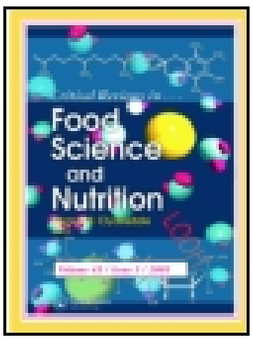

Critical Reviews in Food Science & Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Water activity, water glass dynamics, and the control of microbiological growth in foods

Jorge Chirife , María del Pilar Buera & Dr. Theordore P. Labuza

To cite this article: Jorge Chirife , María del Pilar Buera & Dr. Theordore P. Labuza (1996) Water activity, water glass dynamics, and the control of microbiological growth in foods, Critical Reviews in Food Science & Nutrition, 36:5, 465-513, DOI: 10.1080/10408399609527736

To link to this article: https://doi.org/10.1080/10408399609527736

Critical Reviews in Food Science and Nutrition, 36(5):465–513 (1996)

Water Activity, Water Glass Dynamics, and the Control of Microbiological Growth in Foods

Jorge Chirife and María del Pilar Buera[1]

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, (1428) Buenos Aires, Argentina

Referee: Dr. Theordore P. Labuza, Dept. of Food Scl. and Nutrition, Ciudad Univ., Nunez, Buenos Aires, Argentina

[1] Member of Consejo Nacional de Investigaciones Científicas y Técnicas, República Argentina.

ABSTRACT: Water is probably the single most important factor governing microbial spoilage in foods, and the concept of water activity (a_w) has been very valuable because measured values generally correlate well with the potential for growth and metabolic activity. Despite some drawbacks (e.g., solute effect), the concept of a_w has assisted food scientists in their effort to predict the onset of food spoilage as well as to control food-borne disease hazards in food products. In the last decade the concept of a_w has been challenged. It has been suggested that reduced-moisture food products (e.g., low and intermediate) may be nonequilibrium systems and that most of them are in the amorphous metastable state, which is very sensitive to changes in moisture content and temperature. It has been proposed that the glass transition temperature T_g (temperature at which the glass-rubber transition occurs), is a parameter that can determine many product properties, the safety of foods among them. The concept of water dynamics, originating in a food polymer science approach, has been suggested instead of a_w to better predict the microbial stability of intermediate-moisture foods. The usage of a_w to predict microbial safety of foods has been discouraged on the basis that (1) in intermediate-moisture foods the measured water vapor pressure is not an equilibrium one, and because a_w is a thermodynamic concept, it refers only to equilibrium; and (2) the microbial response may differ at a particular a_w when the latter is obtained with different solutes.

This review analyzes these suggestions on the basis of abundant experimental evidence found in the literature. It is concluded that nonequilibrium effects (e.g., inability of water to diffuse in a semimoist food) appear to be in many cases slow within the time frame (food's shelf life) of the experiments and/or so small that they do not affect seriously the application of the a_w concept as a predictor of microbial stability in foods.

The claims that a food science polymer approach to understanding the behavior of aqueous sugar glasses and concentrated solutions may be used to predict the microbial stability of food systems is not substantiated by experimental evidence. This approach does not offer, at the present time, a better alternative to the concept of a_w as a predictor of microbial growth in foods.

It is also recognized that a_w has several limitations and should be always used carefully, and this must include precautions regarding the possible influences of nonequilibrium situations. This aspect may be summarized by simply saying that anyone who is going to employ the term *water activity* must be aware of the implications of its definition.

KEY WORDS: water activity, water glass dynamics, microbiological growth.

I. INTRODUCTION

Water is probably the single most important factor governing microbial spoilage in foods and the concept of water activity (a_w) has been very valuable in physiological studies of microorganisms principally because measured values generally correlate well with the potential for growth and metabolic activity (Gould, 1985). The a_w concept has assisted food scientists and microbiologists in their efforts to predict the onset of food spoilage as well as to identify and control food-borne disease hazards that might exist in various food products. The usefulness of a_w has been somewhat diminished by the fact that measured a_w levels may not always be totally predictive of

1040-8398/96/$.50

microbial growth because the microbial response may differ at a particular a_w when the latter is obtained with different solutes. Despite this occasional behavior, the a_w concept has remained useful and the manipulation of a_w is an important factor for the control of microbial growth in food. Thanks to this concept, many processes could be successfully adapted and new products could be designed (van den Berg, 1991).

Despite its usefulness, the concept of a_w as a determinant of microbial growth in foods has been challenged (Slade and Levine, 1987; Franks, 1991a,b; Slade and Levine 1991a,b). It has been suggested over the last decade that certain food products (mainly of the low- and intermediate-moisture type) are nonequilibrium systems and that most of them are maintained or brought into a state of thermodynamic metastability during processing. However, pseudostability, because it may last longer than the normal lifetime of the product, would be preferable (van den Berg and Bruin, 1981). This approach focused increasing attention on the dynamic aspects of food systems, rather than on equilibrium properties (Slade and Levine, 1991a). In many foods and biological materials the solids (either biopolymers or low-molecular-weight carbohydrates) are in an amorphous metastable state that is very sensitive to changes in moisture content and temperature (Slade and Levine, 1987; Levine and Slade, 1992; Roos and Karel, 1991a). This amorphous matrix may exist either as a very viscous glass or as a more liquidlike, rubbery structure, as shown in Figure 1. The characteristic temperature, T_g, at which the glass-rubber transition occurs has been proposed as a physicochemical parameter that can determine product properties, such as stability and safety of foods (Slade and Levine, 1987, 1991a,b, Levine and Slade, 1992). Slade and Levine (1987) proposed new guidelines for more

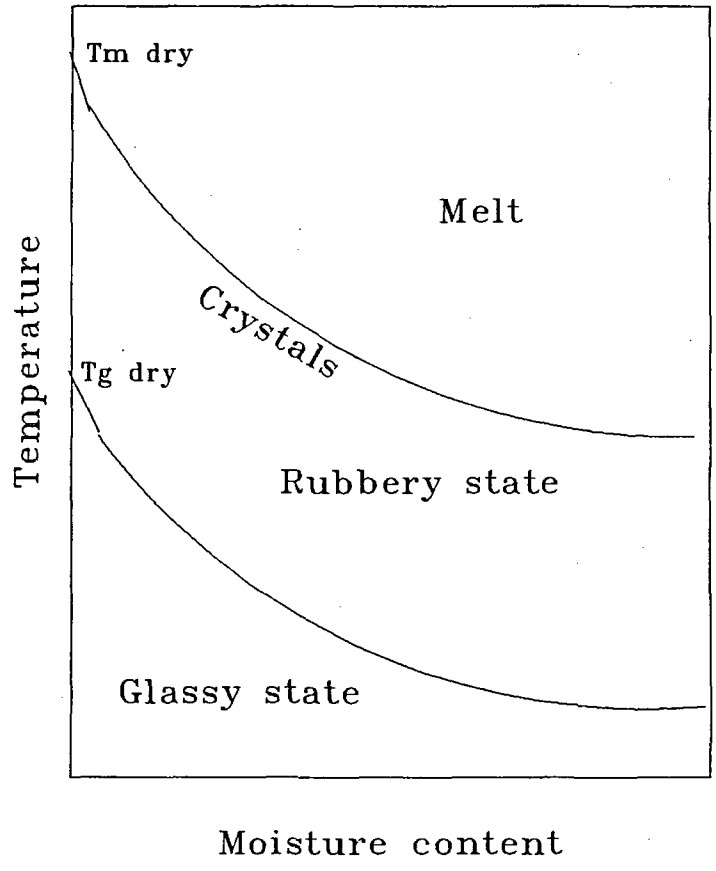

FIGURE 1. A portion of a phase diagram showing the melting temperatures (T_m) and glass transition temperatures (T_g) of a crystallizing component as a function of moisture content.

credible criteria of stability and viability in intermediate-moisture systems to replace a_w by using their concept of water dynamics. Franks (1991a) stated that the assumption of a good correlation between measured a_w and potential for microbial growth has led to "disastrous consequences in the reformulation of intermediate moisture foods to some predicted 'safe' a_w value, merely because glucose was replaced by fructose."

Slade and Levine (1987) and Franks (1991a) stated that in many product situations, equilibrium thermodynamic descriptions are inappropriate, because the measured physical properties are time dependent; thus, a_w must not be used to describe the attributes of these systems. The measured a_w relies on the existence of a liquid-vapor equilibrium; this situation may not occur when the substrate is a quasi-solid dispersion such as a low-moisture or even a semimoist food product. The high viscosity of the food system is related to a much slower rate of attainment of equilibrium; what is measured in such cases is a nonequilibrium relative vapor pressure (RVP) and not a_w. van den Berg and Bruin, 1981 suggested that in food systems true equilibrium may not exist, but rather some pseudoequilibrium exists, which is not clearly definable, but which may remain for months in that condition. As a consequence, the thermodynamic a_w cannot be defined throughout the system, but rather an empirical pseudo-a_w is reflected in the system properties.

