A STUDY OF CERTAIN ADSORPTION PROPERTIES RELATING TO THE LOSS OF DIACETYL FROM SELECTED FOOD SYSTEMS

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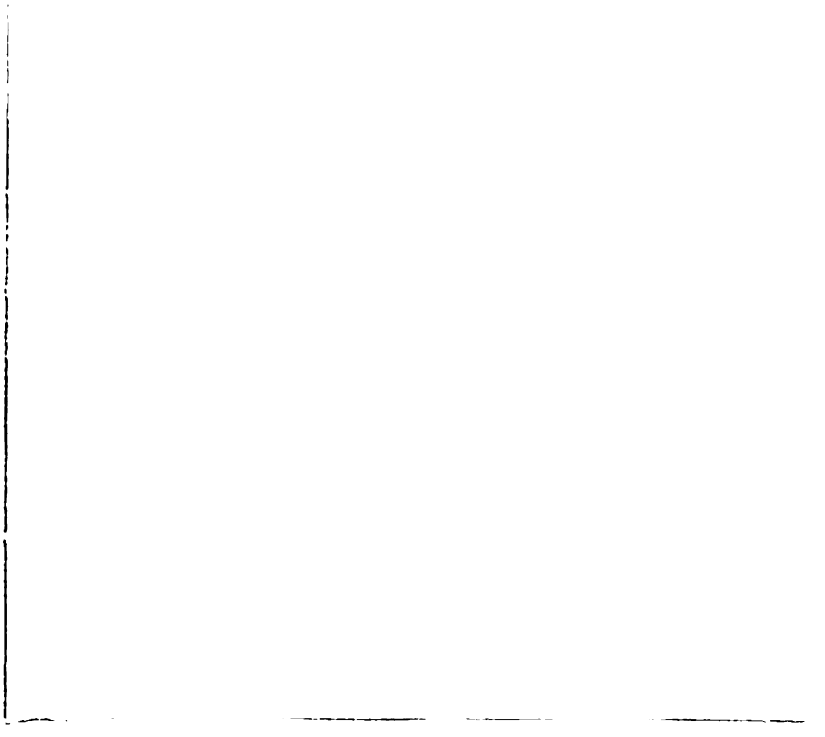

ABSTRACT

A STUDY OF CERTAIN ADSORPTION PROPERTIES
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FROM SELECTED FOOD SYSTEMS

By

Paul Kelly Wheeler

This research study was proposed to investigate the effect of exposure in selected relative humidities upon the retention or loss of diacetyl from a number of food systems. A major segment of this study involved the analysis of moisture adsorptive characteristics of the food systems.

Diacetyl was added to NFDM, casein, sucrose and cream prior to freeze-drying. Samples of the freeze-dried systems were subsequently exposed to various constant relative humidities for a period of time, or until the samples ceased to change in weight. The dehydrated food samples were then removed from the relative humidity environments and were analyzed for diacetyl to ascertain what losses, if any, occurred during moisture equilibration. Studies were also made to evaluate the capacities of the four products to adsorb water under these conditions.

Data acquired from this study allow the four food systems to be compared on the bases of:

(a) rates of water adsorption at various relative humidities;

(b) characteristics of their respective moisture adsorption

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And lastly, to my wife, Susan, for her support and unselfish hours of assistance in the preparation of this manuscript, this thesis is respectfully dedicated.

isotherms and conventional BET analyses; and

(c) their respective diacetyl desorption curves.

It was found that both casein and NFDM adsorbed a significant amount of moisture over the relative humidity range from 11% to 90%. Sucrose and freeze-dried cream, however, adsorbed substantially smaller amounts of moisture.

All products exhibited characteristic sigmoid moisture adsorption isotherms, with monolayer moisture values corresponding to approximately 12% relative humidity and from 2% to 5% moisture (dry basis).

A BET analysis of sucrose and cream exhibited values for $1/V_mC$ approaching zero and prohibited any reliable conclusions. Both NFDM and casein gave positive values for the monolayer moisture and the constant C. When the monolayer moisture values for NFDM and casein were compared on an equal surface area basis they were found to be equal, indicating that the protein fraction of NFDM plays a major role in determining the sorptive properties and stability of that product.

All of the food systems tended to desorb the added diacetyl with increasing relative humidity. No positive relation between the adsorption of moisture and the desorption of diacetyl was indicated for any of the four food systems. The data would indicate that for 75% retention of added diacetyl in products such as NFDM, casein, or sucrose, storage conditions should be no greater than 10% relative humidity. However, in high fat products such as freeze-dried cream, a comparable retention of diacetyl may be obtained at much higher relative humidities, perhaps as high as 40%. These higher humidities would seldom be desired, however, as microbic growth and various degradative reactions would then be encouraged.

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INTRODUCTION

This research study was proposed to investigate the effect of exposure in selected relative humidities upon the retention or loss of diacetyl from a number of food systems. A major segment of this study involved the analysis of the moisture adsorptive characteristics of the food systems.

This relation between a food product and its moisture content is of great value in predicting the behavior of the food in storage. Moisture contents determine the minimum levels at which microorganisms may grow and the relative rates of such reactions as Maillard browning and oxidation. Recent research has further suggested that the properties of various hygroscopic materials in equilibrium with the atmosphere influence the retention or loss of volatile materials.

An attempt was therefore made in this study to relate the water adsorptive capacities of the selected food systems to the loss, if any, of diacetyl. Diacetyl was chosen as it is found in numerous food products, including cheese, sour cream, butter, coffee, cocoa, beer, and it is often included in many artifical flavor compounds.

REVIEW OF LITERATURE

Introduction

Recent research in food science and technology has included an increasing amount of attention to the sorptive properties of foods. Investigation in the area of sorption phenomena has been especially concerned with examining the storage stability of dehydrated foods, retention and release of volatile food flavor compounds, and determination of various parameters related to the diffusion of water vapor in food systems.

This study was undertaken to investigate the retention or loss of diacetyl in a number of food systems, as influenced by the adsorption of various amounts of moisture.

Flavor is recognized as one of the main acceptability attributes of a food. Consequently an understanding of the mechanism of formation of food flavors from their precursors is important.

However, another equally important factor is the subsequent release or retention of flavor compounds in foods. For example, in many food processing operations a high degree of flavor retention is essential for the product to be acceptable.

Several studies have measured the loss of food volatiles during the concentration of some food products. Saravacos and Moyer (1968) measured the loss of aroma compounds during the vacuum drying of aqueous solutions of pectin and glucose. The retention of volatile compounds

during spray drying (Menting and Hoogstad, 1967) and centrifugal film evaporation (Mälkki and Veldstra, 1967) have also been investigated.

Gray and Roberts (1970) investigated factors controlling the adsorption and retention of volatile food flavor compounds on selected substrates. Parameters such as the activation energy of desorption and heats of preferential sorption were determined.

The relation between a food product and its moisture content is of great value in predicting the behavior of the food in storage. Labuza (1968) mentioned the importance of an adequate knowledge of sorptive properties for prediction of the stability of a dehydrated product. Similarly, numerous authors have studied the relation of sorption phenomena in foods to lipid oxidation, browning reactions, growth of microorganisms, enzyme activities, and various physiochemical reactions influencing the stability of food products.

Several models which describe the adsorption-desorption phenomenon in foods are now presented. Those models most applicable to foods have been stressed and the relationship of water activity to the stability of foods is pointed out.

Sorption Theories and Models

In view of the extensiveness of sorption phenomena, an exhaustive coverage of the subject is not intended. Rather, the principal models or theories which have been advanced to account for the sorptive behavior of food materials, or from which such theories are directly or indirectly derivable, will be presented.

Labuza (1968) in a review of sorption phenomena in foods, points out that the theoretical treatment of sorption can be classed into three modelistic frameworks, namely:

(a) The Kinetic Concept of Langmuir,

(b) Polanyi's Adsorption Potential Theory,

(c) Zsigmondy's Capillary Condensation Theory.

The Kinetic Concept

Langmuir (1918) proposed the classical kinetic concept based on his belief that adsorption was induced by unbalanced chemical forces on the surface of crystals leading to a unimolecular layer of the adsorbate. Assuming that: (a) adsorbed molecules are localized, (b) colliding adsorbate molecules are reflected elastically, and (c) the heat of adsorption for every adsorbate molecule striking the bare surface of a solid adsorbent is the same and equal to the heat of vaporization, Langmuir equated the rate of evaporation to the rate of condensation at the surface under conditions of equilibrium. The resulting isotherm equation is usually expressed in the form:

$$P/V_m = 1/bV + P/V_m,$$

where V is the volume adsorbed in cc/gram solid or gram/gram solid at vapor pressure P, V_m is the volume adsorbed at the monolayer point, and b is a constant dependent on both the heat of adsorption and the isotherm temperature.

Labuza (1968) has pointed out that for most food materials, the Langmuir model does not hold for the following reasons:

(a) heat of adsorption is, in fact, not a constant, but dependent on specific reaction sites,

(b) adsorbed molecules undoubtedly interact, whereas Langmuir predicts no such interaction,

(c) maximum adsorption is much larger than the theorized unimolecular layer.

Freundlich (1926) in attempting to improve upon the Langmuir model, postulated that adsorption involves a series of monolayers on a surface composed of heterogeneous sites.

Young and Crowell (1962) concluded that the models of Langmuir and Freundlich are best suited for predicting the adsorption of gases on metals, glass an other such solids, and are limited to explaining only the adsorption of a single layer of molecular moisture in food systems.

In spite of its inherent limitations, the Langmuir isotherm equation is perhaps the most important single equation in the field of adsorption, serving in many cases as the starting point in the derivation of other equations. Perhaps its greatest merit lies in the fact that it forms the basis of the more universally accepted BET equation of multimolecular adsorption proposed by Brunauer, Emmett, and Teller in 1938.

Stitt (1958) has summarized the assumptions of the BET model as follows:

(a) interactions of the adsorbate on the solid surface of an adsorbent are products of Van der Waal's forces,

(b) more than one layer of sorbate molecules may be present on the surface,

(c) the heat of sorption for all molecules in the first layer is constant and equal to the total heat of vaporization,

(c) the energy of sorption for molecules in all other layers is equal to the heat of condensation for the pure sorbate.

The most familiar form of the BET equation is derived from a thermodynamic basis (Adamson, 1963):

$$V/V_m = [Ca/(1-a)] \cdot [1 + (C-1)a],$$

where V and V_m are respectively the volume adsorbed and the monolayer value, a is the water activity, and where

$$C = k \exp (Q_s/RT),$$

Q_s being the heat of sorption.

The thermodynamic basis has been extensively applied to the studies of sorption by foodstuffs and provides a flexible analytical tool for a better understanding of the mechanism of the sorptive process. Young and Crowell (1962) and Brunauer (1945) have explained that adsorption of gaseous molecules serves to partially restore the unbalanced forces which normally exist at the surface plane.

Adsorption decreases the free energy of a system, which is a measure of the work done to accomplish the adsorption process. The heat of sorption indicates the binding energy between adsorbed molecules, such as water, and the surface of the adsorbent (Chung and Pfost, 1967a).

Free energy and heat of sorption of water vapor by proteins and high polymers have been evaluated by Dole and McLaren (1946). Bull (1944)

studied the sorption isotherms of a number of proteins and nylon, and calculated the free energy. The heat and free energy of adsorption of water vapor by cellulose have been calculated by Babbitt (1942).

The heats of sorption of water vapor on wheat flour, starch, and gluten were determined from experimental data by Bushuk and Winkler (1957). Becker and Sallans (1956) obtained similar values on wheat. Heats of hydration have been reported by Winkler and Geddes (1931) and by Schrenk et al. (1949). Heats of vaporization have been determined for shelled corn by Rodriguez-Arias et al. (1963). A detailed study relating sorptive processes by cereal grains to free energy changes have been reported by Chung and Pfost (1967a).

Sophisticated techniques for determining isosteric heats of adsorption employing frontal analysis chromatography were developed by Beebe et al. (1966). Gray and Roberts (1970) employed a Stanton thermal balance and a flow microcalorimeter to measure activation energies and heats of preferential sorption in a pectin:ethylamine model system.

The BET equation is commonly rearranged to the following linear form:

$$a/(1-a)V = 1/V_m C + [a(C-1)/V_m C],$$

(Labuza, 1968; Chung and Pfost, 1967b; Karel and Nickerson, 1964; and Salwin, 1959).

A plot of $a/(1-a)V$ vs. a, gives a straight line. From the intercept and slope of this line, the monolayer value and the factor C can be calculated. Caution, however, must be observed in the BET plotting of sorption data.

Many authors have exclaimed the BET equation is "unrealistic" (Pickett, 1945). Hill (1960) pointed out that BET's assumption of localized multilayers leads to erroneous entropy values. Cassel (1944) has written that the BET model falsely predicts infinite adsorption at saturation.

These two main errors have led to the general belief that the BET equation is applicable only to relative humidities of 50% or less (Becker and Sallans, 1956; Bushuk and Winkler, 1957; Dole and Foller, 1950; Hall and Rodriguez-Arias, 1970; Mellon et al., 1947; Pauling, 1945; and Smith, 1947).

However, data acquired from the 50% or less relative humidity range are sufficient to calculate a monolayer coverage of water. Further, the water surface area can be measured assuming the area of a water molecule to be 10.6 $\mathring{A}^2$. The surface area S_o, in $(meter)^2/gram$ of solid, is determined by the following equation:

$$S_o = V_m \cdot 1/M_w \cdot N_o \cdot A_w,$$

where V_m is the monolayer value, M_w is the molecular weight of water, N_o is Avagadro's number, and A_w is the area of a water molecule (Labuza, 1968).

Surface area values determined by water adsorption analyses usually bear little relation to those determined by nitrogen BET methods. Berlin et al. (1966), using low temperature (-195C) adsorption data reported surface areas for vegetable, fruit, meat, and fish products less than one square meter per gram. Conventional all-glass volumetric adsorption apparatus was used to measure the nitrogen adsorption

(Barr and Anhorn, 1959; Orr and Dalla Valle, 1959). Fox et al. (1963) showed similar results for milk powders.

In contrast, water surface areas are in the realm of 100-300 M^2/g, several orders of magnitude higher. Stitt, in 1958, explained this difference, pointing out the unique ability of a water molecule to structurally alter long chain polymers, exposing interior sites for adsorption. Further, the water molecule is smaller and is able to enter smaller pores than the nitrogen molecule.

Various modifications of the BET model have been developed to account for its obvious shortcomings. Hüttig's (1948) modification is expressed in the following equation:

$$V/V_m = (C \cdot a)/[1 - C \cdot a)(1 + a)].$$

A plot of this equation produces a straight line beyond 50% relative humidity.

However, in view of the fact that the previously mentioned criticisms apply to Hüttig's equation and indeed to the various modifications of the BET equation, the opinion of Gorter and Frederikse in 1949 that

> the kinetical BET theory gives a simple and valuable first picture of the phenomenon of adsorption, but it seems difficult to correct its obvious shortcomings without destroying the simplicity which perhaps constitutes its chief attraction,

seems very appropriate. Halsey (1950) concludes that the BET isotherm should be regarded merely as convenient algebraic tools for locating "point B," which he feels marks the monolayer stage. Labuza (1968) has called the BET equation the most useful in predicting the monolayer value and the heat of adsorption, which are of concern to processing and storage.

The Potential Model

At approximately the same time that Langmuir developed his monomolecular theory, Polanyi (1914, 1916, 1920) formulated an entirely different concept known as the potential theory. This concept recognizes the existence of multilayer adsorption, and considers that there is a potential field at the surface of a solid into which adsorbate molecules fall. The adsorbed layer thus resembles the atmosphere of a planet, being "compressed" most at the surface, and decreasing in density outward. Polanyi defined the adsorption potential at a point on the solid, as the work done in bringing an adsorbate molecule from the vapor phase to that point. It is fundamental to Polanyi's theory that the adsorption potential at any given point is characteristic of the adsorbent alone and is temperature independent, being unaffected by the presence of foreign molecules.

Therefore, since the adsorbent-adsorbate complex remains a puzzle, functions derived under this model can only be considered approximate. The major disadvantage is that it cannot be used to predict the monolayer value which is of prime importance to the food field.

Harkins and Jura in 1944 attempted to modify the Polanyi model by considering the distribution of surface forces to cause the adsorbed film to behave as a two-dimensional liquid. While this method has the advantage of not having to assume the area of a water molecule (as does BET), the basic equation does not hold for relative humidities above 40%.

Considering this limitation, the original formalism of Polanyi and its subsequent modifications including those by Frenkel (1946),

Halsey (1948), Macmillon and Teller (1951), and Harkins and Jura (1944), must be regarded as somewhat primitive in specific applications.

The Capillary Condensation Model

It has long been recognized that the vapor pressure over the meniscus of a liquid contained in a narrow capillary is lower than the vapor pressure of the free liquid at the same temperature. In other words, a vapor is liable to condense in a capillary at a lower pressure than it would on a plane surface (McLaren and Rowen, 1951). This phenomenon is called capillary condensation.

The quantitative relationship between the vapor pressure, P, over a liquid confined in a capillary, and the corresponding saturation vapor in a free space at the same temperature was given by Lord Kelvin (1871) in the form:

$$a = P/P_o = \exp\left[(-2\sigma\cos\theta V_o)/rR_gT\right],$$

where σ is the surface tension of the liquid, V_o is the molecular volume of the adsorbate, r is the radius of the capillary, R_g is the universal gas constant, T is the temperature in degrees Kelvin, and θ is the contact angle.

Zsigmondy (1911) and later Foster (1932) applied the capillary condensation theory to relationships between adsorption and prestructure in porous adsorbents. They argued that in porous structures, the same relation between vapor pressure and meniscus radius exists as in the case of ordinary capillaries. As the equilibrium relative humidity is increased in an adsorption experiment, condensation occurs in

successively larger pores. This rationalization leads to the calculation of pore size from adsorption data. Examples of these equations have been described by Adamson (1963), Labuza and Rutman (1967), and Roberts (1967).

Labuza (1968) has mentioned that knowledge of pore distribution has limited usefulness in the food field. Problems encountered include:

(a) The Kelvin equation is not applicable when the pores are of molecular dimension.

(b) There is difficulty in accurately determining θ, the contact angle.

(c) The surface tension in the pores of foods is variable and not the same as that of pure water.

Kuhn (1964) attempted to correct for the weak features of the Kelvin equation by combining the capillary theory with portions of other theories, establishing an empirical isotherm equation. To this date no practical application using his theory has been reported in the literature.

