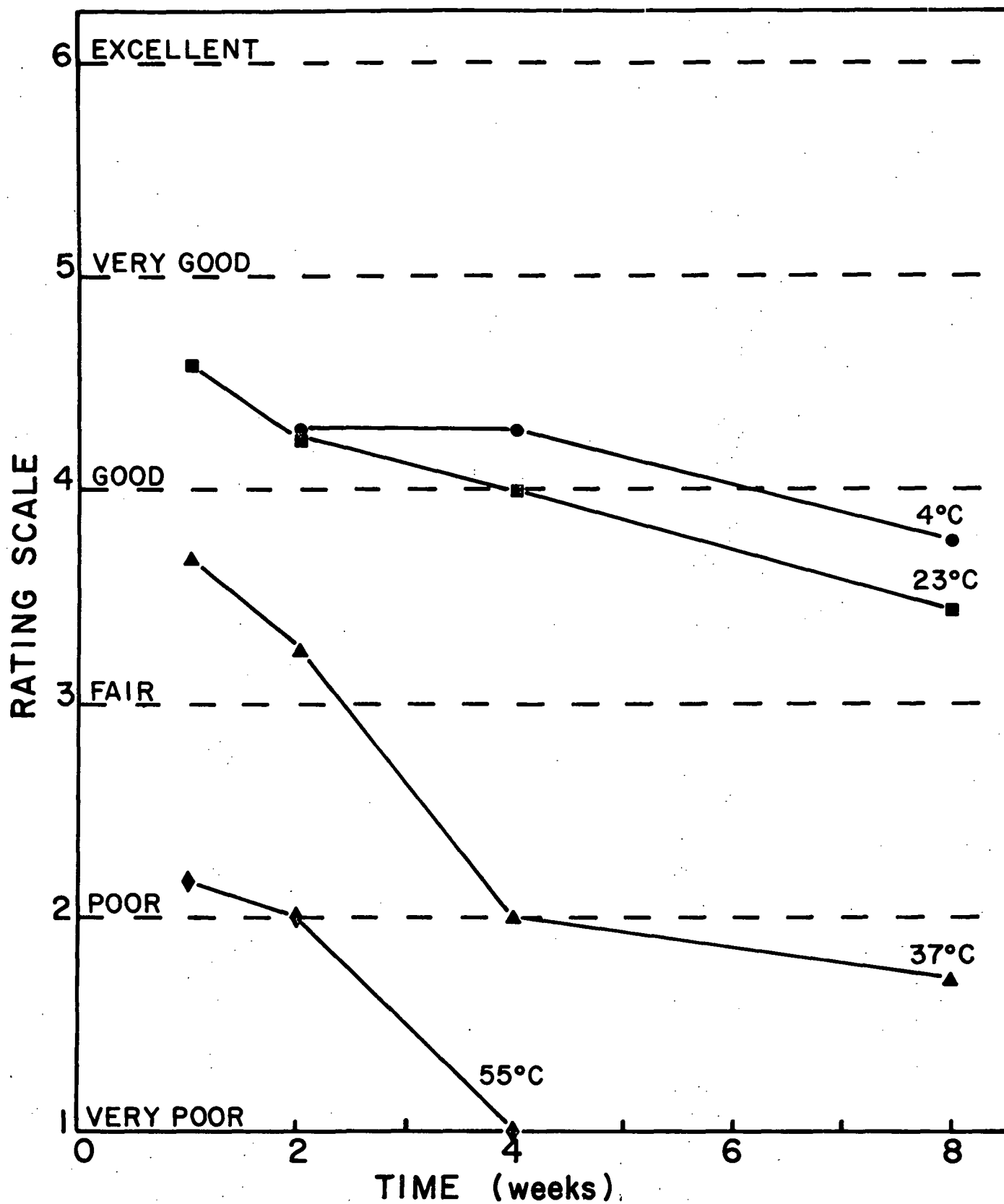Figure 2

7.   Summary of Results

a)   Microscopic observation allows evaluation of effects of drying rate on ice front geometry.

b)   In a complex food gel the phase separation effects during freezing were similar to those observed in simpler systems. Drying behavior, however, showed more complexity with microscopically observable formation of dry "islands" ahead of the drying front. Volatile retention, however, was similar to the simpler systems.

c)   Quantitative studies on retention of ^{14}C-ethyl acetate indicate equivalence of retention behavior between the freeze drying microscope and laboratory driers.

d)   Microscopic studies on freeze drying of o/w emulsions indicate that the solute in the aqueous phase has a decisive influence on the distribution of the oil phase (between free and entrapped oil) after freeze drying.

e)   Browning at elevated temperatures in dehydrated foods and model systems was independent of presence of air, provided moisture changes were avoided.

f)   Effects of water activity on browning of non-fat milk and egg were determined in the temperature range of 70°C to 110°C.

g)   Browning rates become large enough to become significant during drying for certain critical combinations of temperature and water activity. For instance:

32% E.R.H. and 80°C, or 11% E.R.H. and 90°C.

h) Activation energies for browning depended on product and on water activity. For milk they were 47, 53 and 33 kcal/mole for water activities of 0, 0.11 and 0.32 respectively. For egg, activation energy was 23 kcal/mole for the dry product.

i) Fluorescence development in heated egg appears related to intermediate formation and at high temperatures does not correlate with visual evaluation of browning. Spectrophotometric analysis of chloroform extract of egg appears to correlate better with browning.

j) Visual assessment of color depended on simultaneous presentation or absence of controls, and were also affected by processing related differences in particle geometry and surface structure.

k) A literature survey indicates the potential for control of structure using novel freeze drying processes involving non-aqueous solvents.

l) An artificial gel system based on calcium alginate was prepared and evaluated and showed fruit like characteristics when freshly prepared, but the desirable properties were not retained through freezing and drying.

m) A number of simple and complex polymeric matrix formers including: dextrins; starch; cellulose; a complex food gel containing guar, locust bean gum, carrageenan, and agar; bovine serum albumin; and pepsin

showed volatile retention behavior conforming to theories developed previously on the basis of work with sugars and with PVP.

n) It was shown that matrix disruption and volatile release are intimately related, and may be achieved by sorption of water or of selected polar solvents. Very high temperatures will also achieve the disruption and release.

o) It was shown through organoleptic tests for a number of freeze dried fruit products that control of solids content and of freezing rate can result in superior quality.

p) Cantaloupe melon was shown to be among the highly ranked fruit products and a 5 lb sample was prepared and sent to NASA/MSC.

q) Storage tests on the cantaloupe show deterioration of quality in elevated temperature storage.