This review examines various aspects: (1) the suggestions of Slade and Levine (1987, 1991a) that water dynamics may be used instead of a_w to predict the microbial stability of concentrated and intermediate-moisture food systems, (2) the suggested failure of a_w as a predictor of microbial stability when glucose is replaced by fructose, and the behavior of fructose as an a_w-lowering solute in foods, and (3) the practical consequences of nonequilibrium situations on a_w values of foods in the microbiological growth range.

II. EQUILIBRIUM IN WATER SORPTION MEASUREMENTS

The main criticism of the utilization of a_w in the food area may be summarized as follows: In low-moisture and intermediate-moisture foods, the concept of a_w becomes meaningless because the measured vapor pressure of water is no longer the equilibrium vapor pressure. At best, a stationary state is reached under a given set of environmental conditions and mistaken for equilibrium. A recorded low value for water vapor pressure is likely to be due to the inability of water to diffuse rapidly (within the observation period) throughout the substrate and to equilibrate with the vapor above it (Slade and Levine, 1991a; Franks, 1991a). In moisture sorption studies the situation may be further complicated if the amorphous material undergoes a glass-rubber transition during the course of the measurement (Franks, 1991a). This is because the rates of diffusion-limited changes (e.g., moisture equilibration) are more rapid in the rubbery liquid state than in the glassy solid state (Slade and Levine, 1991a). The analysis of various literature data may throw some light on these aspects. However, in order to evaluate the literature data, it is first necessary to have an idea of the usual precision in the experimental determination of sorption isotherms. In a comprehensive collaborative study within the framework of the European Corporation in the Field of Scientific and Technical Research (COST), precision data (e.g., repeatability/reproducibility) in the determination of sorption isotherms were determined. In a study with 24 laboratories the following precision data were obtained for the adsorption isotherms of microcrystalline cellulose (MCC) and potato starch. In an a_w range of interest to microbial growth (0.60 to 0.90) the average standard deviation of all data was 2.6% on the equilibrium moisture content for MCC and 3.8% for potato starch (Wolf et al., 1984). The repeatability (tests performed at short intervals at one laboratory by one operator using the same equipment) was 2.0% for both MCC and potato starch. The reproducibility (tests performed at different laboratories, which implies different operators and different equipment) was 8.2 and 12.9% for MCC and potato starch, respectively. Other authors determined the repeatability of their isotherm data for different foods and reported similar values (Andrieu et al., 1985; Macchia and Bettelheim, 1964; Jayas and Mazza, 1991).

The definition of the equilibrium between food samples and the water vapor source is a critical

aspect in performing sorption isotherms. Practically, the sorption process could be stopped when the weight difference after the final equilibrium becomes less than the sensitivity of the balance used. But even this stage may require a very long waiting time; therefore, an apparent equilibrium state is commonly defined in terms of a maximum tolerable weight change during an arbitrary time (Gal, 1981). This is the procedure usually followed in the literature. It is noteworthy that the equilibrium time will depend not only on water diffusion characteristics in the substrate, but also on mass transfer characteristics in the head space over the sample (Spiess and Wolf, 1985). The heat and mass transfer within the equipment used to determine the isotherms may or may not be lower than in the product itself (King, 1968).

Lomauro et al. (1985) reported on a study to test an objective criterion for determining the equilibrium moisture content when sorption isotherms are prepared. Foods of different physical characteristics (flour, freeze-dried apple, freeze-dried turnip, freeze-dried ground beef, oatmeal cookie, shredded wheat, and raisins) were exposed to an atmosphere of 75.2% RH at 25°C in nonevacuated desiccators and the moisture content recorded as a function of time. These authors considered that a pseudoequilibrium was reached when the moisture content (dry basis [d.b.]) did not change more than 0.5% during three consecutive sample periods at not more than 7-d intervals. This criterion for equilibrium moisture content was compared with the values obtained after 6-month storage in closed mason jars, which were considered to be very close to the equilibrium moisture content. Lomauro et al. (1985) concluded that the foods tested reached (or were very close to) equilibrium within 1 month, based on the above criterion. Also, the values compared well (differences between 0.5 and 3.4%) with the 6-month-storage study. The differences between the 1- and 6-month studies are in the range reported for the repeatability of isotherm determinations, as noted before.

Various authors reported their equilibrium times for isotherm determinations of different food systems using the gravimetric, static, method over saturated salt solutions. Most authors utilized a small amount of sample (1 to 3 g) to diminish water transport resistances in the substrate and their equilibrium times ranged mostly between 1 and 3 weeks, depending on temperature and relative humidity (Lagoudaki et al., 1993; Valdez-Niebla et al., 1993; Hill and Rizvi, 1982; Engels et al., 1987; Hansen, 1971; Wedzicha and Quine, 1983; Lima and Cal-Vidal, 1983; Kumar, 1974; Rasekh et al., 1971; Chhinnan and Beuchat, 1985; Hutchinson and Otten, 1984; Mazza and Jayas, 1991; Piñaga and Lafuente, 1965; Tsami et al., 1990; Bolin, 1980; Saravacos et al., 1986; Ayranci et al., 1990; Hayakawa et al., 1978). The data reported by Bizot et al. (1985) for starch are relevant to the present analysis. Bizot et al. utilized a practical equilibration time of about 7 d (±0.02% water per 24 h) for a 1-g sample, but also stored their starch samples over saturated salt solutions for 2 years. They noted a slow drift of desorption pseudoequilibria, but it was only 1% water content (d.b.) after such a long time. On the other hand, adsorbing samples remained stable. van den Berg (1991) reported that a 0.15-g sample of starch in a sorption balance under high vacuum exhibited equilibration times of 0.5 to 3 d, producing states that were steady for up to several months.

From the above considerations it may be assumed that sorption determinations performed with the usual precautions regarding a practical weight constancy are likely to be close to equilibrium. The differences are probably within the uncertainties associated with the experimental determination of isotherms (Wolf et al., 1984).

III. SORPTION ISOTHERMS OF FOODS CONTAINING CRYSTALLIZABLE SUGARS

Dried fruits (e.g., raisins, figs, prunes, etc.) are good examples of nonequilibrium, intermediate-moisture systems. Sugars (glucose, fructose, and sucrose) constitute about 50 to 83% of the dry matter (Tsami et al., 1990) and in the intermediate-moisture range they are likely to be in an amorphous, metastable, rubbery state when stored at microbial incubation temperatures (Roos, 1987, 1993; Chirife and Buera, 1994a). Sorption iso-

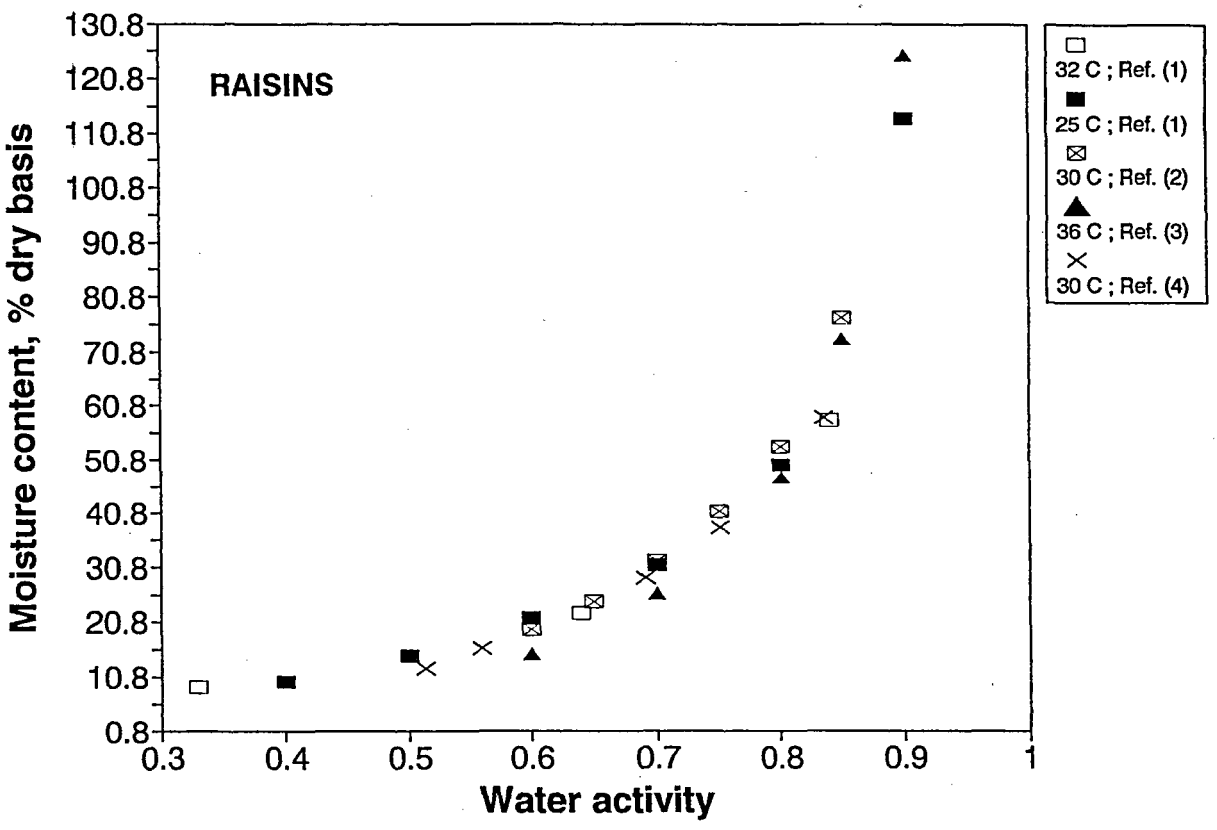

FIGURE 2. Water adsorption isotherms in raisins: comparisons of several literature data. References: (1) Bolin, 1980; (2) Saravacos et al., 1986; (3) Ayranci et al., 1990; (4) Tsami et al., 1990. (From Chirife, J. and Buera, M. P., 1995. *J. Food Eng.*)