Secondary Sorption Theories and Models

The models to be taken up in this section of the review are designated secondary in so far as they are subordinate to one or more of the primary models previously discussed.

Although as early as 1882, Muller had proposed an equation to predict the adsorption of water by textile fibers. His equation turned out to be quite valueless (Swan and Urquhart, 1927) because his arbitrary assumptions--(a) a linear relation between the amount adsorbed and the relative vapor pressure, and (b) zero adsorption at the boiling point of water--were unsound.

The Smith Equation

It was not until 1947 that a partially successful treatment of water sorption by biological materials was formulated by Smith who recognized the existence of two principal classes of sorbed moisture: (a) bound moisture, M_b, and (b) normally condensed moisture, M_c. He assumed that the relation between M_b and a can be approximated by the Langmuir equation. Accepting the basic concepts of the BET theory for the framework of M_c, he derived the following expression for M_c:

$$M_c = -V'\ln(1-a),$$

and summed M_b and M_c to obtain:

$$M = M_b + M_c = M_b = V'\ln(1-a),$$

where V' is the absorbed mass in a monolayer of condensed moisture, and M is the total adsorbed mass.

While the Smith equation has been used to fit experimental isotherm data (Becker and Sallans, 1956), it has been shown by other authors (Strohman and Yoerger, 1967; Chung and Pfost, 1967b) to be basically empirical.

The Henderson Equilibrium Equation

Perhaps the best known and most widely used equation for predicting the equilibrium moisture content of food materials is the semiempirical equation of Henderson (1952). Henderson derived an

equation which can account for the temperature dependence of the experimental curve. His equation is of the form:

$$(1-a) = \exp(-KTM^n),$$

where T is absolute temperature in degrees Rankine, K and n are empirical constants, and M is the per cent equilibrium moisture content, dry basis.

Henderson evaluated the two constants, n and K, on the basis of two experimental points arbitrarily chosen at about 20% and 75% relative humidity. He applied his equation to a series of 18 miscellaneous hygroscopic products.

For most of these, satisfactory agreement was obtained between the derived theoretical curves and experimental observations. When appropriate values of the parameters K and n are available, the Henderson equation or its modifications by Day and Nelson (1965) and Thompson et al. (1967) have been found to fit isotherm data for cereal grains fairly well (Rodriguez-Arias, 1956).

However, at extreme relative humidities, Henderson's experimental data deviated significantly from calculated theoretical curves for corn, sorghum and other products. Karel and Proctor (1955) applied Henderson's equation to the calculation of theoretical curves for shredded coconut, starch, rice, gelatin dessert, and flaked oats.

These authors also observed significant deviations between the derived experimental curves and observed experimental curves, and suggested that more than two curves be employed for the evaluation of the constants.

Other authors (see for example, Pickler, 1956; Bakker-Arkema, 1961; and Day and Nelson, 1965) also found the Henderson equation inadequate for certain biological products.

Rockland, in 1957, recognized that Henderson's equation could be converted to the more useful linear form:

$$\log \log (1-a) = n \log M + K.$$

The constants n and K could be more easily and precisely evaluated by graphical or least squares analysis.

However, a straight line was seldom obtained; rather, the experimental points generally described three straight lines. This led Rockland to formulate his "local isotherm" concept which suggests that moisture sorption may not necessarily be a uniform, continuous process, but a series of two or more independent processes.

Rockland (1969) concluded that Henderson's initial success in fitting observed data to theoretical isotherms was due to "fortuitous choices of hygroscopic systems and the unavailability of reliable data..."*

The Young-Nelson Equation

An effort to construct a theory of adsorption for biological materials to reflect their basic cellular nature was made by Young and Nelson (1967). These investigators considered the cell as the ultimate basis for adsorption and recognized the existence of three modes of adsorbed moisture: (a) unimolecular-bound moisture of Langmuir,

*The main deficiency of the Henderson equation is that it is totally based on thermodynamics, it is therefore not founded on a model and gives no information about the nature of the adsorbent or its surface.

(b) normally condensed or multi-layer moisture of Brunauer, Emmett, and Teller, and (c) adsorbed moisture which results from a diffusion of moisture into the inner cell and which, due to the irreversibility of the diffusion process, is responsible for the occurrence of hysteresis. Although these authors developed a comparatively straightforward representation of hysteresis, the simplifications and reasoning leading to their explicit expression for the adsorbed moisture effectively destroyed their model. If the ultimate cell of a biological product is taken as the basis of sorption, it appears logical that moisture transfer across the semipermeable cell wall can take place only as a result of osmosis. Ngoddy (1969) presented proof that moisture transfer across the membrane cannot be justified prior to saturation, due to the osmotic pressure which could not be exceeded or even balanced at lesser relative humidities.

The Chung-Pfost Equation

The general framework of the potential theory was utilized by Chung and Pfost (1967b) to develop an isotherm equation for cereal grains and their derivatives. The equation is of the form:

$$\ln (a) = -A/R_g R \exp (-BM),$$

where M is the specific adsorbed mass, and the parameters A and B are respectively product and temperature dependent constants. Bradley (1936) earlier developed a related equation, also based on potential theory:

$$\ln (a) = K_2 K_1^{a},$$

where K_2 and K_1 are constants, and a is the amount adsorbed at a specific pressure. Hoover and Mellon (1950) found Bradley's equation fitted experimental data for relative humidities from 6% to 90% for a variety of high polymers.

Moisture Sorption in Foods

Since derived moisture sorption isotherms serve a number of useful functions, moisture sorption data have been obtained for a wide variety of food materials. They may be employed to: define limits for the dehydration of various foods (Makower and Dehority, 1943); estimate moisture content changes under any given condition of temperature or humidity (Makower and Myers, 1943); evaluate processing variables and to distinguish differences between grades of varieties of agricultural commodities (Houston and Kester, 1954; Karon and Adams, 1949); aid in the selection of packaging materials (Houston and Kester, 1954; Makower and Dehority, 1943); and, define moisture or humidity conditions under which product deterioration (Brockington et al., 1949; Cuendet et al., 1954; Henderson, 1952; Salwin, 1963) and microbial growth can be inhibited (Breese, 1955; Mossel, 1955; and Mosel and van Kuijk, 1955).

A plot of the amount of water adsorbed as a function of the relative humidity of the vapor space around the material, describes a sorption isotherm (Labuza, 1968). Most foods exhibit a sigmoid isotherm described as Type II Isotherm according to the classification of Brunauer (1945). (See Figure 1.)

Figure 1 shows a general moisture isotherm. The isotherm can be divided into several regions: Area I in Figure 1 represents the

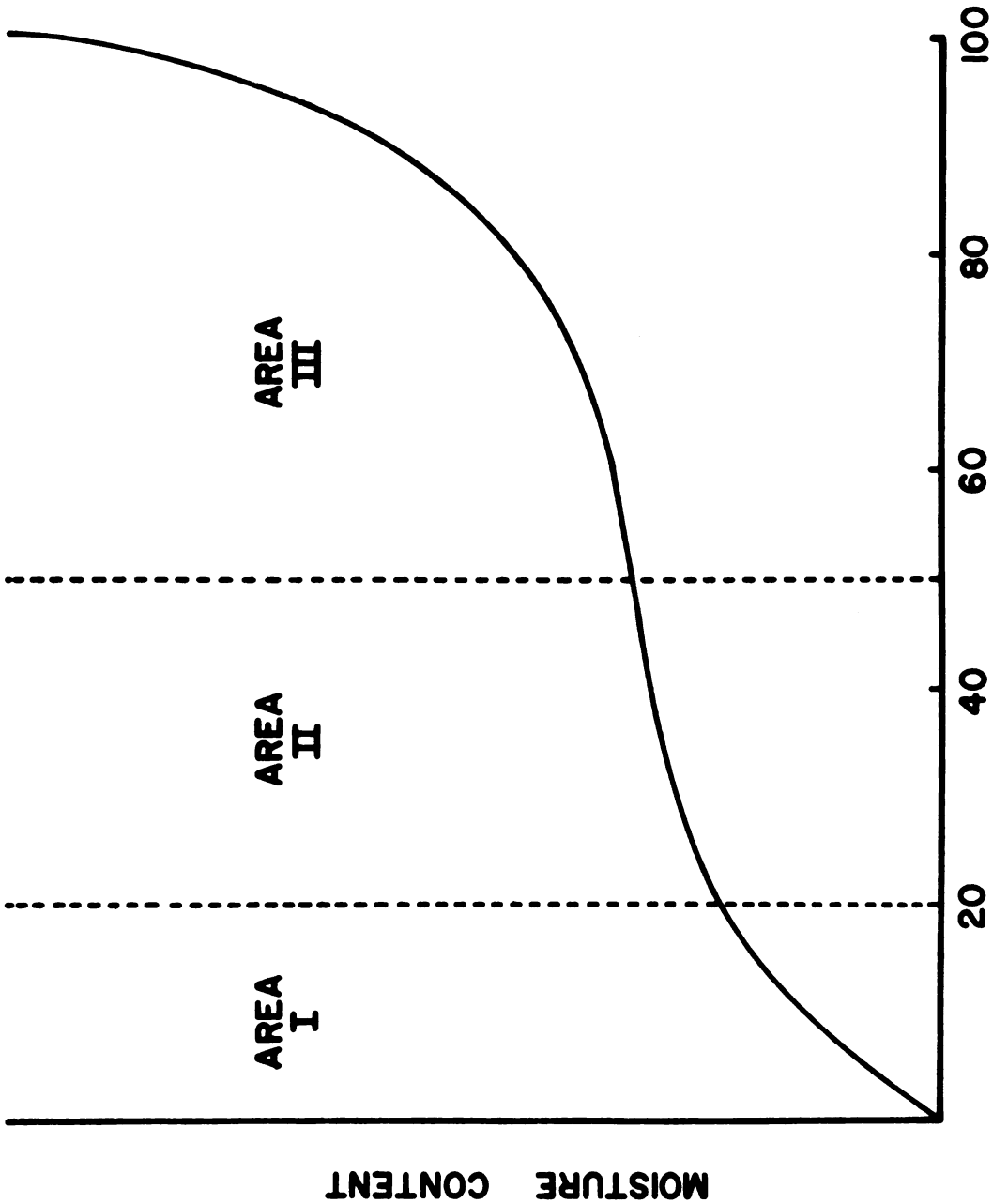

Figure 1. General moisture adsorption isotherm.

adsorption of a unimolecular film of water; Area II corresponds to the adsorption of additional layers of water; and Area III represents the condensation of water in the pores of the material. No definite relative humidity can be pinpointed to represent crossover from one area to the next; indeed, they may overlap as evidenced by the desorption curve.

Procedures for obtaining water vapor isotherms for foods have been described in detail by Taylor (1961), Stitt (1958), Karel and Nickerson (1964), and Mossel and van Kuijk (1955).

In general, these methods utilize one of three techniques:

(a) weighing bottle and humidity chamber,

(b) direct measurement,

(c) high vacuum measurement.

Sorption Isotherm Techniques

Weighing bottle and humidity chamber

Most of the moisture equilibrium data available for foods have been obtained by allowing samples to reach a constant weight when exposed to an environment in which the temperature and humidity are fixed within narrow limits. (See Makower and Dehority, 1943; Issenberg et al., 1968; and Wink, 1946).

Either saturated salt solutions (Rockland, 1960; Wink and Sears, 1950), or sulphuric acid solutions (Stokes and Robinson, 1949) are usually used for maintaining constant relative humidity in a closed vessel at atmospheric pressure or vacuo.

Examples of collecting adsorption data using salt solutions include: Mellon et al. (1947b), with proteins; Karel and Nickerson (1964), with dehydrated orange juice; and Wink and Sears (1950), using instrumentation as a control. Sulphuric acid solutions were employed by Bull (1944) using proteins, Chung and Pfost (1967c), using cereal grains, and Makower and Dye (1956) using various sugars.

Many days or even months may be required to reach equilibrium, but the time required to transfer water through the vapor phase may be reduced by evacuation or continuous circulation of the conditioned atmosphere over the sample. (See Bull, 1944.) Reduction of particle size is one method of reducing the time required for transfer of water within the material, and wetting the sample followed by freeze-drying may also produce a material which equilibrates more rapidly (Stitt, 1958).

Direct measurement

Another method of obtaining moisture equilibrium data consists of determining the water vapor pressure of samples of various moisture contents. This can be done in a suitable vacuum apparatus in which the vapor pressure is measured directly (Makower and Myers, 1943; Taylor, 1961), or by determining the relative humidity of the atmosphere in moisture equilibrium with the sample by use of a suitable hygrometer (Karel and Nickerson, 1964; Mossel and van Kuijk, 1955; and McBain and Bakr, 1926). A single determination requires, at most, about one hour, provided equilibrium moisture within the sample has been previously attained. The relative speed of this method is clearly advantageous for reducing thermal degradation reactions during measurements at elevated temperatures.

High vacuum measurement

In a third approach, which has been used in some of the most careful studies of sorption isotherms, the material is confined to a high vacuum system (as low as 10^{-7} mm Hg), and the gain or loss of moisture brought about by the admission or removal of water to the system is followed by use of a quartz-spiral balance (Bushuk and Winkler, 1957; Karel and Nickerson, 1964; and McBain and Bakr, 1926).

One disadvantage of the vacuum sorption balance is the loss of time in calibration due to frequent spring breakage (McLaren and Rowen, 1951).

Moisture Sorption and Stability

It was implied previously that many food products exhibit maximum stability within an optimum moisture range. Referring back to Figure 1, this optimum condition generally corresponds to that portion of a product's moisture sorption isotherm characterized by Area II. It is of interest that the lower limit of Area II corresponds with the "BET monolayer" (Brunauer et al., 1938).

Salwin (1959) suggested that the "BET monolayer" may be a satisfactory specification for the lower limit of equilibrium relative humidity in dehydrated foods. He concluded that the monolayer may be regarded as a protective film which protects the particles of food from attack by oxygen. There is a mass of evidence in the literature that oxidation and rancidity are aggravated by drying to very low moisture levels. Attack by oxygen is also responsible for pigment instability

and loss of vitamins, and sometimes, the initiative in Maillard browning reactions (Stadtman, 1948; Karel and Nickerson, 1964; Sharp, 1962). Salwin has further stated that the moisture vapor pressure at the monolayer point represents a partial pressure of water vapor, which is competitive with the oxygen partial pressure to the extent of being protective.

It seems reasonable that this protection should extend to the water holding capacity of the dehydrated food. According to Klotz and Heiney (1957), the attachment of an oxygen molecule to a binding site of a protein would produce an incongruity in the aqueous covering sheath which could disturb the hydration structure of neighboring sites.

Competition with oxygen is not the sole basis for explaining the protective effects of water. The bond energy of the adsorbed water would inhibit interactions between polar groups on adjacent carbohydrate or protein molecules, and thereby preserve the rehydratability and texture of the food (Salwin, 1959).

Rockland (1957) has shown that above and below a critical moisture of 5.5%, darkening and development of off-flavors in walnut kernels is accelerated. Maloney et al. (1966) has confirmed that, in model systems, lipid oxidation increases significantly below the monolayer value. However, Martinez and Labuza (1968) reported that freeze-dried salmon is more stable at moisture levels considerably higher than the "BET monolayer."

At lower moisture levels within Area I, free radicals by irradiation or oxygen adsorption may potentiate rancidity development (Rockland, 1969).

Area III appears to define the region in which free water has a dominant influence on food product stability. Unfavorable enzyme reactions are accelerated within Area III (Acker, 1969). Block (1953), Mossel and van Kuijk (1955), and Scott (1957) have demonstrated that microbial growth proliferates at high relative humidities generally observed within Area III.

Sorptive Properties of Food Systems

To complete this review, examination of some specific sorptive properties of a number of food components and composite food systems remains.

Nickerson and Dolby (1971) examined the effect of chemical composition, particle size, and previous heat treatment on the ability of a number of sugars to adsorb diacetyl. They reported that the regular lactose α-hydrate adsorbed 18-34 mg/kg diacetyl under standard test conditions. Spray drying, producing an amorphous lactose glass, reduced diacetyl adsorption to 11 mg/kg; heating to produce the regular anhydride increased adsorption to 156-306 mg/kg. Converse results were obtained when heating glucose and sucrose, reducing their diacetyl adsorption from 82 and 39 mg/kg to 36 and 10 mg/kg, respectively.

Salwin (1963) concluded that foods high in carbohydrates had monolayer moisture values which markedly increase with moderate increases in temperature.

Bushuk and Winkler (1957) reported on the sorption of water vapor on wheat flour, starch and gluten. They found that typical sigmoid isotherms were produced. However, at relative humidities of 90% or greater, the flour and glutens were physically altered and the

sorption was no longer reversible. A 24-hour heat treatment of the flour at 100C reduced its sorptive capacity of water vapor by 20%. The authors further concluded that adsorption of water vapor by flour appears to be a bulk property of the material and not dependent on particle size.

Various investigators have studied the hysteresis phenomenon with respect to cereals and their products. Hubbard et al. (1957) and Hart (1964) indicated that repeated wetting and drying tends to decrease hysteresis slightly. Rao (1941) showed disappearance of the hysteresis loop of isotherms of rice, after successive adsorption and desorption cycles. This observation was also reported by Chung and Pfost (1967c).

The subject of moisture sorption by proteins has been extensively reviewed in the literature. Dole and McLaren (1946) gave extensive treatment to the thermodynamics of moisture sorption by proteins and high polymers. Free energies and heats of hydration were tabulated and results similar to those found by Bull (1944) were reported.

In a now classic paper, Bull (1944) evaluated BET constants for a number of proteins and concluded that water is adsorbed between coherent planes of linked protein molecules whose exposed surfaces are hydrophilic.

Examining the various protein components, Mellon et al. (1946) reported a three-step procedure in the adsorption of water by proteins. The first step seems to be a sharing of one molecule of water between two amino groups, below 6% relative humidity. The second step is a linear increase in adsorbed water with increases in relative humidity. Equations were presented for this increase between zero and 60% relative

humidity. The third step is a rapidly increasing amount of adsorption with increasing relative humidity, which appeared to be a condensation of water on water molecules already attached to the amino groups. The authors concluded that 25% to 33% of the water adsorbed by casein is adsorbed by the amino groups.

It was later reported by Mellon et al. (1947a) that hysteresis was entirely independent of the content of free amino groups.

Examining the subject of water sorption, Mellon et al (1948) reported that peptide groups were responsible for about 45% of the water adsorption by casein and 70% by zein. However, in 1945 Pauling wrote that peptide groups usually do not bind water due to their hydrogen-bond formation with carbonyl and imido groups, but that water is bound by non-hydrogen bonding carbonyl groups.