therm data for raisins (glucose + fructose = 83% d.b.) as reported by different workers are compared in Figure 2. The agreement between all data is good (considering the usual precision in the reproducibility of isotherms) despite the fact that raisins constitute a nonequilibrium system with a high sugar concentration. Slade and Levine (1987) suggested, and Roos and Karel (1991a) confirmed, that sugar (sucrose) crystallization is possible only above the T_g, and that the rate of crystallization depends on the difference between the storage temperature and T_g ($T-T_g$). Crystallization of amorphous sugars results in release of moisture and this increases product a_w (Karel and Roos, 1993). However, the absence of discontinuities in the isotherm of raisins is noticeable, suggesting that sugars remained amorphous, even at very large $T-T_g$, which would correspond to moisture-temperature conditions for the microbiological growth range (Chirife and Buera, 1994). This may be attributed to the presence of high-molecular-weight compounds in the raisins matrix, which delay (or inhibit) crystallization (Iglesias and Chirife, 1978).

Sorption isotherms of other fruits reported in the literature do not show discontinuities (Tsami et al., 1990), suggesting the absence (in the time of observation) of sugar crystallization. Bolin (1980) reported a_w and equilibrium moisture values for raisins sealed in glass jars and held at 21 or 32°C for up to 12 months. His data for 21°C are plotted in Figure 3; it can be seen that storing raisins in sealed jars for several months had little effect on the moisture isotherm. This suggests that nonequilibrium effects (inability of water to diffuse rapidly) are very slow (in the time frame

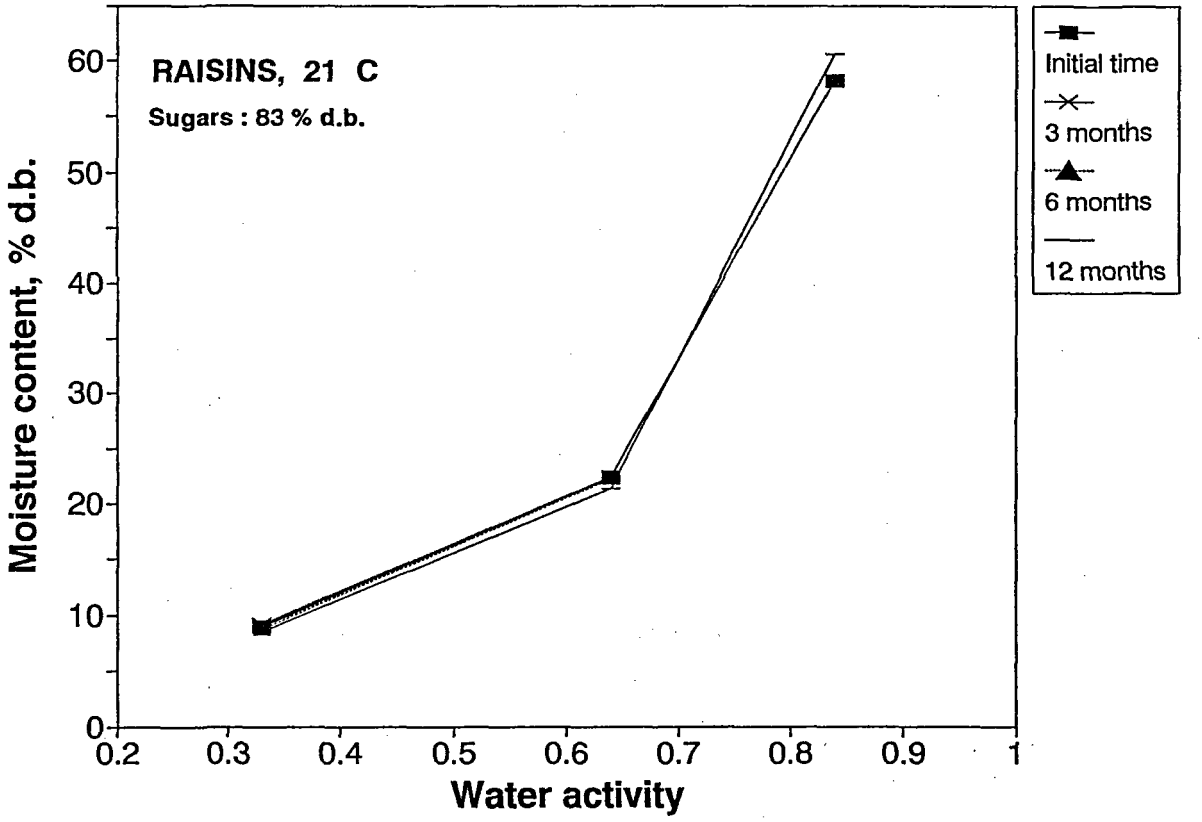

FIGURE 3. Effect of storage time at 21°C on the adsorption isotherm of raisins. Based on data from Bolin, 1980. (From Chirife, J. and Buera, M. P., 1995. *J. Food Eng.*)

of these experiments) and/or small. Cañelas et al. (1993) stored raisins (initial $a_w = 0.61$) at 4, 11, and 23°C for up to 11 months. Samples stored at 11 and 23°C showed an increase in a_w from 0.61 to 0.65 after 11 months. The sample stored at 4°C had a lower variation, and after 8 months of storage it remained constant. It is noteworthy that any observed time dependency of measured RVP in semimoist systems may not necessarily indicate the inability of water to diffuse rapidly within the food. It may be also due to chemical changes that may occur during the time of observation. Cañelas et al. (1993) stated that in stored raisins, the sugars (glucose and fructose) are involved in the Maillard reaction, and consequently a decrease in sugar content was observed during storage. This would increase the a_w; moreover, during the browning reaction, water is formed and this could also increase the a_w. Because Maillard reactions have a strong temperature dependence (Hendel et al., 1955; Resnik and Chirife, 1979; Buera et al., 1987) it is expected that at 4°C reactions would be slower and a_w would remain almost constant, as was experimentally observed.

IV. PREDICTION OF a_w IN FOODS IN THE MICROBIOLOGICAL GROWTH RANGE

Franks (1991a) and Slade and Levine (1991a) stated that the high viscosity of certain semimoist systems causes a very slow rate of attainment of equilibrium, and what is measured in such cases is a nonequilibrium RVP and not a_w. Franks (1991a) noted, however, that in simple solutions (liquid systems) the assumption of equilibrium (and hence the use of a_w) can be valid, because the diffusion rate of water molecules is high compared to the time scale of the measurement of

vapor pressure. Thus, it is interesting to compare experimentally determined a_w in some semimoist foods (nonequilibrium values) with predictions made from knowledge of the a_w of simple solutions (equilibrium values) representative of their main soluble substances. In semimoist foods containing a relatively high proportion of soluble substances (usually NaCl and/or low-molecular-weight sugars), a_w is mainly determined by the molality of solute(s) in the aqueous phase (Lupin et al., 1981). Predicted and measured a_w values in raisins in the intermediate-moisture range are compared in Figure 4. The sugar content of raisins was (dry basis) 85.3% (Tsami et al., 1990), and the main sugars were glucose and fructose. Predicted values were calculated by applying the Ross equation (Ross, 1975; Chen, 1990) to the data reported by Chirife et al. (1981a) for the a_w of glucose and fructose solutions. Good agreement between prediction and reality is observed. The goodness of this agreement should be judged on the basis of experimental error in the determination of sorption values. The comparisons were extended to a wide variety of semimoist foods comprising many physical states: solids (fish), pastes (ketchup and milk jam), jellies (fruit preserves and marmalades), emulsions (mayonnaises), and very concentrated liquid food solutions (sugar molasses and honey). The results are shown in Figure 5. A satisfactory agreement is observed; slight discrepancies may be attributed, among other factors, to some inaccuracies in the use of electronic hygrometers mostly used for the experimental determination of a_w in semimoist foods (see below). The observed agreement between a_w measurements in semimoist foods