Mellon et al. (1950) further reported that guanidino groups of proteins are responsible for about 10% of the total water adsorption.

The same authors in 1948 subjected a number of proteins to severe heat treatments which altered the internal structures and concluded that the sorption of water by proteins has little dependency on their physical structure.

Quantitative Determination of Diacetyl

Earliest attempts to measure the amount of diacetyl present in foods usually involved measuring diacetyl in the presence of its biochemical precursor acetylmethylcarbinol (Barnicoat, 1935). This gravimetric method involved determining acetylmethylcarbinol plus diacetyl as nickel dimethylglyoximate, and was only satisfactory when

fairly large amounts were present. However, due to the low solubility of the derivative, attempts to measure 1 to 2 mg of diacetyl in 100 ml of distillate resulted in very low recovery.

Most methods which are used in the microdetermination of diacetyl are based on the quantitative formation of colored compounds.

Testoni and Ciusa (1931) appear to have been the first to use a colorimetric method for diacetyl. They oxidized nickelous dimethylglyoximate and obtained a soluble red complex.

Barnicoat (1935) dissolved the precipitated nickel dimethylglyoximate in chloroform and used the resulting yellow solution for comparisons. Mohr and Wellm (1937) improved this method by extracting with chloroform the nickel complex which was left in solution.

Pien, Baisse and Martin (1936) reacted the diacetyl with certain O-diaminobenzene derivatives and made use of the yellow colors which the resulting diaminobenzene derivative exhibits in the presence of strong acid as the basis of their procedure.

Prill and Hammer (1938) developed a sensitive colorimetric method for determining very small amounts of diacetyl. The method is based on the formation of the intensely colored mono-ferrous dimethylglyoximate. The diacetyl is first converted into dimethylglyoxime; the excess dimethylglyoxime is fixed with acetone in a phosphate buffered solution; the ammonium hydroxide, a large amount of tartrate, and finally, a small amount of ferrous sulfate are added. The resulting rose-red color develops very rapidly.

A simple but sensitive colorimetric method of diacetyl determination was developed by Westerfeld (1945). This method is based on

the Voges-Proskauer reaction as modified by Barritt (1936). This reaction was shown by Harden and Norris (1911) to be due to the reaction between diacetyl and a guanidino group in the presence of alkali. Attempts to increase the sensitivity of this reaction led to the addition of creatine by O'Meara (1931) and naphthol by Barritt (1936). The resulting rose-red color was found to give a linear relation over the range of diacetyl concentrations and was stable up to thirty minutes (Westerfeld, 1945).

Titrimetric methods for diacetyl are also possible, such as that of Ruehe and Corbett (1937), but it is doubtful whether these methods could be as sensitive and specific as colorimetric methods (Prill and Hammer, 1938).

METHODOLOGY

Introduction

In this study, diacetyl was added to four different food systems prior to freeze-drying. Samples of the freeze-dried systems were subsequently exposed to various relative humidities for a period of time, or until the samples ceased to change in weight. The dehydrated food samples were then analyzed for diacetyl to ascertain what losses, if any, occurred during moisture equilibration at a given relative humidity. Studies were also made to evaluate the capacities of the four products to adsorb water under these conditions.

Preparation of Samples

The food systems employed in this study included a low-heat spray-dried non-fat dry milk (NFDM), commercially manufactured sodium caseinate, commercial grade sucrose, and fresh pasteurized whipping cream. The systems were chosen to represent respectively foods rich in carbohydrate and protein, an essentially pure protein, a relatively pure carbohydrate, and a food containing a large amount of lipid.

Each system was incorporated with a small amount of diacetyl (from 5 to 11 ppm). Solutions of approximately 10%, by weight, were prepared from the NFDM and casein, and a 50% solution was prepared from sucrose. The cream was used as obtained from a retail supplier. Known

amounts of diacetyl were then thoroughly mixed into each of the liquid systems.

Accurately weighed amounts of each food system were placed in aluminum pans (9x12x1-1/2 in.) to be frozen overnight at -10F. An iron-constantan thermocouple was secured to the bottom of the pan with the sensing portion of the thermocouple in the center of the product.

The frozen samples were freeze-dried in a Repp Model FFD42WS freeze-drier equipped with a Honeywell Electronic Multipoint Strip Chart Recorder. The condenser temperature was maintained at -60F and the platen temperature was set at 100F. Vacuum in the freeze-drying chamber was maintained at 4 microns of Hg. When the center temperature of the product reached that of the platen, the run was terminated, and the chamber vacuum released with air.

After removal from the freeze-dryer, each product was accurately weighed, placed in brown glass air-tight jars, and stored in a refrigerator. Samples of each of the four products were prepared in duplicate by the method outlined above.

Exposure to Selected Relative Humidities

Relative humidity (RH) environments at room temperature were prepared using saturated salt solutions (Rockland, 1960). (See Table 1.)

Approximately 100 ml of the saturated salt solutions were placed in glass dishes in sealed standard laboratory desiccators. A small amount, ca. 25 grams, of the solid salts were added to their respective saturated solutions in the desiccators to allow for any gain or loss of moisture due to the initial humidity within the chamber. Seven days

Table 1. Saturated salt solutions used to produce selected relative humidities.

Desiccator Number	Salt	Relative Humidity (%)
1	Lithium chloride	11
2	Magnesium chloride	33
3	Sodium bromide	57
4	Sodium chloride	75
5	Potassium bromide	83
6	Barium chloride	90

were allowed for the saturated solutions to reach moisture equilibrium with the atmosphere in the sealed desiccator.

The data in Table 1 show the saturated salt solutions used to produce the selected RH within each sealed desiccator. After equilibrium RH had been established within each of the six chambers, 1-2 gram samples of the freeze-dried food system were accurately weighed into specially prepared plastic petri dishes. (Each dish had numerous small holes in the top cover to allow free movement of moisture and diacetyl to and from the product.)

Four such sample dishes of the freeze-dried food system were placed in each of the six desiccators previously described. Two of the sample dishes contained product from an initial sample preparation and two from a duplicate preparation.

Each sample dish was accurately weighed every second or third day, until the samples ceased to change in weight.

The sample dishes were covered with a fitted plastic top during transporting to and from the analytical balance and during the weighing procedure to prevent gain or loss of either moisture or diacetyl. After constant weight was attained by the sample in each desiccator, the samples were carefully removed from the plastic dishes and analyzed for diacetyl content. This experimental procedure was followed in duplicate for each of the four food systems.

The loss of diacetyl from NFDM during prolonged exposure in the RH environments was investigated in a supplementary experiment. Four samples of freeze-dried NFDM, treated as previously described, were placed in each of the six desiccators, and allowed to reach constant

weight. One sample from each desiccator was removed and analyzed for diacetyl at the end of one, two, three and four weeks, after constant weight was attained.

Analytical Procedures

Moisture

The freeze-dried systems (NFDM, casein, cream, and sucrose) were examined for moisture within a few hours after removal from the freeze-dryer by an A.O.A.C. method appropriate for each product.

Any weight gained or lost by the food systems during exposure in the various RH environments was assumed to be due to moisture. In any case, weight losses or gains resulting from a change in diacetyl content would be too small to measure on a standard laboratory analytical balance.

Fat

The samples of cream, both liquid and freeze-dried, were analyzed for fat by the official Roese-Gottlieb wet extraction procedure (A.O.A.C. 16.052).

Diacetyl

The samples of each food system were analyzed for diacetyl both after freeze-drying and after attainment of constant weight during exposure in the various relative humidities.

The method of Westerfeld (Westerfeld, 1945) was modified as follows:

(a) The α-napthol was dissolved in sodium hydroxide under a rapid stream of nitrogen, to minimize the development of an unwanted yellow color (additional step).

(b) Two to three drops of Dow Anti-Foam silicone solution were added during the steam distillation of NFDM, casein, and cream to minimize foaming (additional step).

(c) The test tubes containing distillate and reagents were held in a 30C water bath for 10 minutes to allow consistent color development (rather than holding at room temperature).

(d) Absorbance of the reaction product was measured at 510 nm (rather than 530 nm) using a Beckman DU-2 spectrophotometer because the absorbance maximum occurred at this wavelength.

RESULTS AND DISCUSSION

Examination of Recovery and Analysis of Diacetyl

To test the suitability of the method of Westerfeld, a standard curve was prepared in the range from 1 to 12 ppm of diacetyl. As can be seen from the graph in Figure 2, the relation of diacetyl concentration to absorbance is linear, obeying Beer's Law.

To determine the amount of diacetyl lost during the analysis, a recovery test was conducted. Loss of diacetyl during steam distillation amounted to about 3%, as 97+% recovery was possible (Table 2).

The method could not be applied to a sample weighing less than 3.0 grams due to the dilution of the condensate which occurred during steam distillation. Therefore, to enable small samples of the equilibrated products to be accurately analyzed, such samples were fortified with 3.0 g of a standard NFDM containing a known amount of diacetyl prior to steam distillation. The addition of this extra material, for which the diacetyl content had been previously obtained, allowed the analysis of very small quantities of diacetyl.

To examine the sensitivity of the analytical method to detect small variances of the amount of diacetyl in the samples to be analyzed, a further test of the method was conducted. Samples of 3.5, 4.0 and 4.5 grams of NFDM were accurately weighed and analyzed in triplicate. As can be seen from the data in Table 3, the analytical method is sufficiently sensitive to determine a range of 0.3 ppm in the amount of diacetyl present.

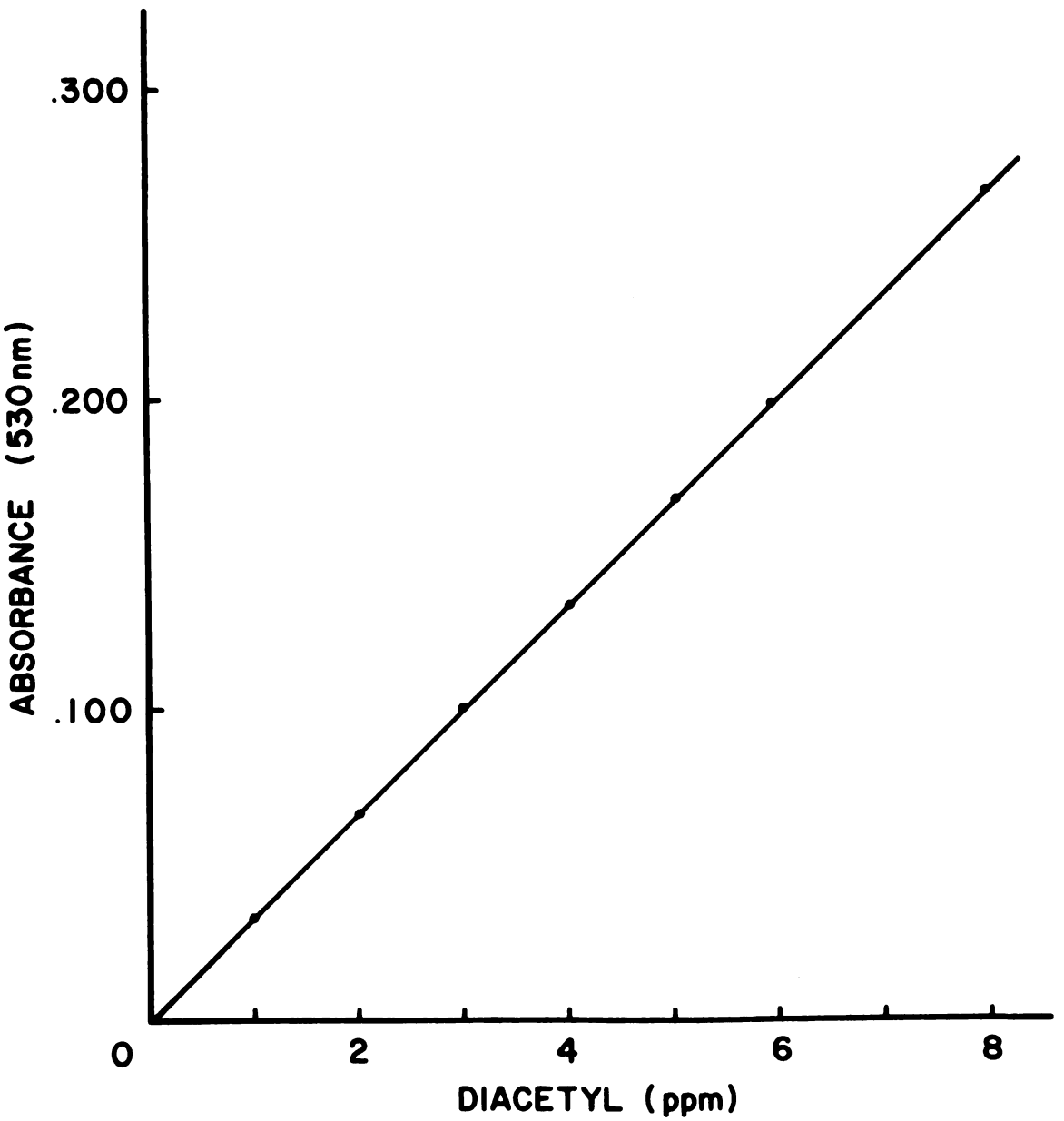

Figure 2. Standard curve for diacetyl.

Table 2. Recovery of diacetyl using the method of Westerfeld.

NFDM (g)*	Diacetyl Added (µg)	Distillate (ml)	Diacetyl Recovered (µg)	Recovery (%)
2	30	72	28.8	97
2	30	56	28.5	97
2	25	63	24.2	98

*All samples presented here and in subsequent experiments were accurately weighed to five significant figures.

Table 3. The ability of the method of Westerfeld to detect small variances in the amount of diacetyl present in the sample (Diacetyl concentration in the samples of NFDM equals 7.7µg/g NFDM).

Sample Size NFDM (g)	Yield (ml)	Diacetyl Content (ppm)	Mean
3.5	70	7.6	
3.5	75	7.5	7.6
3.5	75	7.8	
4.0	65	7.8	
4.0	71	7.7	7.7
4.0	68	7.6	
4.5	58	7.7	
4.5	57	7.8	7.7
4.5	83	7.7	

Preliminary Analyses

Shortly after the products were freeze-dried they were analyzed for diacetyl to determine the loss due to freeze-drying.

As shown by the data in Table 4, NFDM, casein, and sucrose showed relatively little difference in the amount of diacetyl lost. Cream, however, lost a significantly greater amount (73%) of the added diacetyl during freeze-drying.

The presence of ample binding sites in NFDM, casein, and sucrose could account for the rather moderate loss of added diacetyl during the freeze-drying of these products. The presence of far fewer binding sites is consistent with the high loss of diacetyl from freeze-dried cream (Mellon et al., 1948).

The four food systems were tested for moisture content shortly after freeze-drying. Moistures, calculated on a wet-basis (wb), were similar for the four freeze-dried products, ranging from 3.4 to 4.1%. The samples of cream were further examined for fat content (Table 5).

Primary Data

The four food systems were exposed to various RH environments for a number of days. The samples of the food system were weighed periodically until no further change in weight was observed. When the samples attained constant weight, they were removed from the RH environments and analyzed for loss, if any, of diacetyl.

Data acquired from this study allows the four food systems to be compared on the bases of:

Table 4. Loss of diacetyl from selected food systems during freeze-drying (FD).

Amount of Product (g)	Amount of Solute (ml, water)	Amount of Diacetyl		Diacetyl Loss (%)*
		Before FD	After FD	
NFDM:				
100	900	0.63	0.32	49
100	900	0.63	0.31	51
CASEIN:				
100	900	2.00	1.13	44
100	900	2.00	1.17	42
SUCROSE:				
500	1000	1.00	0.51	49
500	1000	1.00	0.56	42
CREAM:				
214	---	2.33	0.63	73
214	---	2.33	0.61	74

*Each percentage is the mean value of three determinations.

Table 5. Moisture content (wb) and fat content (cream only) of selected food systems after freeze-drying.

Product	Moisture Content, %(wb)		Fat Content, %	
	Trial 1	Trial 2	Trial 1	Trial 2
NFDM	4.10	4.12		
	4.11	4.11		
	4.10	4.11		
Mean	4.10	4.11		
Casein	3.50	3.68		
	3.78	3.73		
	3.79	3.68		
Mean	3.70	3.69		
Sucrose	3.30	3.47		
	3.50	3.39		
	3.35	3.42		
Mean	3.38	3.42		
Cream	3.78	3.79	83.04	83.08
	3.81	3.82	83.03	83.07
	3.80	3.80	83.03	83.06
Mean	3.80	3.80	83.03	83.07

(a) rates of water adsorption at various humidities;

(b) characteristics of their respective moisture adsorption isotherms including conventional BET analyses; and,

(c) their respective diacetyl desorption curves.

Adsorption of Water by Selected Food Systems

Data relating to moisture sorption were calculated on the dry basis to show a relation between the aqueous and solid fractions of the food system. Unless otherwise specified, data in the tables and figures are the mean values of duplicate sample preparations and two replications per preparation.

Initial moisture contents were determined by oven methods; other moisture contents were based on weight gained or lost. Dry basis moisture values for the four food systems are presented in Tables 6 through 9.

NFDM (Table 6) adsorbed the greatest amount of water over the range of relative humidities examined. The moisture content of the NFDM samples increased by a factor of six during exposure in the 11% through the 90% RH environments. Having both the free amino and peptide groups of the casein fraction (Mellon et al., 1948) and the carbonyl and hydroxyl binding sites of the lactose fraction (Nickerson and Dolby, 1971), NFDM would be expected to participate in active water adsorption.

Casein (Table 7) adsorbed moisture in amounts similar to NFDM over the range of RH. As in the NFDM, a six-fold increase in per cent moisture (db) was observed from the 11% through 90% relative humidity

Table 6. Dry basis moisture values for NFDM equilibrated in controlled relative humidity environments (25C).

Relative Humidity (%)	Mean Sample Weight Before[1] (g)	Mean Sample Weight After[2] (g)	Changes in Weight (as water) (g)	Amount of Water in Sample Before (g)	%M Before (db)	%M After (db)
11	1.0206	1.0247	.0041	.0418	4.27	4.68
33	1.0015	1.0369	.0364	.0411	4.28	8.07
57	1.0020	1.0507	.0487	.0411	4.28	10.00
75	1.0022	1.1138	.1116	.0411	4.28	15.88
83	0.9987	1.1395	.1408	.0409	4.27	18.97
90	1.0066	1.2612	.2546	.0413	4.28	30.65

[1]Before exposure in controlled RH environment

[2]After reaching constant weight in controlled RH environment

Table 7. Dry basis moisture values for casein equilibrated in controlled relative humidity environments (25C).

Relative Humidity (%)	Mean Sample Weight Before[1] (g)	Mean Sample Weight After[2] (g)	Changes in Weight (as water) (g)	Amount of Water in Sample Before (g)	%M Before (db)	%M After (db)
11	1.0001	1.0082	.0081	.0370	3.70	4.68
33	1.0020	1.0528	.0508	.0371	3.70	9.11
57	1.0005	1.0791	.0786	.0370	3.70	11.99
75	1.0007	1.1562	.1555	.0370	3.70	19.98
83	1.0057	1.1933	.1876	.0372	3.70	23.21
90	1.0095	1.2597	.2502	.0375	3.70	29.57

[1]Before exposure in controlled RH environment

[2]After reaching constant weight in controlled RH environment

Table 8. Dry basis moisture values for sucrose equilibrated in controlled relative humidity environments (25C).

Relative Humidity (%)	Mean Sample Weight Before[1] (g)	Mean Sample Weight After[2] (g)	Changes in Weight (as water) (g)	Amount of Water in Sample Before (g)	%M Before (db)	%M After (db)
11	1.9991	1.9716	-.0275	.0676	3.50	2.08
33	2.0030	1.9761	-.0242	.0676	3.49	2.24
57	1.9976	1.9737	-.0239	.0675	3.50	2.26
75	1.9994	1.9760	-.0234	.0676	3.50	2.30
83	1.9989	2.0070	+.0081	.0676	3.50	3.92
90	2.0242	---	---	.0676	3.50	---

[1] Before exposure in controlled RH environment

[2] After reaching constant weight in controlled RH environment

Table 9. Dry basis moisture values for FD cream equilibrated in controlled relative humidity environments (25C).

Relative Humidity (%)	Mean Sample Weight Before[1] (g)	Mean Sample Weight After[2] (g)	Changes in Weight (as water) (g)	Amount of Water in Sample Before (g)	%M Before (db)	%M After (fat-free db)
11	1.9980	2.0011	.0031	.0759	3.94	24.25
33	2.0032	2.0106	.0074	.0761	3.95	25.54
57	2.0018	2.0143	.0125	.0760	3.95	27.08
75	2.0012	2.0382	.0370	.0760	3.95	34.59
83	2.0021	2.0555	.0534	.0761	3.95	39.62
90	2.0050	2.0835	.0830	.0760	3.95	48.71

[1]Before exposure in controlled RH environment

[2]After reaching constant weight in controlled RH environment

range. According to Mellon et al. (1948), the amino groups of casein are responsible for about 33% of the adsorbed water, while the peptide groups can account for 45%.

Sucrose (Table 8) lost moisture during exposure in 11% through 75% RH. The moisture loss initially was rapid and constant weight was attained within three days. The amounts of moisture lost by the sucrose samples in the 11%, 33%, 57% and 75% RH were very similar. Dry basis moistures of the sucrose samples in the 11% through 75% RH decreased from 3.5% before exposure to an average of 2.2% at constant weight. According to Makower and Dye (1956) crystalline sucrose tends to readily release moisture, even at higher RH, to yield an essentially anhydrous material. During exposure to 83% RH, the sucrose samples adsorbed a small amount of water. The dry basis moistures of the sucrose samples increased an average of 0.4% at 83% RH. It is suggested by the author that at this high RH, the sucrose crystals were very slowly forming an aqueous solution. If the samples were exposed in the 83% RH environment for a much longer period of time, a saturated sucrose solution would probably result.

The tendency of sucrose to form an aqueous solution at very high humidities was most evident in the 90% RH environment. After one day exposure the smaller sucrose crystals had adsorbed sufficient moisture to be dissolved. The adsorption of water was sufficiently rapid at this RH to preclude accurate weight measurements.

The samples of freeze-dried cream adsorbed the least amount of moisture over the range of relative humidities examined in this study (Table 9). Being largely free fat and not associated to any

great degree with either protein or carbohydrates, the adsorptive capacity of the freeze-dried cream would be expected to be quite low. Moisture contents for the samples of cream were calculated on a non-fat dry basis.

Rates of Water Adsorption of Selected Food Systems

The rates of adsorption of water by NFDM, casein, sucrose and freeze-dried cream are shown by the graphs in Figures 3 through 6, and are tabulated in the Appendix.

All of the products examined exhibited a rapid change of weight within the first few days of exposure in the various relative humidities. A decelerated weight change was then observed during the next several days of exposure followed by a constant weight phase. The major exception to this generality is sucrose (Figure 5), which experienced a rapid and virtually linear exposure in 90% RH.

The data in Figure 3 demonstrate an increase in moisture content of NFDM during exposure in each of the relative humidities. In each RH, the samples of NFDM closely approached constant weight by the fourth day of exposure. While a measurable increase in weight of the NFDM samples was observed from the fourth to the eighth day of exposure in the relative humidities, the gain was so slight as to produce virtually a horizontal linear relation.

The graph in Figure 7 examines the rates of water adsorption of NFDM presented in Figure 3. Log $\frac{M_e - M}{M_e - M_o}$, is plotted versus time in days, where M_e is the equilibrium moisture content (db) of NFDM after four days of exposure, M is the observed moisture content (db) and M_o is the

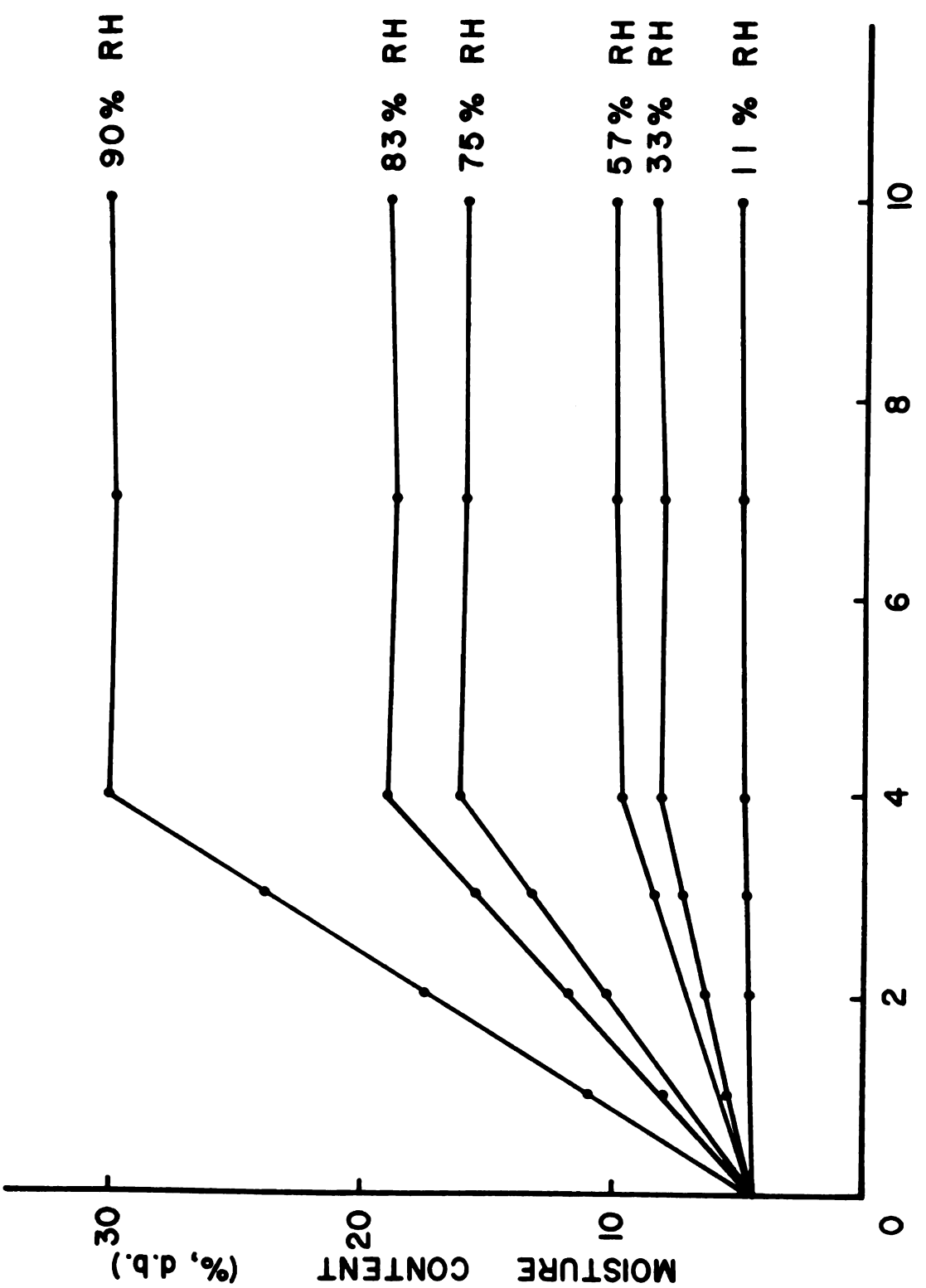

Figure 3. Water adsorption by NFDM at various relative humidities (25C).

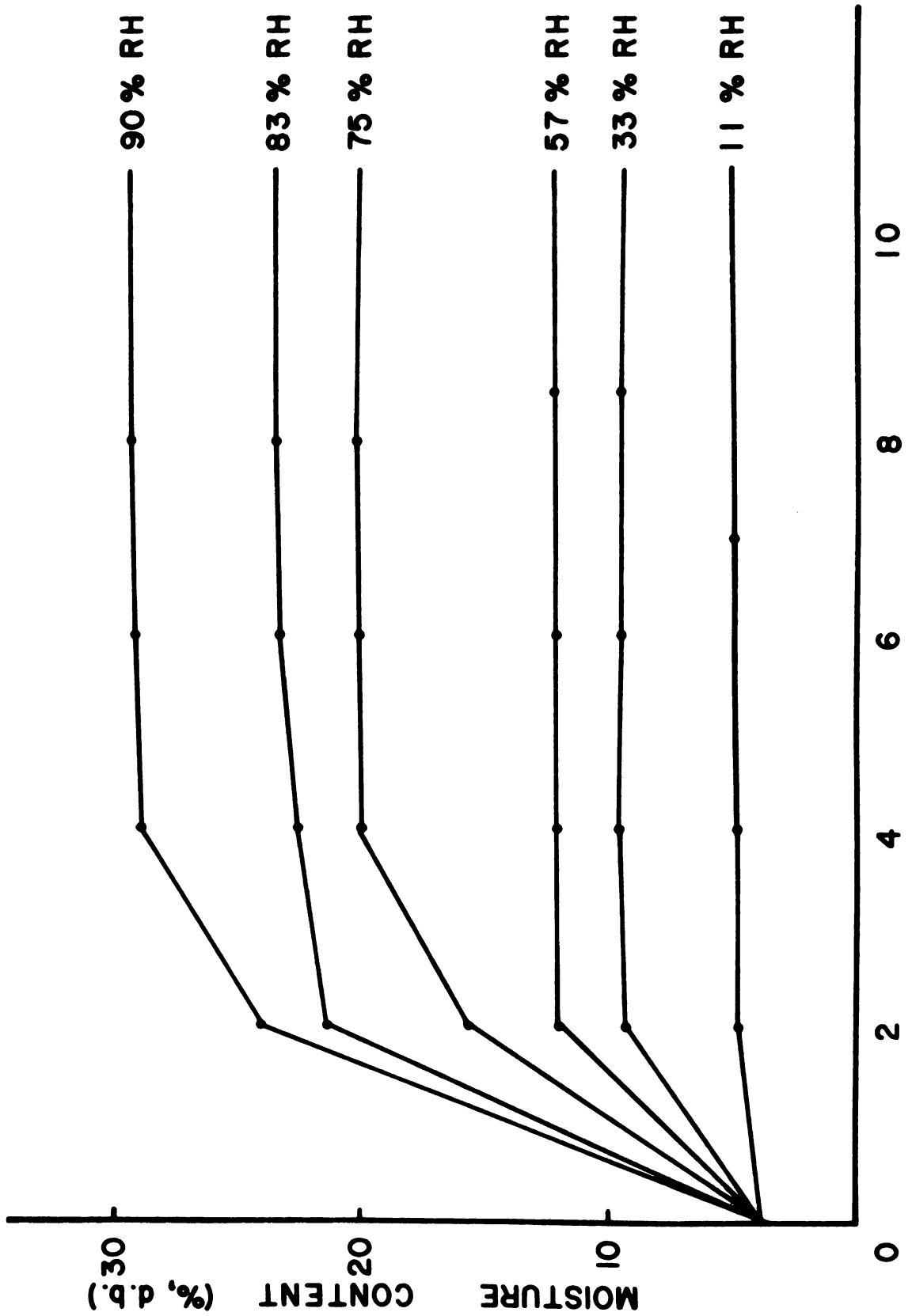

Figure 4. Water adsorption by casein at various relative humidities (25C).

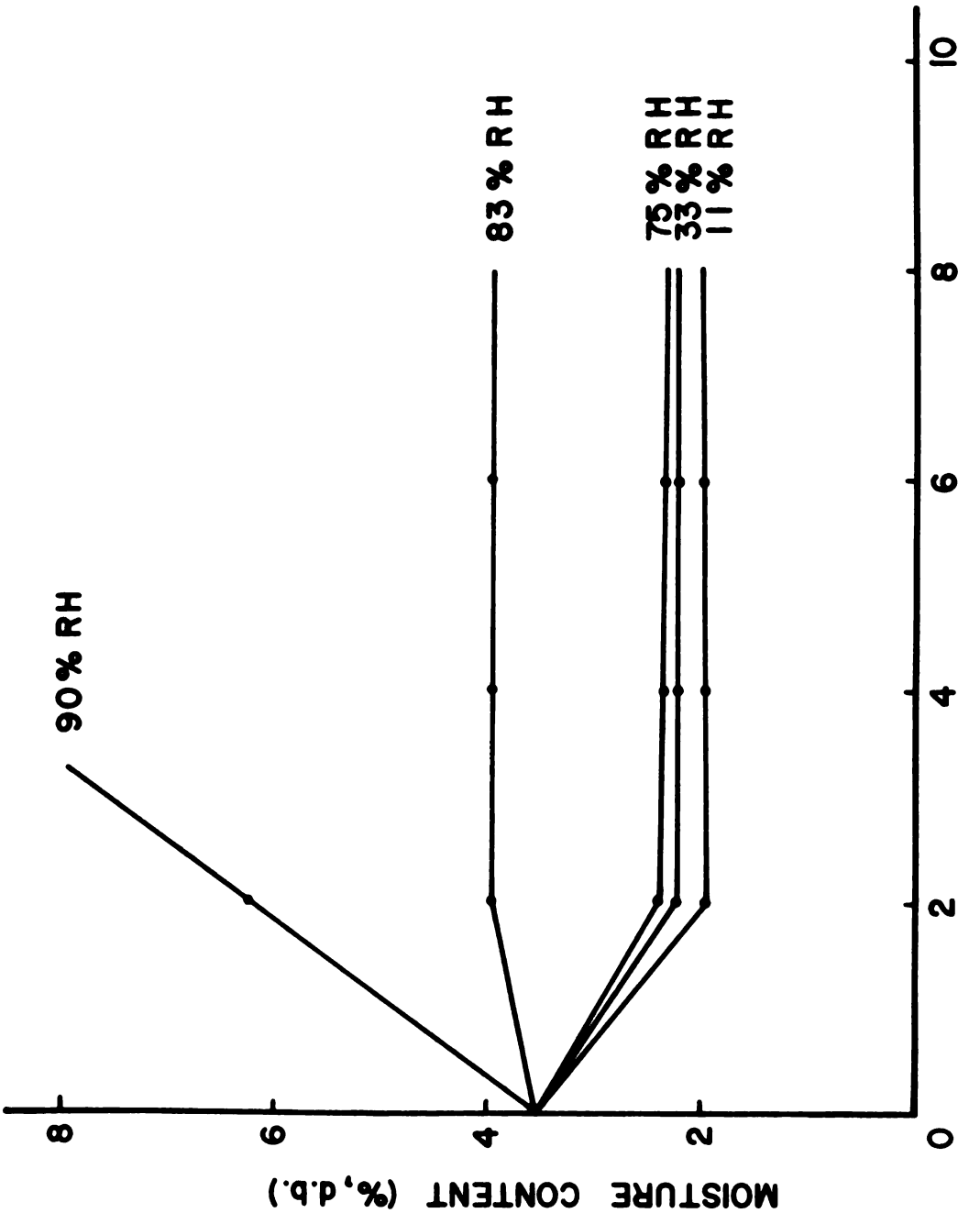

Figure 5. Water sorption by sucrose at various relative humidities (25C).