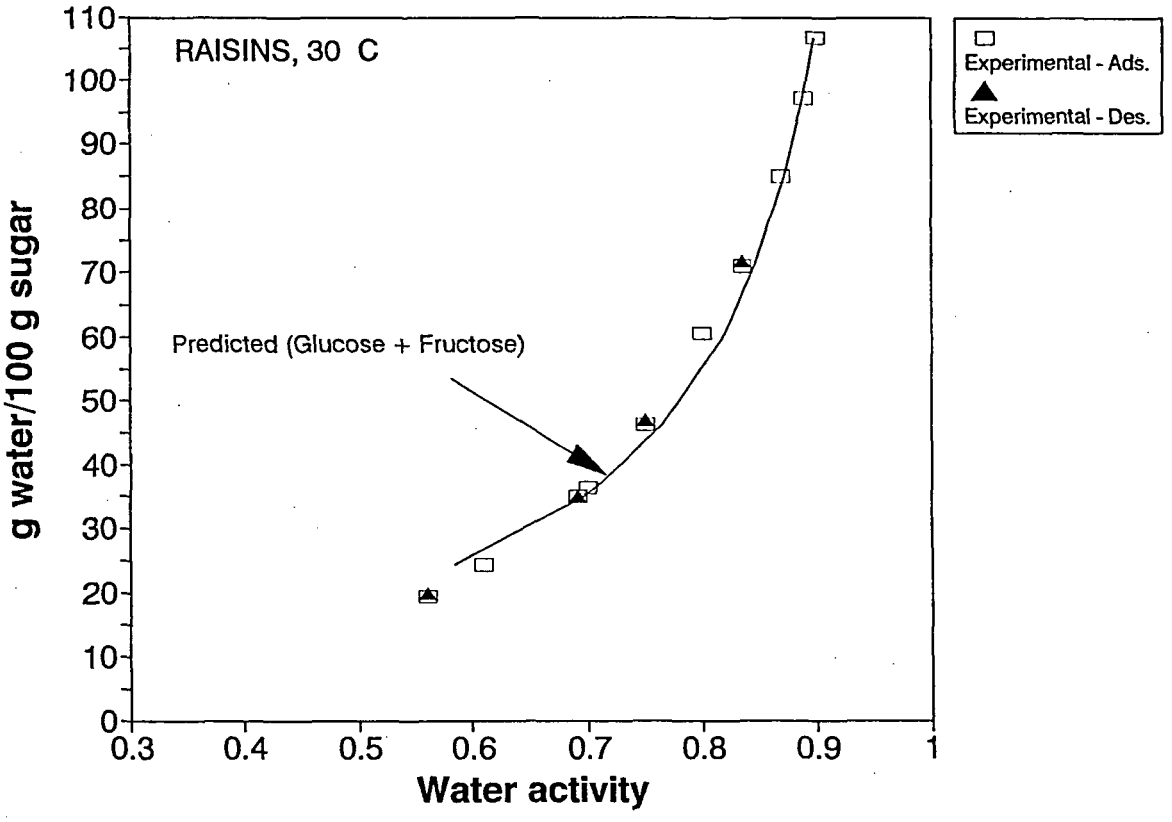

FIGURE 4. Comparison of predicted and measured water sorption in raisins at 30°C. (From Chirife, J. and Buera, M. P., 1994. *J. Food Eng.*)

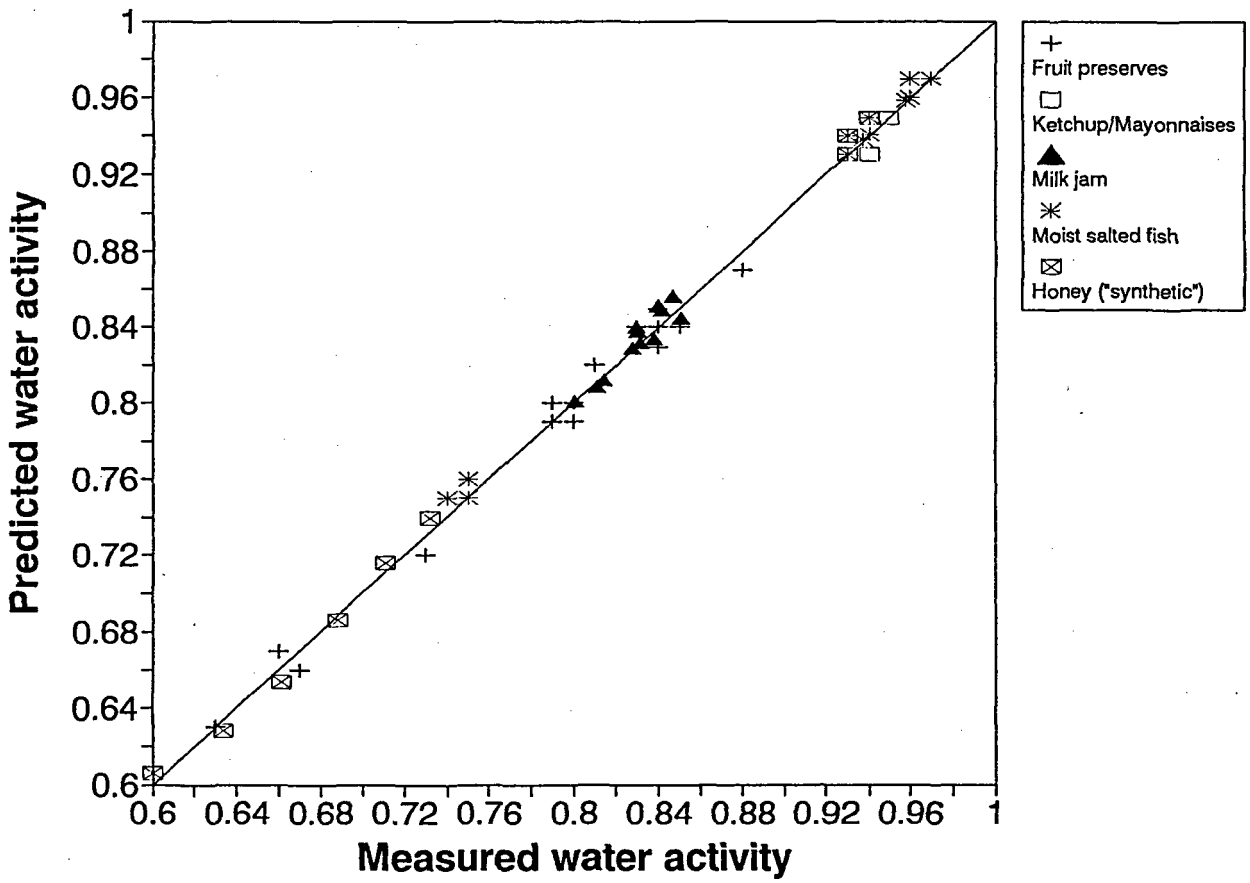

FIGURE 5. Predicted and measured a_w values in various semimoist and concentrated foods. (From Chirife, J. and Buera, M. P., 1995. *J. Food Eng.*)

(nonequilibrium systems) and predictions made from equilibrium situations (liquid solutions) may indicate that nonequilibrium effects (slow rate of water equilibration) are relatively small. At least the variations are comparable with the usual error in the experimental determinations of a_w.

V. STABILITY OF METASTABLE SYSTEMS

Some questions that may arise are (1) why, if the concept of a_w has been misleading, this parameter has been such a helpful tool in predicting microbial stability in foods, and (2) why, if semimoist foods are nonequilibrium systems that may exist in different states of energy at different times, their sorption isotherms also serve as valuable information.

Equilibrium thermodynamics deals with the properties of the macroscopic component in the state of thermodynamical equilibrium. The thermodynamical conditions corresponding to equilibrium lead to a minimum Gibbs free energy (G) and can be expressed by the equations

$$\frac{\partial G}{\partial \eta_G} = 0; \quad \frac{\partial^2 G}{\partial \eta_G^2} \geq 0$$

where η_G = order parameter (any parameter that measures the order of the system).