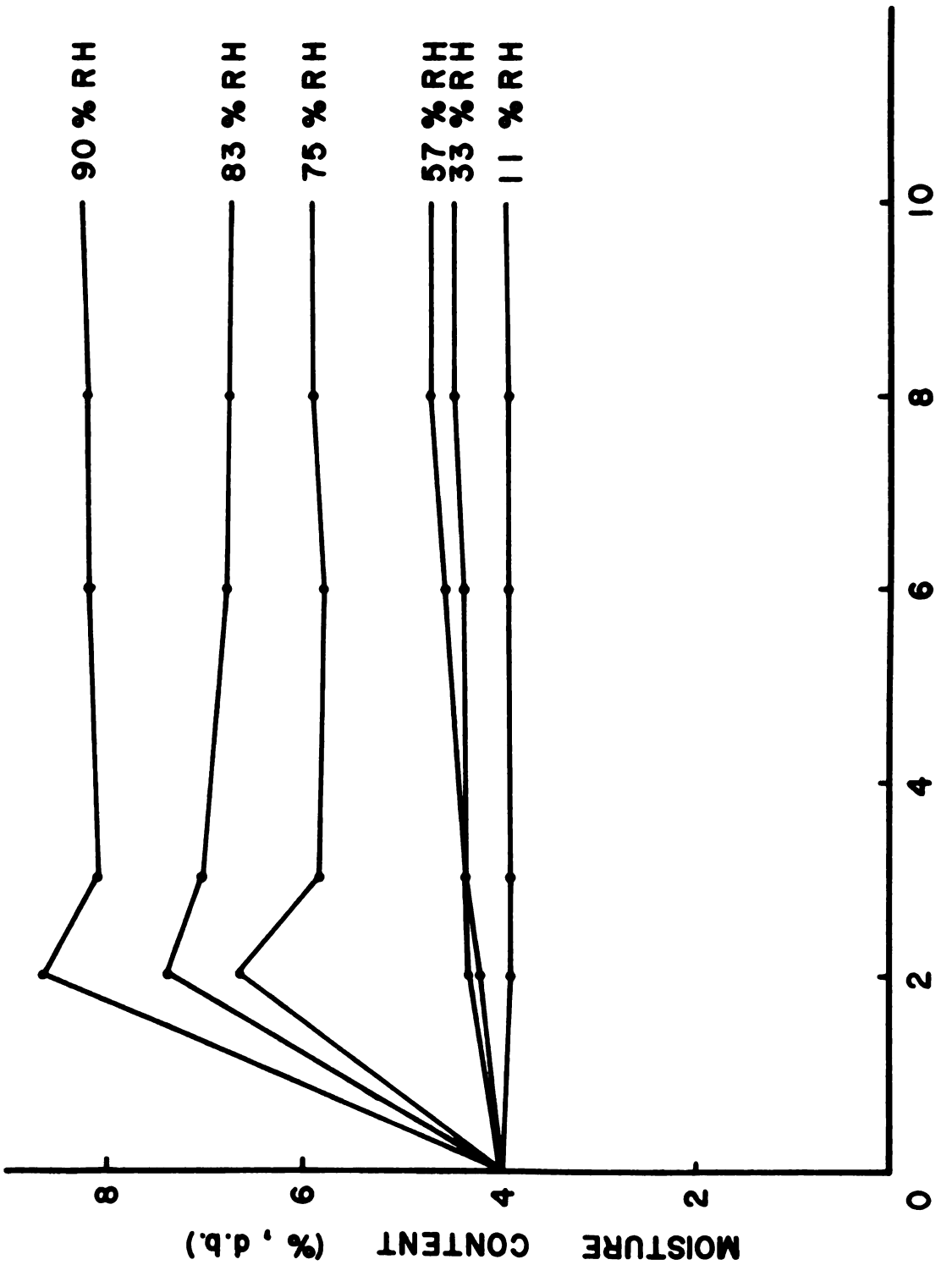

Figure 6. Water adsorption by freeze-dried cream at various relative humidities (25C).

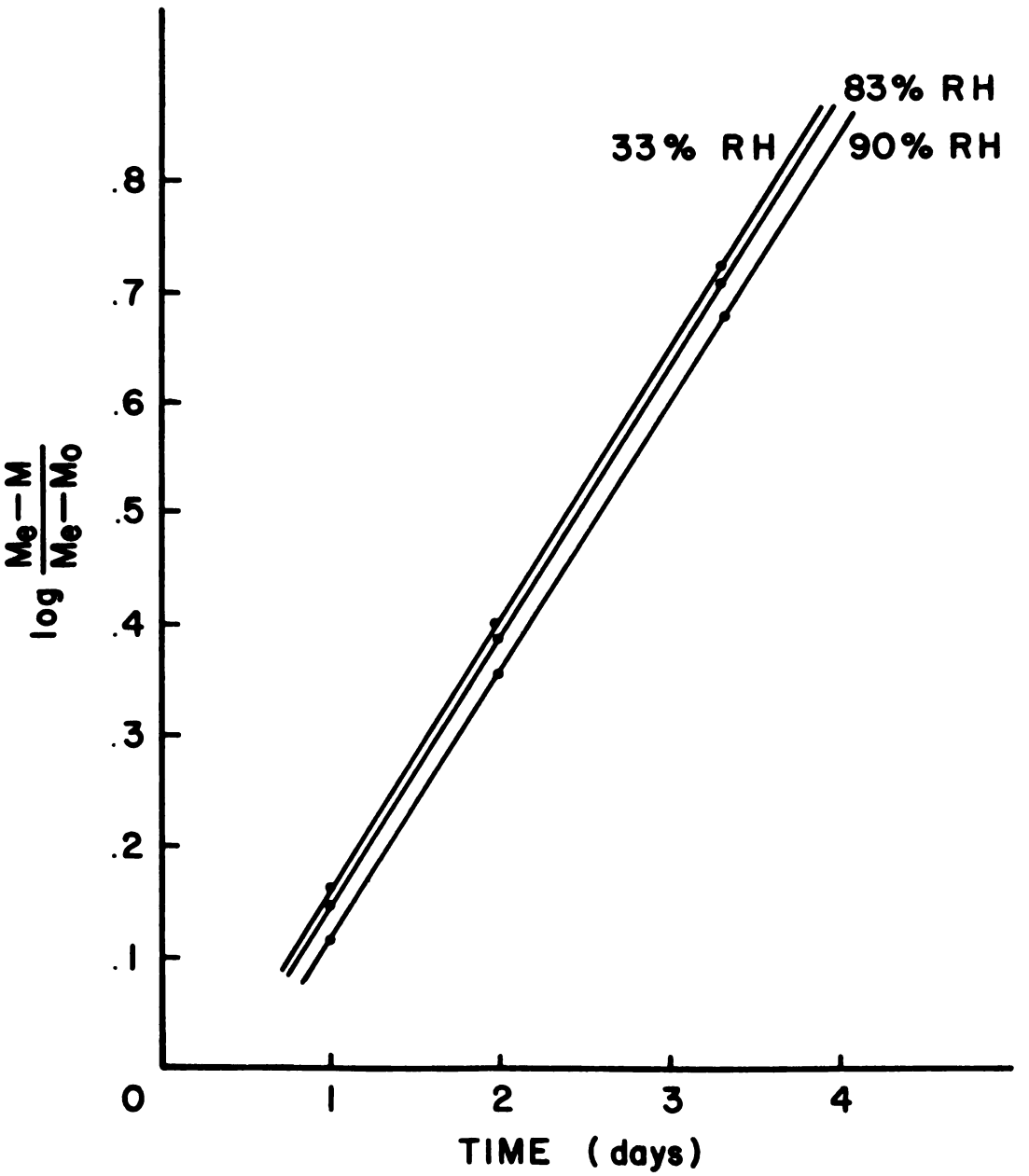

Figure 7. Water adsorption by NFDM at various relative humidities (25C) plotted with semilogarithmic coordinates.

moisture content (db) before exposure. The plots for NFDM in the relative humidities from 11% to 83% are superimposed upon one another, with the plot of the NFDM samples exposed in the 90% chamber being slightly removed from the other lines. From these graphs one could conclude that water adsorption by NFDM is independent of time and is solely a function of the product.

As can be seen from the graphs in Figure 4, the rates of water adsorption by casein are similar to those of NFDM. Most of the gained moisture is adsorbed by the fourth day at all relative humidities studied. The samples of casein at 11%, 33% and 57% RH adsorbed very close to maximum moisture content in two days, with little additional moisture being adsorbed from the second through the tenth days. The samples at 75%, 83% and 90% RH gained the majority of the total adsorbed moisture by the second day; however, a significant amount of moisture was adsorbed, at a decreased rate, from the second to the fifth day of exposure. The constant weight phase was attained by the samples of casein by the fifth day. A $\log \frac{M_e - M}{M_e - M_o}$, versus time plot for casein (Figure 8) shows no relation in the rates of water adsorption at various relative humidities. This would perhaps indicate an altering or rearrangement of the casein structure upon adsorption of large amounts of water. The fact that the plots of the casein exposed to the 11%, 33% and 57% RH are very similar would suggest an alteration of the adsorption capacity of casein during exposure at the higher relative humidities.

As mentioned previously, sucrose tended to lose moisture during exposure in the relative humidities, from 11% to 75%. At higher humidities above 83% rapid adsorption of moisture was observed,

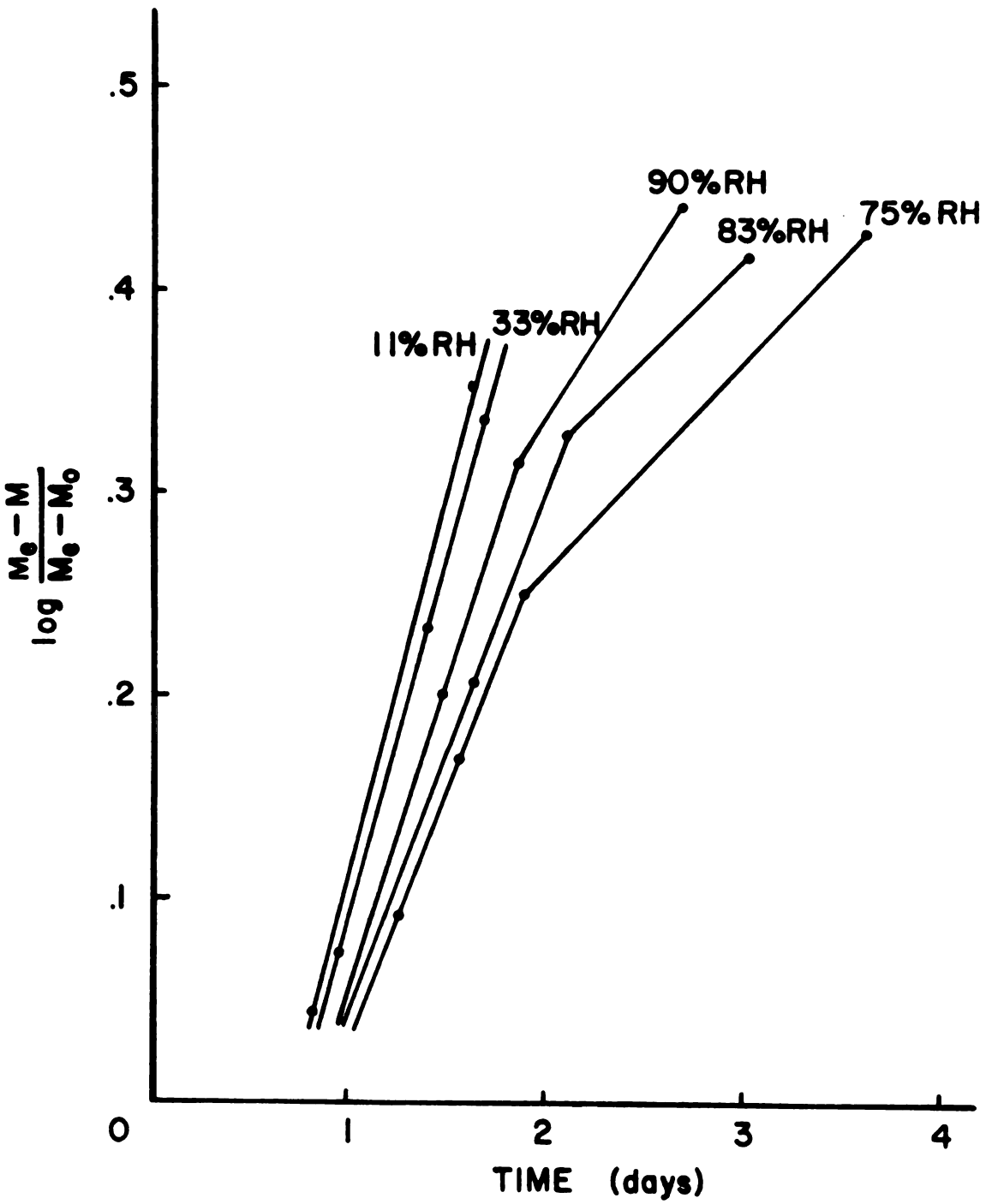

Figure 8. Water adsorption by casein at various relative humidities (25C) plotted with semilogarithmic coordinates.

leading to the formation of a saturated sucrose solution (Figure 5). Losses of moisture from the samples of sucrose occurred at relatively the same rate and to the same degree in 11%, 33%, 57% and 75% relative humidities. Maximum moisture loss was achieved in two to three days in those humidities. A slight gain in moisture by the sucrose samples was experienced in the 83% relative humidity chamber. In the extreme relative humidity of 90%, a rapid linear adsorption of moisture was observed, with the smaller crystals of the sucrose samples going into solution after only a few days of exposure.

As shown by the curves in Figure 6, the samples of freeze-dried cream adsorbed very little moisture during exposure at relative humidities less than 57%. In the atmosphere with 11% relative humidity the cream samples lost a small amount (approximately 0.1% (db)) of moisture. During exposure in atmospheres of 75% RH, and greater, the cream samples rapidly gained moisture for two days. After two days of exposure in 75+% RH, however, the samples of cream lost about 6.5% of the adsorbed moisture. The loss was rapid, occurring in a twenty-four hour period. In all samples of cream exposed to the various relative humidities, constant weight was not achieved until after about the eighth day of exposure. From these data it could be concluded that freeze-dried cream has a limited capacity to adsorb moisture from its environment. Further, at moderate to low relative humidities, freeze-dried cream adsorbs a small amount of moisture, only about 1% (db). The decrease in adsorbed moisture from samples held at the higher RH remains unexplained at this time.

Water Adsorption Isotherms of Selected Food Systems

Water adsorption isotherms for NFDM, casein, sucrose, and freeze-dried cream were obtained by plotting per cent moisture (db), calculated after the samples reached constant weight, versus the per cent RH. These isotherms are shown in Figures 10 through 13, and respective tabular data are presented in the Appendix.

As displayed by the isotherms in Figure 9, the four systems all exhibit characteristic sigmoid isotherms (Labuza, 1968). The isotherms for casein and NFDM are very similar, indicating that the casein fraction of NFDM plays a major role in determining the adsorptive properties of that product. The isotherms for sucrose and cream indicate that these products tend to adsorb relatively little moisture over the range of relative humidities examined. Definite and extended plateaus are seen in the isotherms for cream and sucrose. Had the sucrose samples been initially exposed in the relative humidities as anhydrous materials, it is believed that the isotherm would have commenced with a positive slope.

It is the opinion of many investigators (Salwin, 1959; Rockland, 1957; Stitt, 1958) that Area I (See Figure 1) or the first inflection point of the sigmoid isotherm represents the moisture content at which a product has greatest stability. This moisture content corresponds to the adsorption of a single monolayer of moisture. The monolayer of moisture has been theorized to protect the product from harmful degradative reactions. At moisture levels above this value, destructive chemical, enzymatic and microbial activity reactions are accelerated.

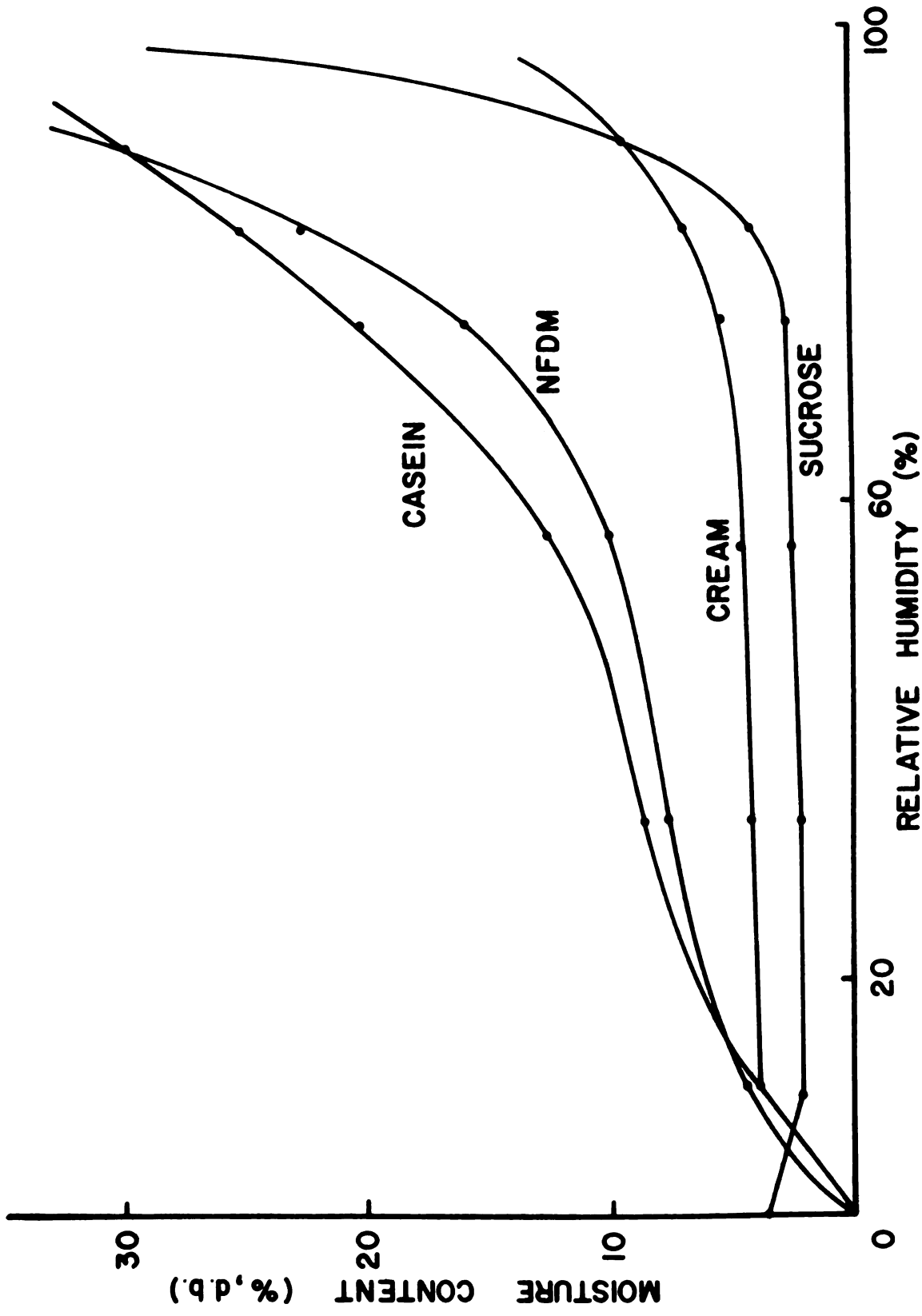

Figure 9. Water adsorption isotherms for casein, NFDM, cream and sucrose (25C).

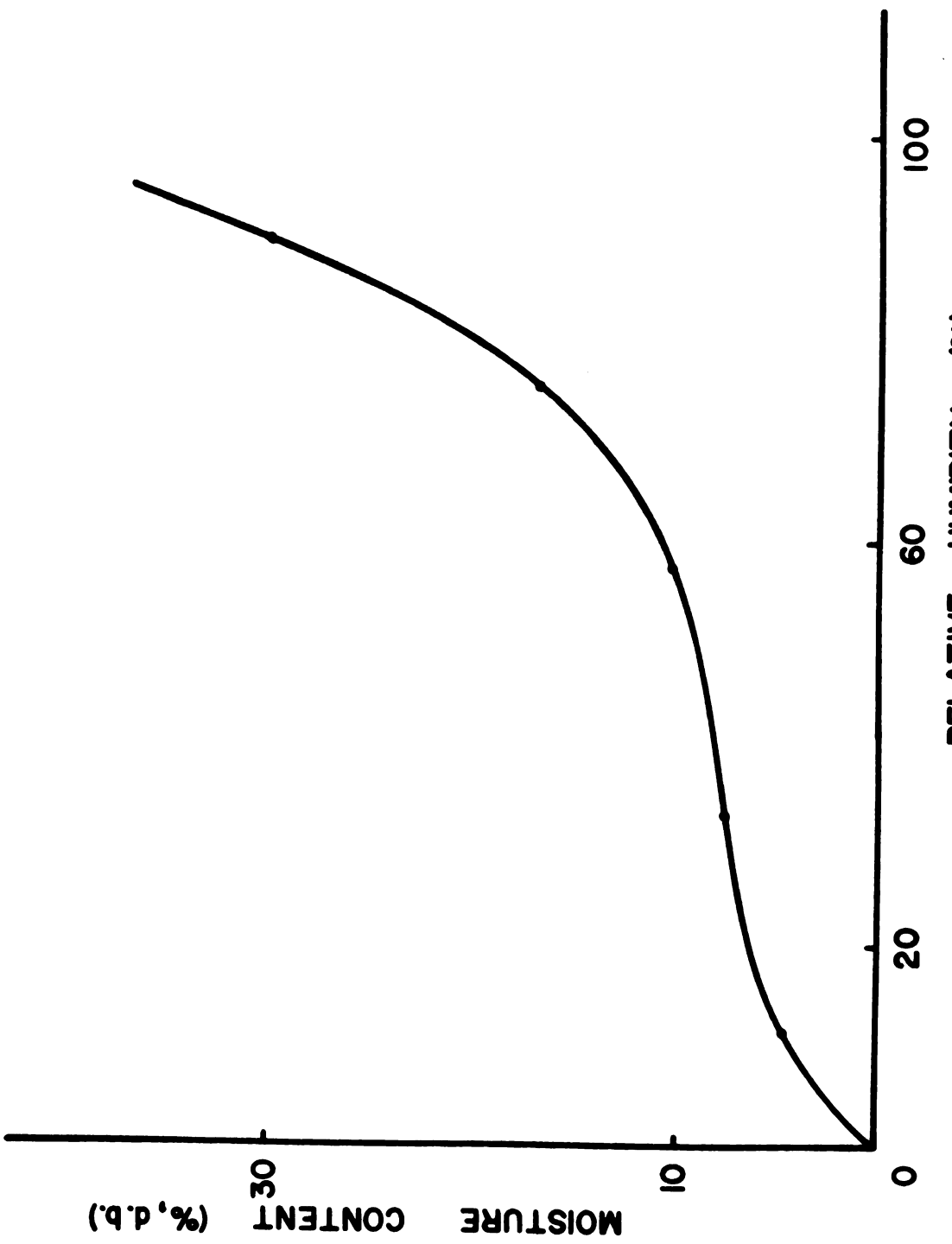

Figure 10. Water adsorption isotherm for NFDM (25C).

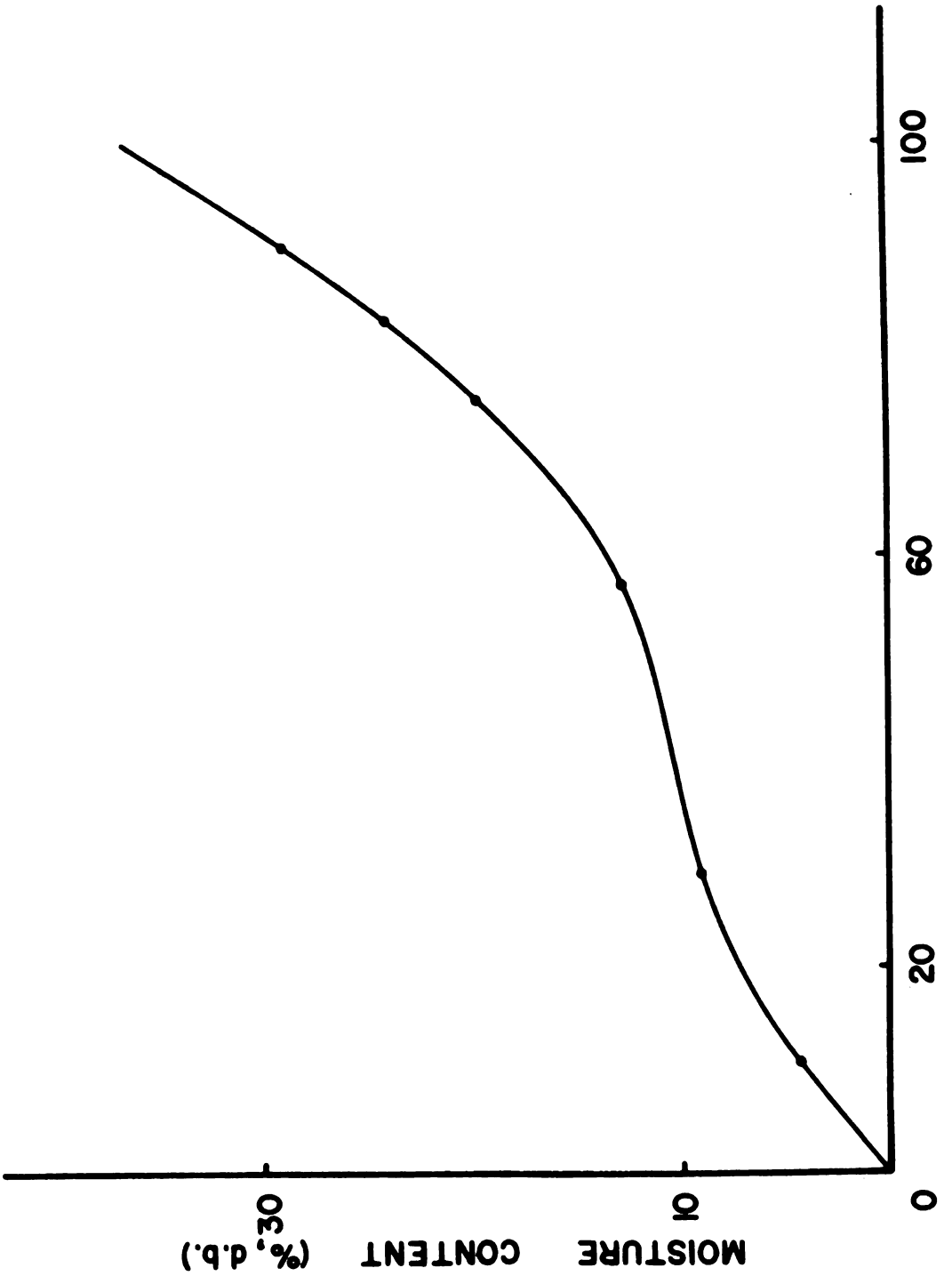

Figure 11. Water adsorption isotherm for casein (25C).

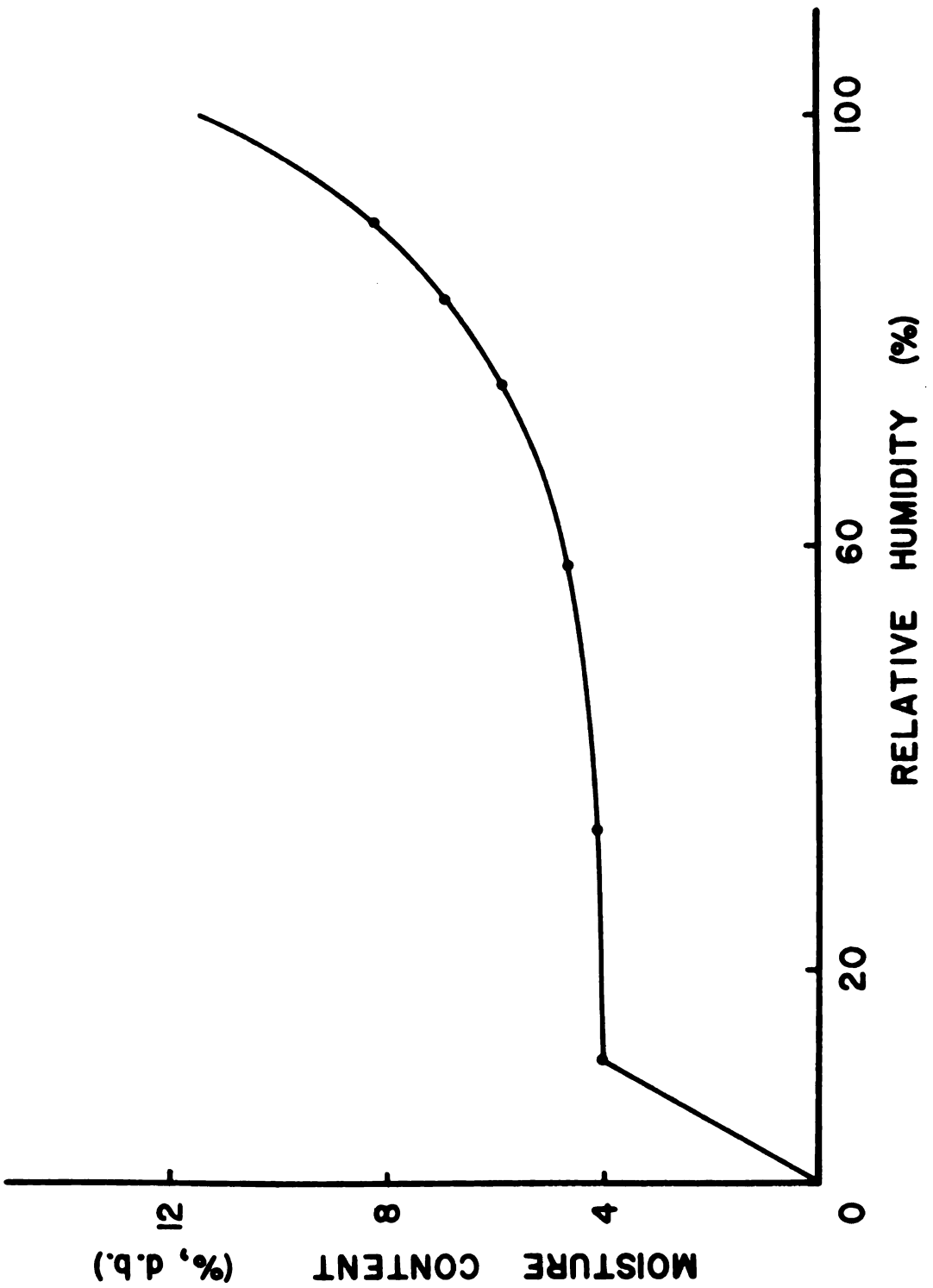

Figure 12. Water adsorption isotherm for FD cream (25C).

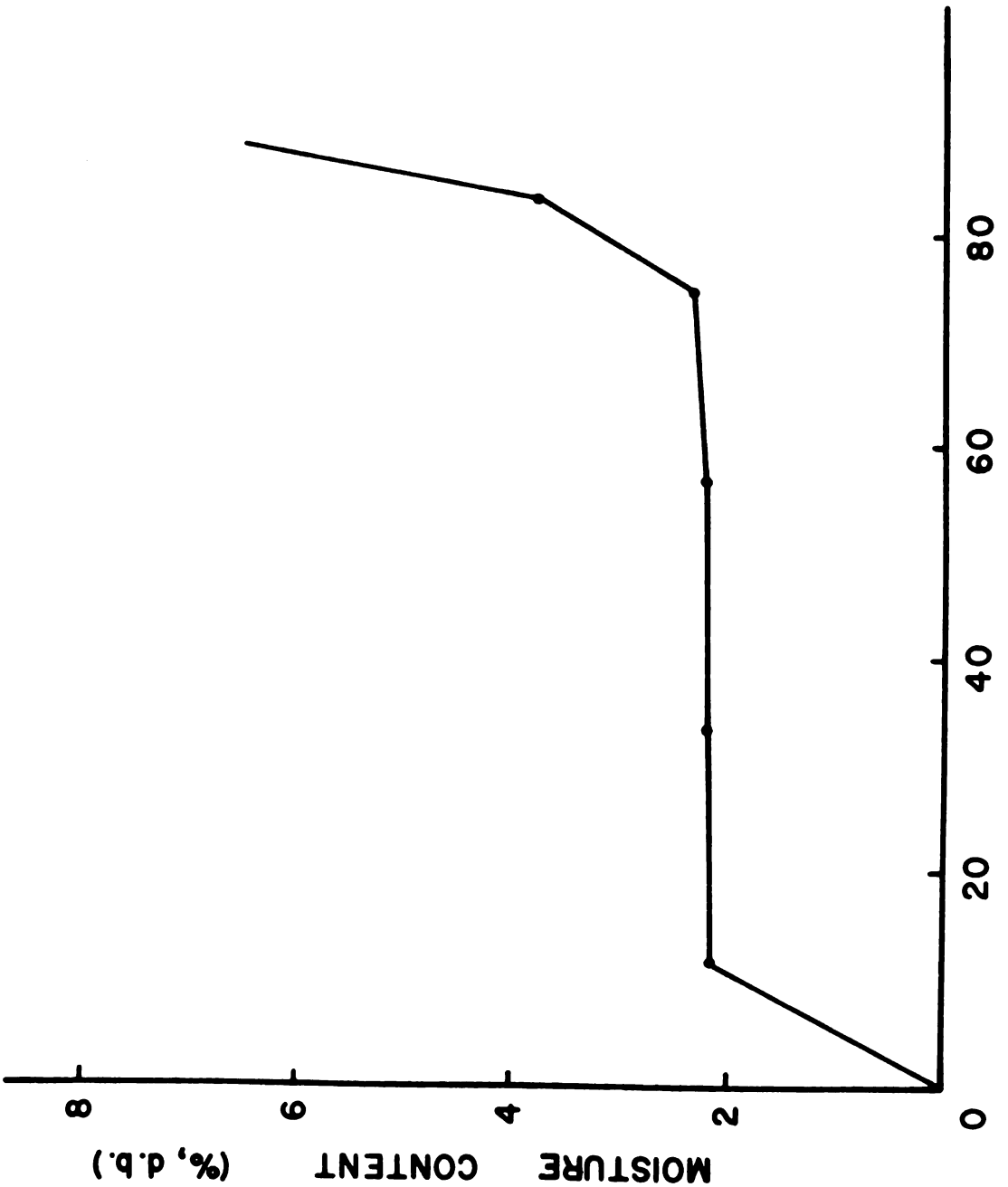

Figure 13. Water adsorption isotherm for sucrose (25C).

At moisture levels below the monolayer moisture value, certain reactions such as oxidation are accelerated (Acker, 1969; Block, 1953; Cuendet et al., 1954)

It would seem logical then that storage specifications for a particular product would tentatively be the same as those relative humidities corresponding to the monolayer moisture value. The monolayer moisture content would also be a good first target in dehydration when specific stability data are lacking (Salwin, 1963).

Both NFDM and casein (Figures 10 and 11) have monolayer moisture values corresponding to about 12% relative humidity and 6% moisture (db). Therefore, these products should be dried to at least 6% moisture (db) and exposed to a maximum relative humidity of 12% during storage for maximum stability.

Also of interest is Area II (See Figure 1) of the isotherm, or the portion between the first and second inflection points of the curve. In this area, hysteresis is most pronounced and the adsorbed water is highly mobile (Salwin, 1959). Area II of the NFDM and casein isotherms corresponds to those relative humidities between 12% and 57%.

Freeze-dried cream (Figure 12) also exhibits a monolayer moisture value corresponding to about 12%. However, the respective moisture content is much lower than either NFDM or casein, being around 4% (db). Very little water is adsorbed in Area II as indicated by the isotherm of freeze-dried cream suggesting that other factors, more important than the presence of moisture may influence the stability of the product.

From the isotherm of sucrose (Figure 13) a monolayer moisture content of approximately 2% db can be observed. This corresponds to a

relative humidity of 10%. There was virtually no difference in the final moisture content for sucrose exposed in relative humidities from 11% to 75%. This would indicate that at relative humidities less than 75%, the monolayer moisture value is approximated by sucrose and significant stability due to its limited adsorptive capacity is attained. At relative humidities greater than 75%, sucrose tends to rapidly adsorb moisture, approaching a saturated solution.

BET Analysis of Water Adsorption Data

The BET theory is most often applied when dealing with foodstuffs because it enables prediction of the monolayer moisture value and the determination of important thermodynamic parameters.

From a plot of a/(1-a)m versus a, where a is the relative humidity and m is the moisture fraction, values for the following can be determined:

(a) the monolayer moisture content,

(b) the net binding energy of the monolayer,

(c) the total binding energy, and

(d) the surface area per gram of solid material.

(Macmillon and Teller, 1951 and Labuza, 1968)

The BET plots for NFDM, casein, cream, and sucrose are presented in Figures 14 through 17, with respective tabular data included in the Appendix.