In the case of the absolute minimum of G, we speak of the stable equilibrium; in the case of a local minimum, we talk about metastable equilibrium. A schematic representation of true equilibrium and metastable conditions is given in Figure 6. The lifetime of metastable phases is related to

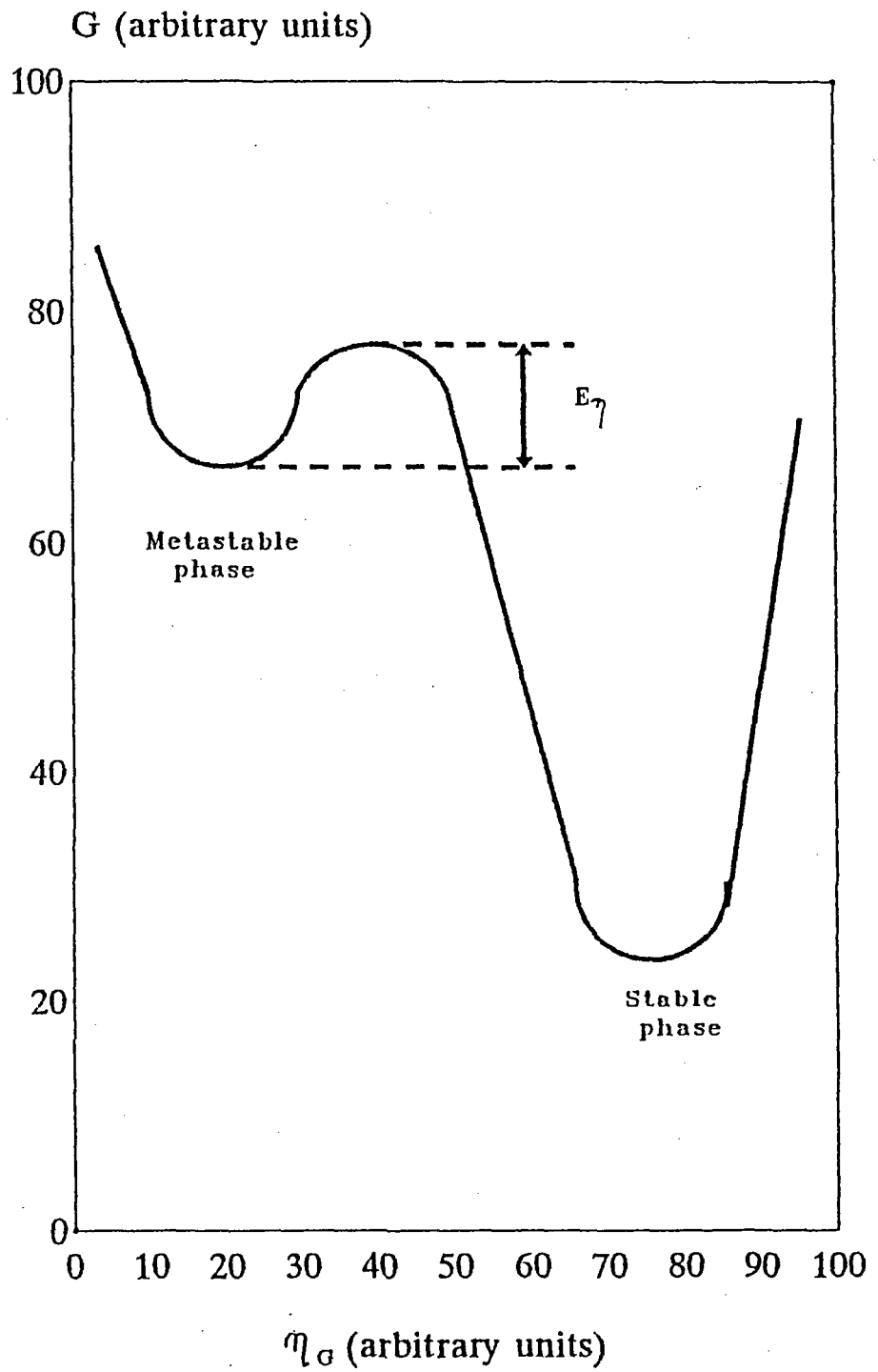

FIGURE 6. Schematic representation of true equilibrium and metastable conditions. G, Gibbs free energy; η_G; order parameter (any parameter related to the order of the system); E_η, energy barrier.

the height of the energy barrier, E_η, that the system must overcome to get from the local extreme to the true equilibrium. In many cases this lifetime can be as long as *thousands of years* (e.g., glasses, frozen metastable phases of solid states; diamond [a metastable phase of graphite]), and metastable phases that have specific physical properties can be used in technical practice. These

states are stable with respect to small fluctuations of external conditions. If the given system is in a nonequilibrium state in consequence of external conditions, those macroscopically observable processes take place within this object, and, thus, the state variables are a function of time and spatial coordinates. The process assuring that the system reaches the state of thermodynamic equilibrium (within a certain interval of time) is called *relaxation*. For various systems and processes the relaxation time may considerably differ, for example from 1×10^{-6} s (realization of the thermodynamical equilibrium in ideal gases) to several years (impurity reached by diffusion in the solid state). The mechanism of relaxation of metastable states is related to relatively large fluctuations and these states are *stable with respect to small fluctuations.* The metastable phase is in a state of partial thermodynamical equilibrium and it does not depend on the history of the system if the following relationship holds:

$$\tau_i = L/V_i << t_{exp} < \tau_n$$

where L is the linear dimension of the system; V_i is the velocity of relaxation to the local equilibrium relative to the i-th parameter, τ_i is the characteristic time of relaxation, t_{exp} is the characteristic observation time of the experiment, and τ_n is the mean value of the creation of a new metastable nucleus (Chvoj and Kozisek, 1991).

The exact and full equilibrium phase diagrams of a given material can exist only at a theoretical level. In fact, the real experimental conditions — maintained within the measured sample with limited accuracy — may differ, more or less, from unambiguous equilibrium conditions required for construction of equilibrium phase diagrams. When the rates of elementary (molecular) processes — which determine the state of the system at the macroscopic level — the principle of local thermodynamic equilibrium is applicable (Chvoj and Kozisek, 1991).

VI. THE a_w BEHAVIOR OF FRUCTOSE: WATER DYNAMICS AND MOLD GERMINATION

Slade and Levine (1987) stated that there is a "controversial, contradictory, and confusing state of the intermediate moisture food (IMF) literature on use of fructose vs. glucose to lower a_w in moisture management applications." They added, "it has not even been possible for agreement to be reached in the IMF literature on the correct relative vapor pressures (RVP) values for 50 and 60% fructose solutions." Chirife and Buera (1994a) recently showed that this is not true. The a_w of fructose and glucose solutions up to about 50 to 60% (w/w) may be considered identical (within the uncertainty [about $\pm 0.005 a_w$] associated with usual experimental determinations of a_w), as conclusively shown in Figure 7.

On the basis of the supposed contradictory state of the literature on the a_w of fructose vs. glucose solutions, Slade and Levine (1987) applied the water dynamics theory to explain experimental results on germination of *Aspergillus parasiticus* in various intermediate moisture solutions. A comparison was made between the inhibitory effects on conidia germination for a series of concentrated solutions of selected glass formers, such as glucose, fructose, mannose, maltose, sucrose, glycerol, and others. The various glass formers were assayed in pairs, deliberately matched with respect to the individual parameters of approximately equal RVP. It was pointed out that the solute system with a lower ratio of melting temperature to T_g (T_m/T_g) in the dry state (0% moisture content; see Figure 1) showed faster germination regardless of RVP values. In addition, due to the inherent lack of mobility of a sugar in its glassy reference state and the lower local viscosity (and so greater translational mobility) in its rubbery state, water availability was greater for fructose (dry $T_m/T_g = 1.06$) than for mannose (dry $T_m/T_g = 1.36$), which was greater than for glucose (dry $T_m/T_g = 1.42$), which in turn was greater than for glycerol (dry $T_m/T_g = 1.62$). Water availability was also greater for maltose (dry $T_m/T_g = 1.27$) than sucrose (dry $T_m/T_g = 1.43$). Slade and Levine (1987) thus indicated antimicrobial stabilization decreased in the order glycerol > glucose > mannose > fructose, and sucrose > maltose, and concluded that their results demonstrated conclusively that the observed rates of germination (as a mechanical relaxation process) could be interpreted by a conceptual approach based on mobility in the concentrated rubbery sugar-water systems. A very unusual

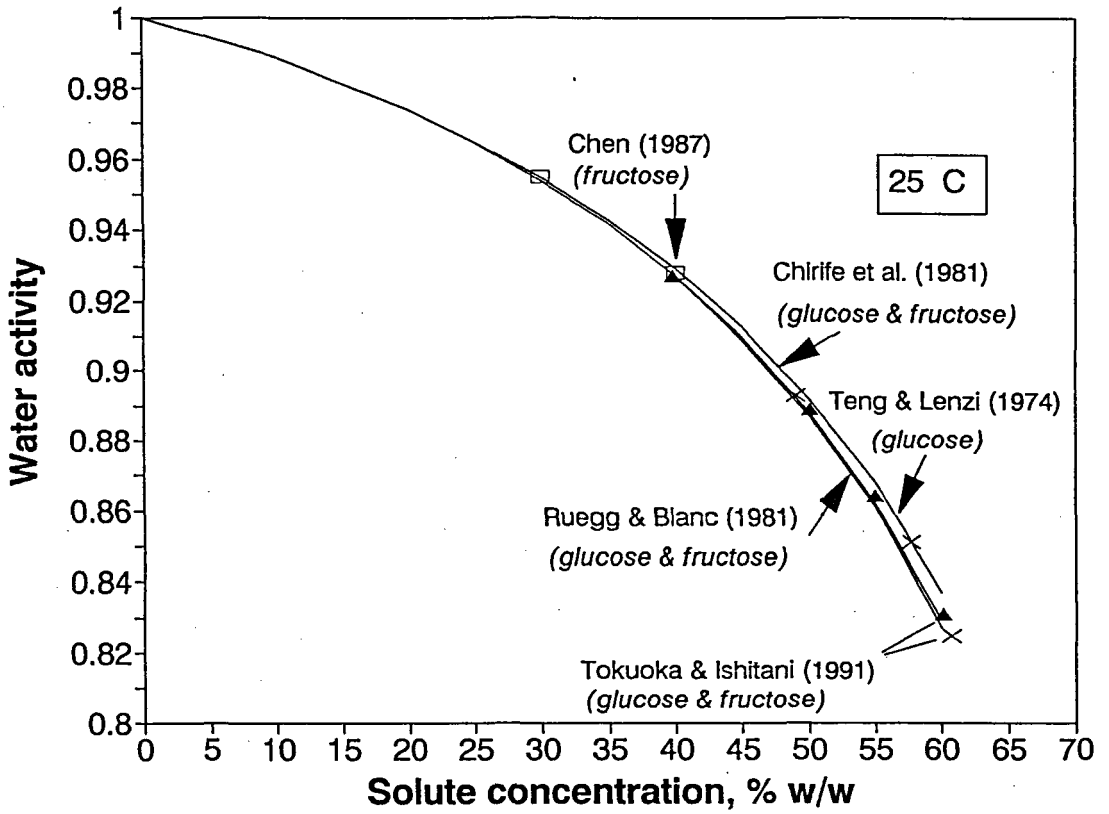

FIGURE 7. The a_w of glucose and fructose solutions at 25°C: comparison of several literature data. (From Chirife, J. and Buera, M. P., 1994. *J. Food Sci.*)

behavior of *A. parasiticus* in concentrated fructose solutions was reported; the same fast germination time (2 d) in solutions of 40 to 70% (w/w) was observed and this was attributed to the extraordinary mobility and water availability of fructose rubbers, as suggested by its low dry T_m/T_g value. Overall, it was concluded that the results of these biological experiments demonstrated conclusively that the observed rates of germination could be interpreted by an approach based on mobility transformations and that water dynamics may be used instead of a_w to predict the microbial stability of concentrated and intermediate moisture systems. Such an all-embracing conclusion about microbial stability (of utmost importance for food preservation) cannot be done on the basis of limited experimental data (e.g., testing of only one mold strain, *A. parasiticus* [Slade and Levine, 1987]). Chirife and Buera (1994a) challenged their results and conclusions using available literature data for microbial stability in IM systems and did not confirm the conclusions of Slade and Levine. For example, germination times at 25°C of *Penicillum implicatum* spores in media with a_w controlled by either glycerol or a 1:1 glucose/fructose mixture are compared in Figure 8. The glucose/fructose mixture evidences greater stability (e.g., slower germination) than glycerol, which is contradictory to the above predictions of Slade and Levine. Figure 9 shows colony diameter of *Monilinia fructicola* after 14 d (25°C) at various a_w regulated by the addition of glucose, fructose, or mannose in a defined medium. At the same a_w and in terms of mobility, inhibition of colony growth diameter should follow the order glucose > mannose > fructose; however, experimental evidence shows the opposite, that is, glucose gave the greatest diameter. Figure 10 shows the effect

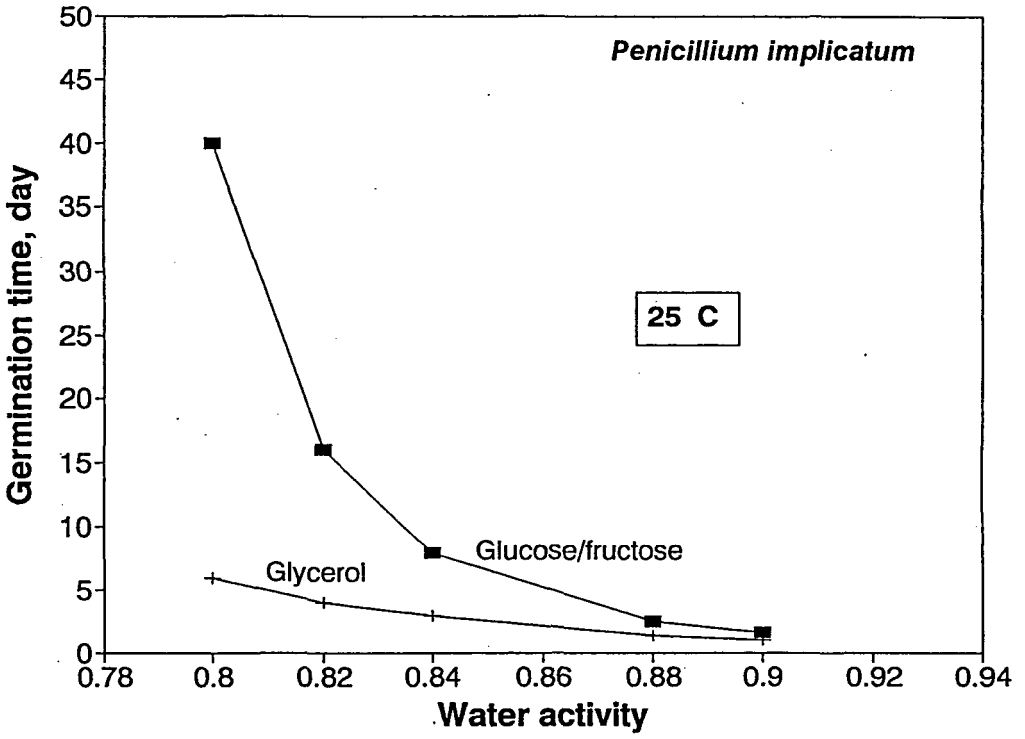

FIGURE 8. Solute effect (glycerol or 1:1 glucose/fructose) on germination time at 25°C of *Penicillum implicatum.* (From Chirife, J. and Buera, M. P., 1994. *J. Food Sci.*)

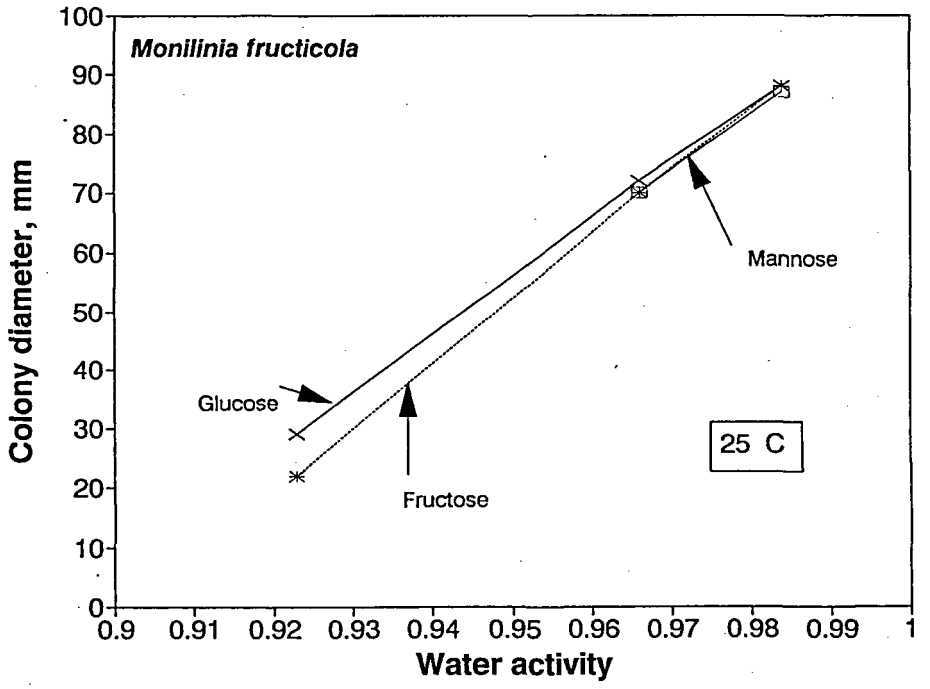

FIGURE 9. Solute effect (glucose, fructose, or mannose) on colony diameter of *Monilinia fructicola* after 14 d of incubation at 25°C. (From Chirife, J. and Buera, M. P., 1994. *J. Food Sci.*)