For relative humidities below 50%, the graph of a/(1-a)m versus a, usually produces a straight line. From the y-intercept and slope of this line the monolayer coverage value can be calculated from the

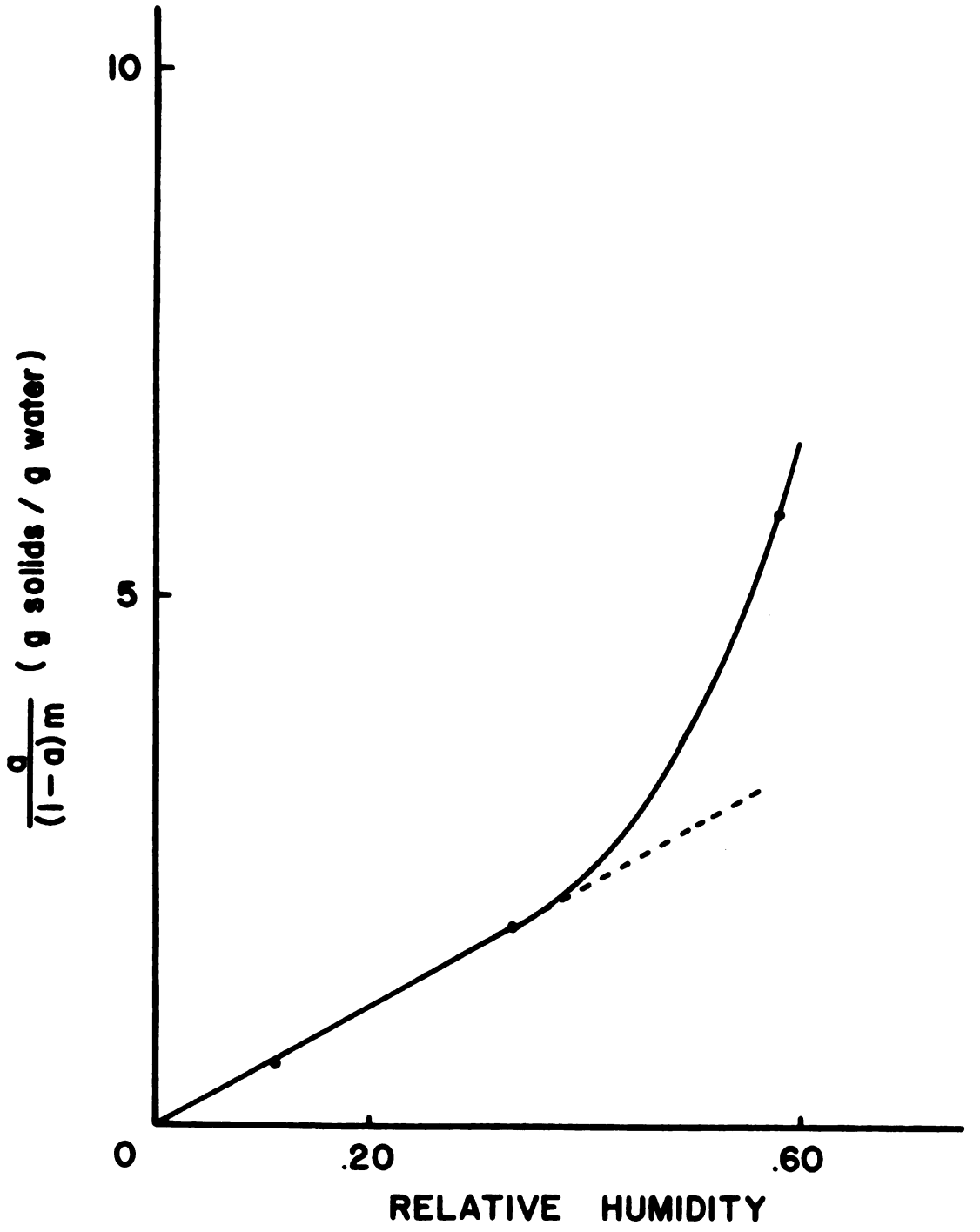

Figure 14. BET plot for sucrose (25C).

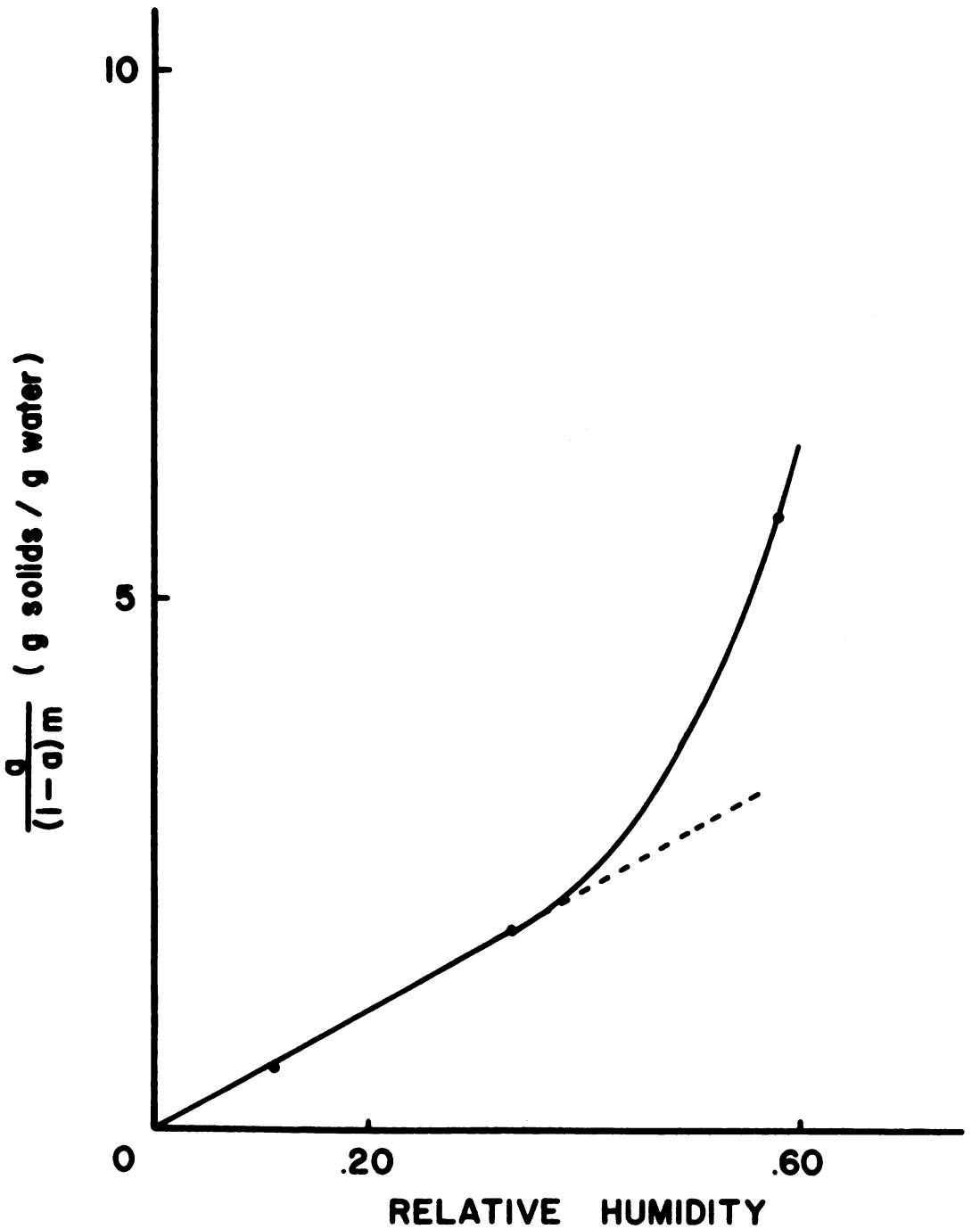

Figure 14. BET plot for sucrose (25C).

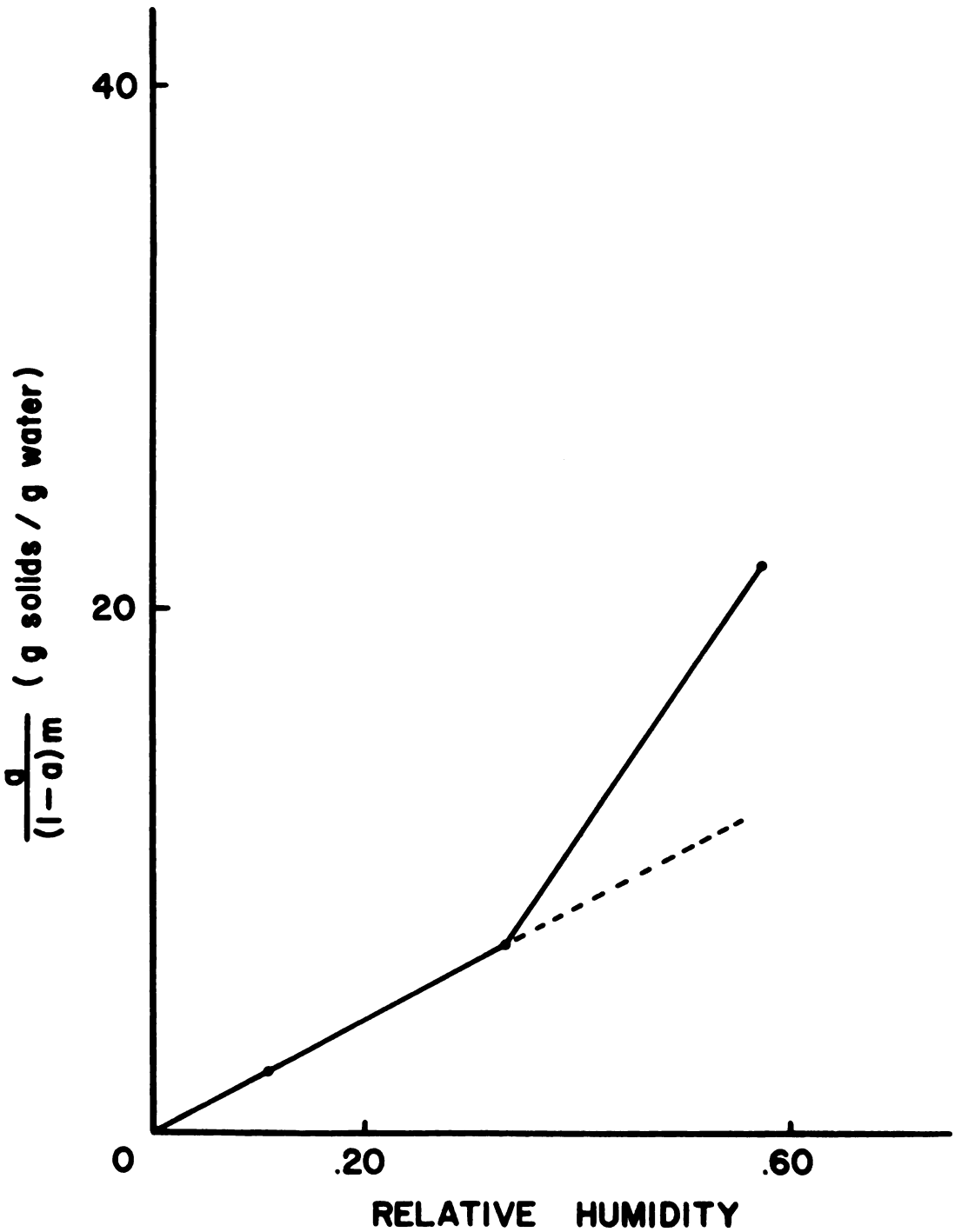

Figure 15. BET plot for FD cream (25C).

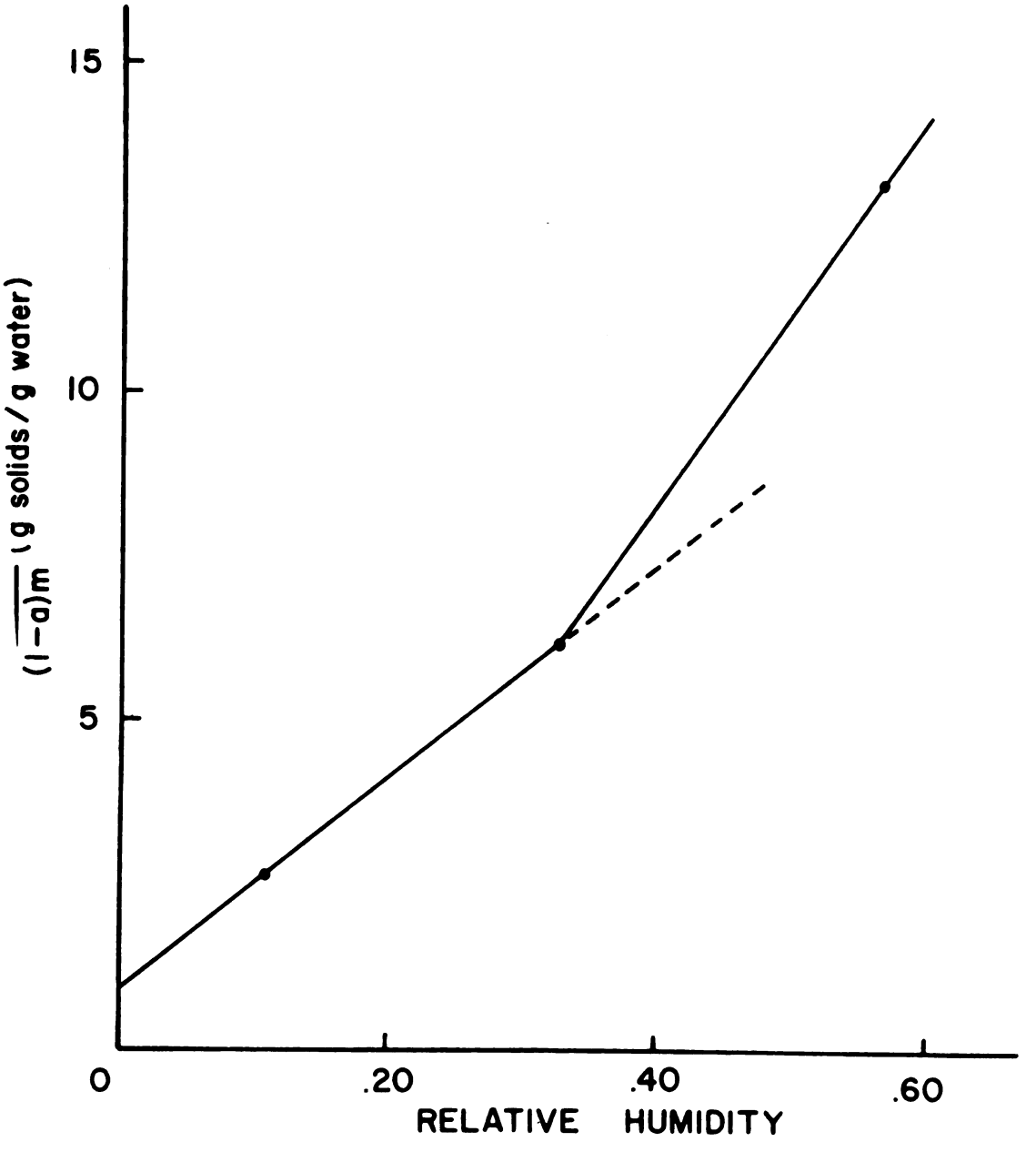

Figure 16. BET plot for NFDM (25C).

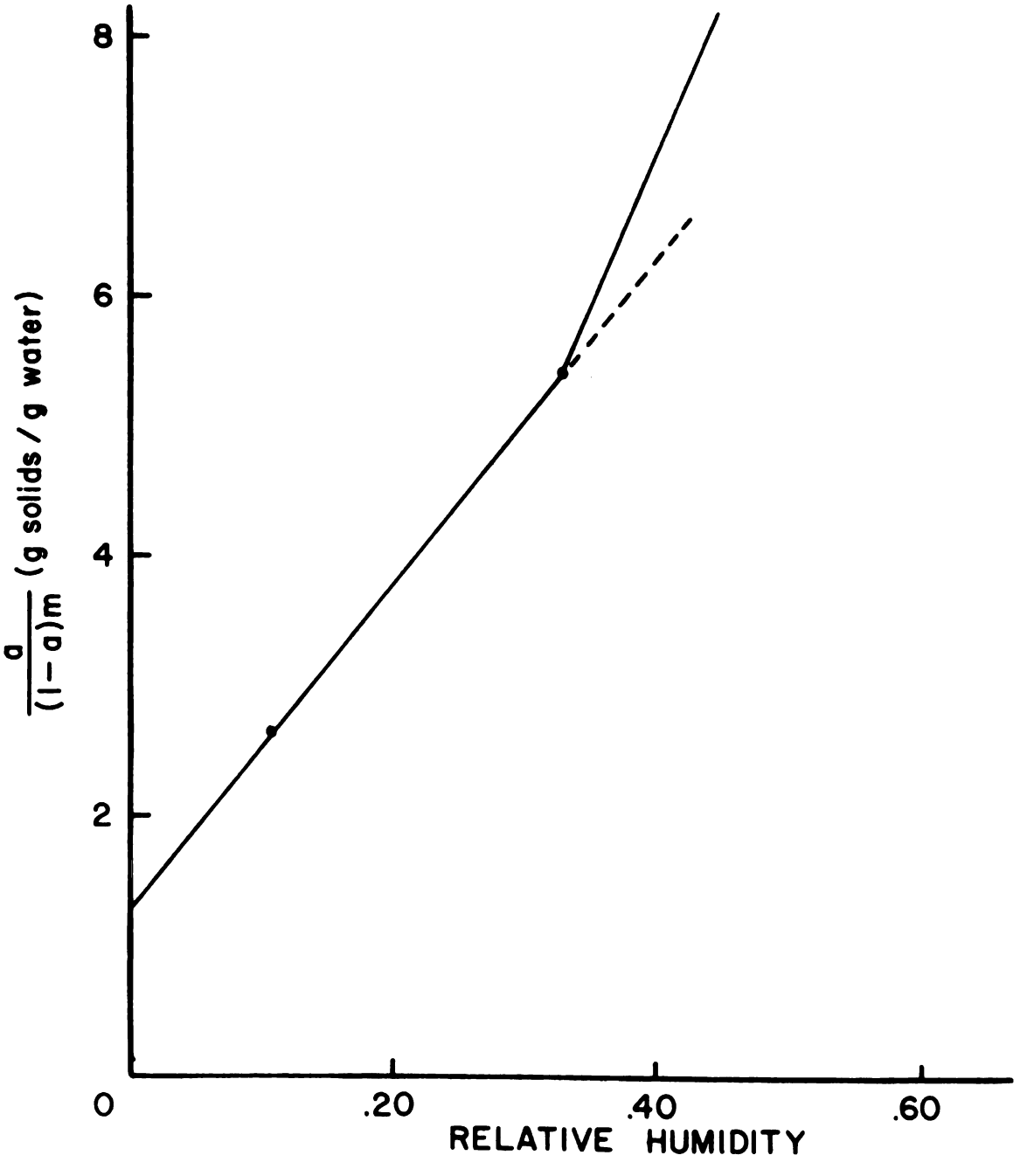

Figure 17. BET plot for casein (25C).

following equation:

$$a/(1-a)m = 1/V_m C + [a(C-1)]/V_m C,$$

where V_m is the monolayer moisture value and C is a thermodynamic constant (Chung and Pfost, 1967b; Rockland, 1969).

The constant C is equal to

$$C = k \exp (Q_s/RT),$$

where Q_s is the net binding energy of the monolayer moisture. (Labuza, 1968)

The heat of adsorption, Q_s, can then be broken down into its component parts thusly,

$$Q_s = E_1 - H_v \ (25C),$$

where E_1 is the total binding energy and H_v is the heat of vaporization of water (Dole and McLaren, 1946).