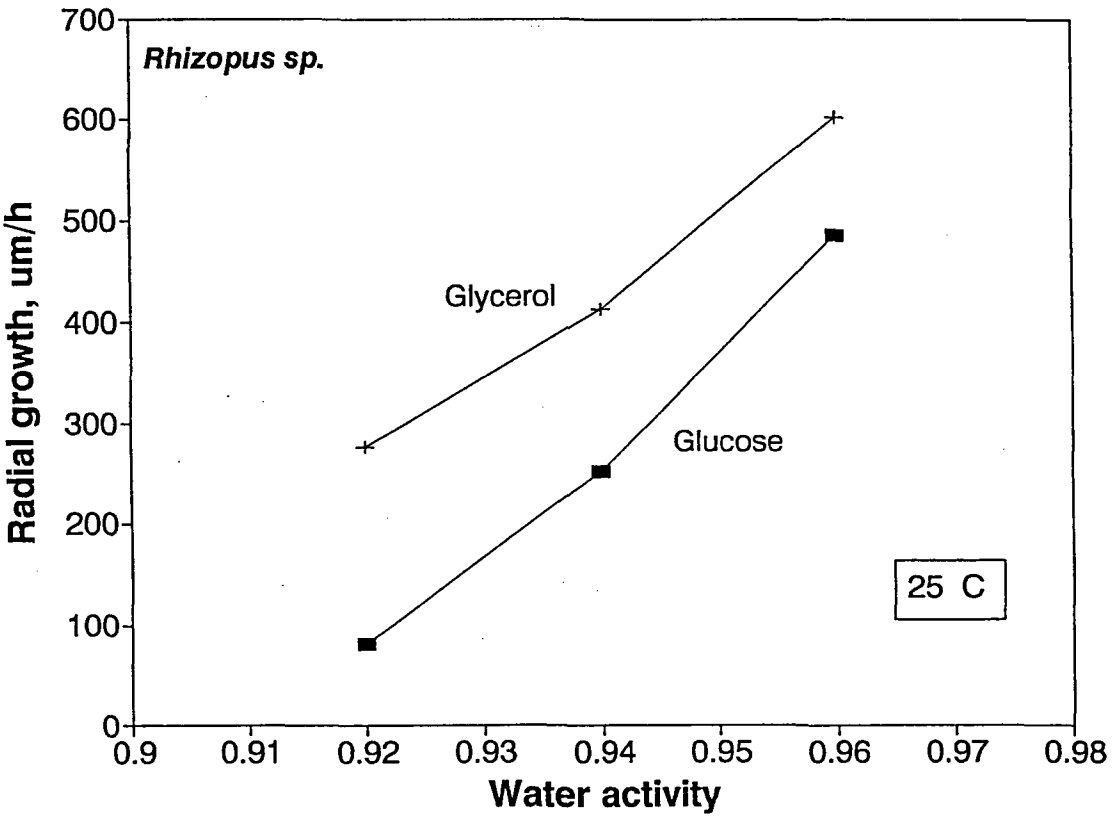

FIGURE 10. Solute effect (glucose or glycerol) on radial growth at 25°C of *Rhizopus sp.* (From Chirife, J. and Buera, M. P., 1994. *J. Food Sci.*)

of different solutes on the radial growth of *Rhizopus* sp. colonies at 25°C in defined media. The growth behaviors of *P. funiculosum* at 25°C on agar malt with the a_w controlled by the addition of either glucose or fructose are compared in Figure 11. None of the results observed agree with the predictions of Slade and Levine; instead, glucose is more inhibitory (in terms of radial growth) than glycerol (Figure 11) and fructose produced an equal or slightly more stable system (slower growth) than glucose (Figure 10). In order to verify the experimental results reported by Slade and Levine (1987) for mold spore germination in IM solutions, Chirife et al. (1994) determined the germination time at 28°C of various mold spores (*A. flavus, A. niger,* and *Eurotium herbariorum*) in concentrated solutions of glucose, fructose, mannose, glycerol, sucrose, maltose, and propylene glycol. The results did not agree with the findings of Slade and Levine; the expected order of antimicrobial stabilization, glycerol > glucose > mannose > fructose, and sucrose > maltose, was not observed. For example, Figure 12 shows the effect of solute type on germination time (at 28°C) of *A. niger*; germination did not occur more quickly in solutions of fructose than in solutions of glucose, mannose, or glycerol, nor did it occur more quickly in solutions of maltose than in solutions of sucrose, as expected from mobility (e.g., dry T_m/T_g values) considerations. Chirife et al. (1994) also determined the effect of fructose concentration on germination time of *A. parasiticus* as compared with data previously reported by Slade and Levine (1987). Their results are shown in Figure 13; a strong influence of fructose concentration on germination time is observed, and at 70% (w/w) concentration, germination was not observed after 70 d of incubation. These results contrast sharply with those of Slade and Levine (1987), who reported the same fast (2 d) germination time for *A. parasiticus* in solutions of 40 to 70% (w/w) fructose and attributed this behavior

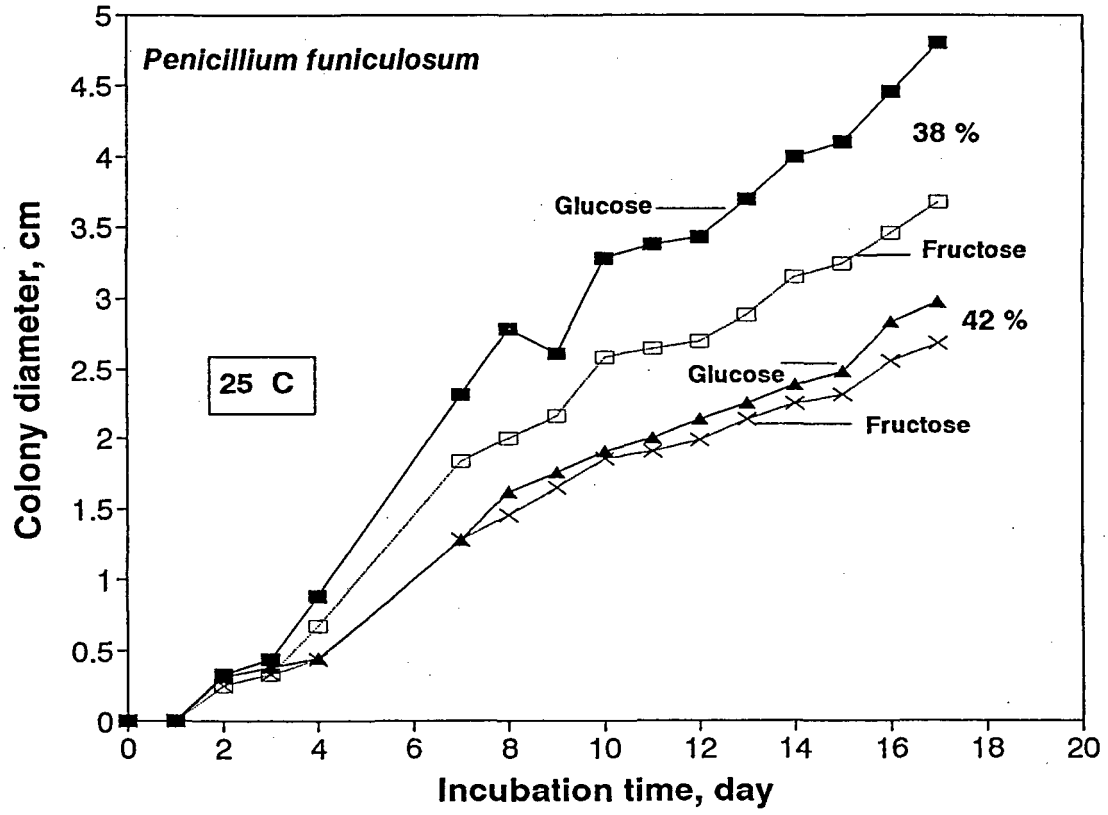

FIGURE 11. Effect of glucose or fructose on radial growth of *Penicillum funiculosum* in malt extract agar at 25°C. (From Chirife, J. and Buera, M. P., 1994. *J. Food Sci.*)

to the extraordinary mobility and water availability of fructose solutions.

VII. REPLACEMENT OF GLUCOSE BY FRUCTOSE AND GLYCEROL BY PROPYLENE GLYCOL AND THE MICROBIAL STABILITY OF INTERMEDIATE-MOISTURE FOODS

Franks (1991a) stated that the assumption of a good correlation between measured a_w and potential for microbial growth has led "to disastrous consequences in the reformulation of intermediate moisture foods to same predicted 'safe' a_w value, merely because glucose was replaced by fructose." However, no reference to substantiate these statements is given. In the same line of thought, van den Berg (1985) stated:

> It is not surprising therefore that in recent years misconceptions have led to some difficulties in the preservation of intermediate moisture products. New products were formulated based on acquired experience, setting safety margins on the basis of a_w. The new product, however, although having the same a_w as its safe predecessor but with a slightly different humectant composition, spoiled on the shelf by microbial action.