In addition to the thermodynamic data, the surface area, S_o, in $(\text{meter})^2$/gram of solid can be found by the following equation:

$$S_o = V_m \cdot 1/(M_{H_2O}) \cdot N_o \cdot A_{H_2O} = 3.5 \times 10^3 V_m,$$

where V_m is the calculated monolayer value, M_{H_2O} is the molecular weight of water, N_o is Avagadro's number, and A_{H_2O} is the area of a water molecule = $10.6 \times 10^{-20} m^2$ (Labuza, 1968).

From examination of the BET plots for sucrose and cream, one can see that the linear portion intercepts the y-axis at the origin giving a value for $1/V_m C$ of zero. While the linear portion appears to

intercept at the origin, the lack of graphical precision can only allow the conclusion that the value for $1/V_mC$ is, indeed, very small and approaching zero. It would seem unlikely that either the monolayer moisture or the heat of binding for either cream or sucrose would equal zero. As demonstrated previously, the monolayer moistures (db) for cream and sucrose were quite small. It is therefore quite possible that a low monolayer moisture value, perhaps coupled with a low heat of adsorption (which is what the low adsorptive capacities of sucrose and cream might indicate) would yield a value for $1/V_mC$ approaching zero.

Considering this, it is very difficult to subject the data for cream or sucrose to a BET treatment with any hope of reliability. Therefore, only the data for NFDM and casein will receive further BET analysis.

As can be seen from the graphs in Figures 16 and 17 the linear portions of the BET plots for NFDM and casein intersect the y-axis above the origin, giving positive values for $1/V_mC$. The values for the BET constants as well as derived values for Q_s and E_1, and S_o for NFDM and casein are presented in Table 10.

As can be seen from the data in Table 10, the values for the monolayer moistures of NFDM and casein are rather similar. When comparing these values, it must be remembered that V_m is expressed on a per gram of solids basis, which is related to the surface areas of the two products.

From the examination of the S_o column of Table 10, it can be observed that the surface area of the casein is 12 times that of

Table 10. BET constants and derived values for NFDM and casein.

Product	V_m (g/g solid)	C (ln Q_s/RT)	Q_s (Cal/mol)	E_1 (Cal/mol)	S_o (m^2/g)
NFDM	0.060	18.35	1,700	12,200	210
Casein	0.072	11.22	1,400	12,000	252

NFDM. Therefore, if the monolayer moisture values for NFDM and casein were recalculated on an equal surface area basis, they would be equal. Without further data as to the influence of the various other components of NFDM on its adsorptive capacity and stability, definite conclusions are difficult. It appears, however, that the casein fraction of NFDM plays a very major role in determining the stability of that product during exposure in various relative humidities.

The very similar values of Q_s and E_1 for NFDM and casein further support the importance of the casein fraction. Within the accuracy of this experiment, the values for the total moisture binding energy of the two products can virtually be regarded as equal. The very low adsorptive capacity of lactose, the other major component of NFDM, would indicate that it has little influence on the stability of NFDM.

Desorption of Diacetyl from Selected Food Systems

The losses of diacetyl from NFDM, casein, sucrose and cream are displayed graphically in Figure 18. Respective tabular data are presented in the Appendix.

Samples of the freeze-dried food systems were exposed in various relative humidities until they attained constant weight. The samples were then removed from the humidity chambers and the loss, if any, of diacetyl from the product was determined.

As can be seen from the graphs in Figure 18, a curvilinear relation is exhibited by NFDM, casein, and sucrose. The loss of diacetyl from cream was linear with increasing relative humidity.

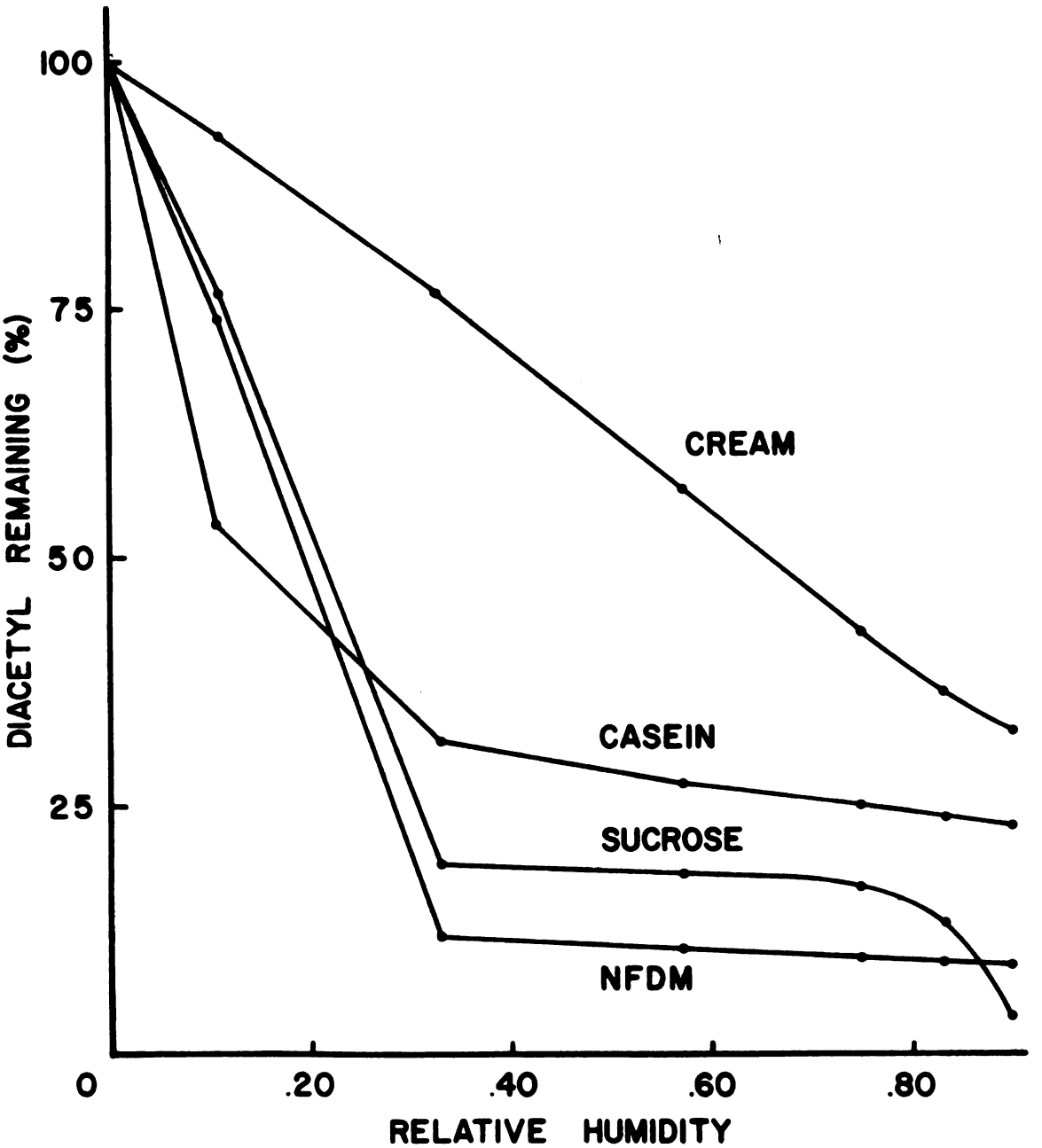

Figure 18. Loss of diacetyl from NFDM, casein, cream and sucrose during equilibration at various RH.

The three curvilinear graphs are composed of a linear slope, where the loss of diacetyl from NFDM, casein, and sucrose was quite rapid with an increase in relative humidity; and a plateau, where the loss of diacetyl was rather constant with increasing humidities.

In NFDM, casein, and sucrose, the plateau phase or attainment of maximum diacetyl loss, was reached around 35% relative humidity. Casein continued to lose small amounts of diacetyl with increasing relative humidities, while sucrose lost virtually all of the added diacetyl in relative humidities greater than 80% due to the formation of a saturated solution.

Maximum loss of diacetyl in 90% relative humidity varied from 70% by the samples of freeze-dried cream to virtually 100% in the sucrose samples.

One may question whether the ability of a food product to adsorb moisture is related to the desorption of diacetyl from that product. This supposition is supported by the data for cream, NFDM and casein. The samples of cream adsorbed little moisture and lost relatively little diacetyl. NFDM and casein adsorbed a substantial amount of moisture and experienced a substantial loss of diacetyl. The data for sucrose, however, would tend to refute this supposition as that product adsorbed little moisture and lost a considerable amount of the added diacetyl.

These data would indicate that for 75% retention of added diacetyl in products such as NFDM, casein, or sucrose, storage conditions should be no greater than 10% relative humidity. However, in high fat products such as the samples of freeze-dried cream, a comparable retention of diacetyl can be obtained at much higher relative humidities,

perhaps as high as 40%. These high humidities, however, would seldom be desired as they are much greater than those indicated from monolayer moisture values.

Effect of Prolonged Exposure in Various Relative Humidities

As a supplementary experiment, four accurately weighed samples of NFDM were exposed in each of three selected relative humidities for a prolonged period of time.

The samples were allowed to reach moisture equilibrium for a period of seven days. One sample from each relative humidity environment was analyzed for diacetyl at the end of 1, 2, 3, and 4 weeks, following moisture equilibrium.

The results of this investigation are included in Table 11. As can be seen from the data, prolonged exposure did not cause further significant loss of diacetyl from the samples of NFDM.

Table 11. Loss of diacetyl from NFDM during prolonged exposure in selected relative humidities (25C).

Time After Moisture Equilibration (days)	Amount of Diacetyl (ppm)		
	11% RH	57% RH	90% RH
0	5.70	0.85	0.70
7	5.70	0.80	0.70
14	5.60	0.80	0.70
21	5.60	0.75	0.70
28	5.60	0.75	0.70

SUMMARY AND CONCLUSIONS

From this study it has been found that the chemical nature of a food system greatly determines its particular water adsorptive capacities. The results would further indicate that the protein fraction of a composite food system such as NFDM greatly influences the ability of NFDM to adsorb water while the influence of the carbohydrate or lipid portion is relatively slight.

In products which were largely protein, carbohydrate, or fat in nature, as well as in a composite food, it was found that moisture equilibrium in any relative humidity was established within seven days. In all food systems examined, a monomolecular layer of moisture was adsorbed at approximately 12% equilibrium relative humidity. This moisture monolayer corresponded to a total product moisture content of ca. 6%. These data would then indicate the approximate storage relative humidity and product moisture content for maximum stability.

In all food systems examined except sucrose, rates of water adsorption were generally very rapid in relative humidities less than 57%, with moisture equilibrium in the higher humidities taking several days longer to be established. Sucrose tended to lose moisture in humidities less than 83% and at higher humidities the crystals of sucrose tended to dissolve and eventually, to form a saturated solution. It was found that with NFDM the rates of water adsorption were a function

of a product and not related to time. This was not the case, however, with the other food systems examined, as the rates of adsorptions generally differed within each relative humidity.

From a BET analysis of the selected food systems, it appears that both freeze-dried cream and sucrose have very high heats of sorption and low values for the monolayer moisture content. The monolayer moisture values for casein and NFDM, calculated on an equal surface area basis, were found to be equal, further indicating that casein largely determines the adsorptive capacity, and, perhaps, the storage stability of NFDM.

No relation was found between the water adsorptive capacity of a food system and the ability of that product to bind the flavor compound diacetyl. The data would indicate that, in products high in protein or carbohydrates, the relative humidity during storage ought to be maintained at less than 10% RH for at least 75% retention of diacetyl. In products high in fat, however, the relative humidity may reach as high as 40% RH with only slight loss of diacetyl.

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APPENDIX

Table 12. Moisture content (db) of NFDM during exposure in selected relative humidities (25C).

Relative Humidity (%)	Time (days) 1	5	7	10
11	4.27	4.54	4.68	4.68
33	4.28	8.05	8.07	8.07
57	4.27	9.83	10.01	10.01
75	4.28	16.13	15.82	15.88
83	4.27	18.93	18.69	18.97
90	4.28	30.18	30.01	30.65

Table 13. Moisture content (db) of casein during exposure in selected relative humidities (25C).

Relative Humidity (%)	Time (days) 1	2	4	6	7	8
11	3.84	4.68	4.68	4.68	4.68	4.68
33	3.84	9.07	9.09	9.09	9.10	9.10
57	3.84	11.83	11.90	11.90	11.90	11.98
75	3.84	15.63	19.99	19.95	19.96	19.98
83	3.84	21.65	22.72	23.07	23.21	23.21
90	3.84	24.19	28.95	29.83	29.56	29.56

Table 14. Moisture content (db) of cream during exposure in selected relative humidities (25C).

Relative Humidity (%)	Time (days) 1	2	3	6	8	10
11	3.94	3.81	3.82	3.86	3.87	3.87
33	3.95	4.22	4.30	4.29	4.33	4.33
57	3.95	4.14	4.14	4.41	4.61	4.60
75	3.95	6.57	5.90	5.75	5.84	5.80
83	3.95	7.37	7.01	6.70	6.73	6.73
90	3.95	8.61	8.12	8.31	8.24	8.26

Table 15. Moisture content (db) of sucrose during exposure in selected relative humidities (25C).

Relative Humidity (%)	Time (days) 1	2	3	5	7
11	3.50	2.07	2.07	2.07	2.08
33	3.49	2.26	2.26	2.24	2.24
57	3.50	2.24	2.26	2.26	2.26
75	3.50	2.27	2.30	2.30	2.30
83	3.50	3.91	3.92	3.92	3.92
90	3.50	6.25	7.29	9.86	---

Table 16. Determination of BET plot for cream.

Relative Humidity (%)	Moisture Content m(db)	$\frac{a}{(1-a)m}$ (g solids/g water)
11	0.236	0.52
33	0.255	1.93
57	0.271	4.89
75	0.346	8.67
83	0.396	12.33
90	0.487	18.48

Table 17. Determination of BET plot for sucrose.

Relative Humidity (%)	Moisture Content m(db)	$\frac{a}{(1-a)m}$ (g solid/g water)
11	2.08	5.94
33	2.24	22.00
57	2.26	58.65
75	2.30	130.43
83	3.92	125.00
90	---	---

Table 18. Determination of BET plot for NFDM.

Relative Humidity (%)	Moisture Content m(db)	$\frac{a}{(1-a)m}$ (g solid/g water)
11	0.047	2.64
33	0.081	6.10
57	0.100	13.20
75	1.588	18.94
83	1.897	25.77
90	3.065	29.36

Table 19. Determination of BET plot for casein.

Relative Humidity (%)	Moisture Content m(db)	$\frac{a}{(1-a)m}$ (g solid/g water)
11	0.047	2.64
33	0.091	5.42
57	0.120	11.05
75	0.200	15.00
83	0.232	21.01
90	0.296	30.41

Table 20. Desorption of diacetyl from NFDM (25C).

Relative Humidity (%)		Amount of Diacetyl After Equilibration (ppm)	Amount of Diacetyl Desorbed (ppm)	Amount of Diacetyl Lost (%)	Mean
11	1)	5.70	2.00	26.0	
	2)	5.60	2.10	27.4	26
	3)	5.70	2.00	26.0	
	4)	5.90	1.80	24.1	
33	1)	0.85	6.85	89.0	
	2)	0.90	6.70	87.0	88
	3)	---	---	---	
	4)	0.98	6.72	87.3	
57	1)	0.85	6.85	89.0	89
75	1)	0.80	6.90	89.5	
	2)	0.75	6.95	90.3	90
	3)	0.80	6.90	89.5	
	4)	0.70	7.00	91.0	
83	1)	0.70	7.00	91.0	
	2)	0.75	6.95	90.3	90
	3)	0.70	7.00	91.0	
	4)	0.80	6.90	89.5	
90	1)	0.75	6.95	90.3	
	2)	0.70	7.00	91.0	90
	3)	0.70	7.00	91.0	
	4)	0.75	6.95	90.3	

Table 21. Desorption of diacetyl from casein (25C).

Relative Humidity (%)	Amount of Diacetyl After Equilibration (ppm)	Amount of Diacetyl Desorbed (ppm)	Amount of Diacetyl Lost (%)	Mean
11	1) 6.21	5.09	45.0	
	2) 5.85	5.45	48.2	47
	3) 5.94	5.36	47.4	
33	1) 3.50	7.80	69.0	
	2) 3.65	7.65	67.7	69
	3) 3.48	7.82	99.2	
57	1) 3.06	8.24	72.9	73
75	1) 2.85	8.45	74.8	
	2) 2.79	8.51	75.3	75
	3) 2.80	8.50	75.2	
83	1) 2.65	8.65	76.5	
	2) 2.59	8.71	77.0	77
	3) 2.58	8.72	77.1	
90	1) 2.60	8.70	76.9	
	2) 2.44	8.86	78.4	77
	3) 2.63	8.67	76.7	

Table 22. Desorption of diacetyl from sucrose (25C).

Relative Humidity (%)		Amount of Diacetyl After Equilibration (ppm)	Amount of Diacetyl Desorbed (ppm)	Amount of Diacetyl Lost (%)	Mean
11	1)	4.10	1.03	20.07	
	2)	3.85	1.28	24.95	23
	3)	3.90	1.23	23.97	
33	1)	1.05	4.08	79.53	
	2)	0.98	4.15	80.90	80
	3)	0.99	4.14	80.70	
57	1)	0.92	4.21	82.10	82
75	1)	0.87	4.26	83.04	
	2)	0.90	4.23	82.45	83
	3)	0.86	4.27	83.23	
83	1)	0.65	4.48	87.33	
	2)	0.67	4.46	86.94	87
	3)	0.68	4.45	86.74	
90	1)	0.32	4.81	93.76	
	2)	0.29	4.84	94.35	95
	3)	0.20	4.93	96.10	

Table 23. Desorption of diacetyl from cream (25C).

Relative Humidity (%)	Amount of Diacetyl After Equilibration (ppm)	Amount of Diacetyl Desorbed (ppm)	Amount of Diacetyl Lost (%)	Mean
11	1) 5.85	0.40	6.40	
	2) 5.92	0.33	5.28	6
	3) 5.80	0.45	7.20	
33	1) 4.78	1.47	23.52	
	2) 4.91	1.34	21.44	22
	3) 4.85	1.40	22.40	
57	1) 3.59	2.66	42.60	43
75	1) 2.69	3.56	56.96	
	2) 2.73	3.52	56.32	57
	3) 2.70	3.55	56.80	
83	1) 2.21	4.04	64.64	
	2) 2.19	4.06	64.96	65
	3) 2.22	4.03	64.48	
90	1) 2.07	4.18	66.88	
	2) 2.15	4.10	65.60	66
	3) 2.06	4.19	67.04	