This statement was not supported by references. Slade and Levine (1991a) also criticized the utilization of a_w to predict the microbiological safety of intermediate moisture foods, saying that "misconceptions have led to some difficulties in the preservation of intermediate moisture foods," and adding:

> For example consider an intermediate moisture pet food product that was originally formulated with a mixture of solutes predominated by glucose and glyc-

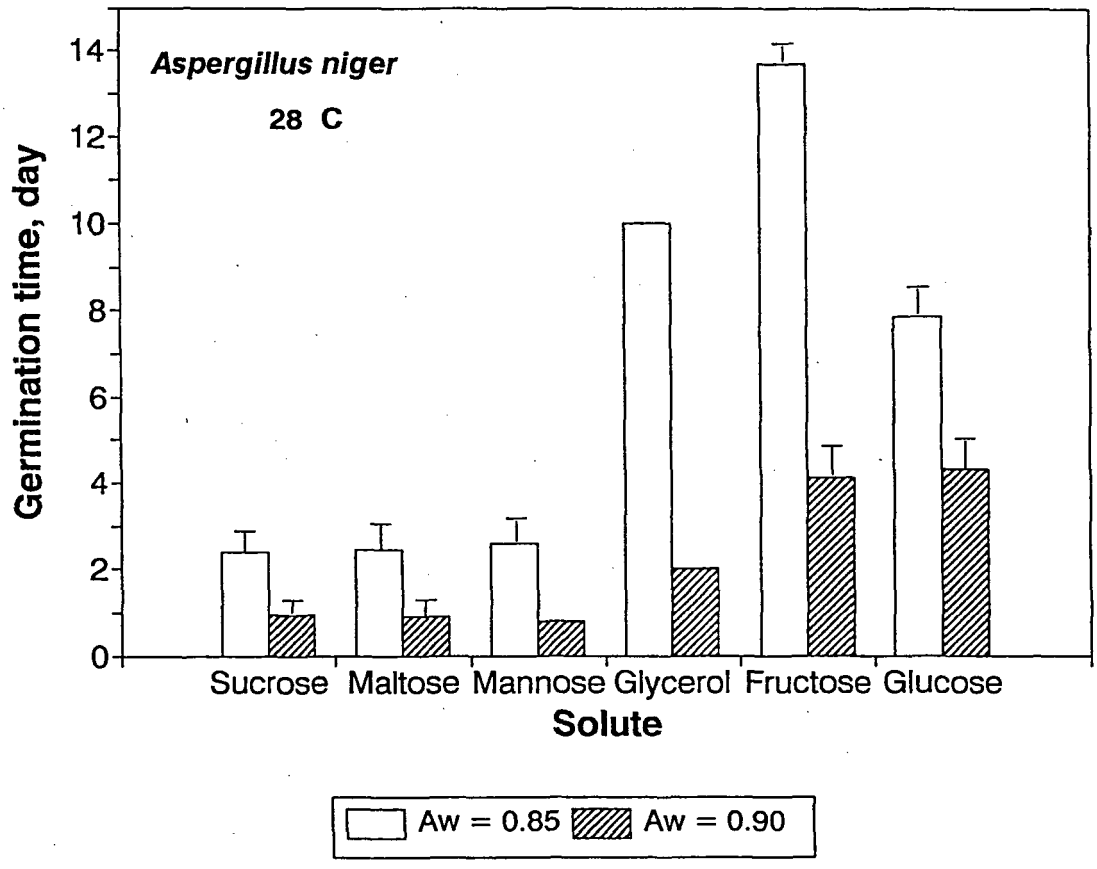

FIGURE 12. Solute effect (sucrose, maltose, mannose, glycerol, fructose, or glucose) on germination time of *Aspergillus niger* at 28°C. (From Chirife, J. et al., 1994. *Food Res. Int.*)

erol. This commercial product was empirically determined to be microbiologically safe and stable at an a_w of 0.92, which was then incorporated as a product specification. Then for the purpose of cost reduction the glucose-glycerol combination was replaced by fructose and propylene glycol, but the a_w specification was not lowered in a corresponding and appropriate fashion but rather naively kept at 0.92. The financially disastrous result required a recall of millions of dollars worth of spoiled product.

However, they did not support these statements with any scientific/technical evidence to demonstrate that solute replacement and no other factors were responsible for the claimed failure. As far as known, a soft-moist dog food formulated to a_w 0.92 using glucose and glycerol cannot be "microbiologically safe and stable" as claimed by Slade and Levine (1991a); it will be spoiled by molds and also by several bacteria, the pathogenic *Staphylococcus aureus,* among them (Haas and Heran, 1978; Boylan et al., 1976). In the food industry, practical control of microbial growth seldomly is achieved by a_w alone. In order to achieve microbial stability, parameters other than a_w need to be utilized and controlled. The parameters include pH, antimicrobials, mild heat treatment, oxygen partial pressure, etc. and may be best visualized, as suggested by Leistner et al. (1981), as hurdles that microorganisms must overcome or, in some way, circumvent in order to cause deterioration of a food. If this were the case, one cannot say that solute replacement was the cause for the failure unless all other hurdles were also checked for any unexpected modification, and this might be reported and substantiated by sound experimental evidence before using this

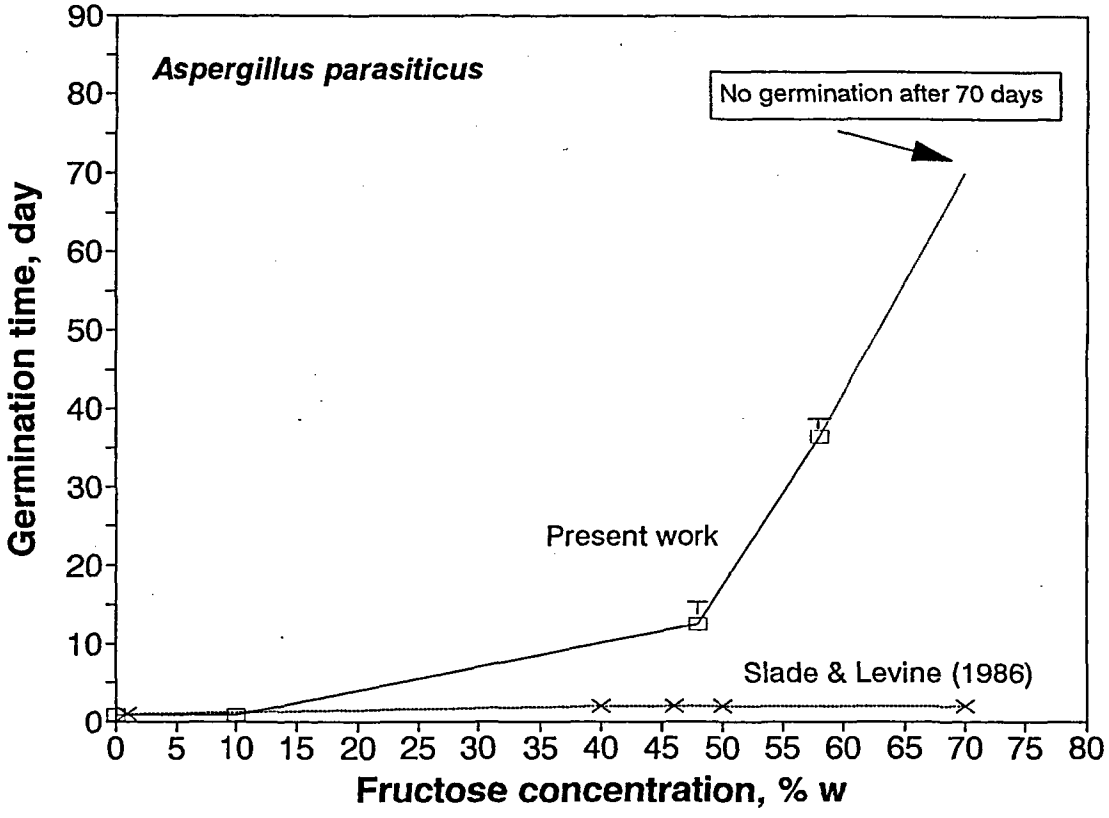

FIGURE 13. Effect of fructose concentration on germination time of *Aspergillus parasiticus* at 28°C. (From Chirife, J. et al., 1994. *Food Res. Int.*)

pet food episode as a demonstration that the concept of a_w was invalid. Nelson (1993) pointed out some potential reasons that may contribute to a difference in microbial stability when different solutes are used to achieve the same a_w, and which do not mean that the concept of a_w is invalid, as suggested by Slade and Levine (1991a). One of the arguments of Slade and Levine (1991a) was that propylene glycol has a much lower dry T_m/T_g ratio (1.27) than does glycerol (1.62), and, therefore, according to their water dynamics approach, a lower RVP (or a_w) at an equivalent solute concentration does not correlate with greater microbiological stability for propylene glycol vs. glycerol. Chirife and Buera (1994) formulated an IM dog food (meat and bone meal, soybean flour, precooked corn, rice and wheat flour, oats, animal fat, minerals, and vitamins) using glucose/glycerol to depress a_w in one case and fructose/propylene glycol in another. The samples were packaged in moisture-proof pouches and stored at 34°C. After 3 d of storage, incipient mold growth was visually observed on the surface of samples formulated with glucose/glycerol and on the fifth day all the samples formulated with glucose/glycerol were totally spoiled by molds. On the contrary, the samples formulated with fructose/propylene glycol remained free of visible molds after 20 d of incubation (Figure 14). These results are exactly the opposite of the predictions of Slade and Levine (1991) made on the basis of the water dynamics theory. Chirife et al. (1994) reported studies on the germination time of various mold spores in solutions of propylene glycol and glycerol at an a_w of 0.90. Water dynamics predicts that a propylene glycol solution would have a poorer microbiological stability than glycerol at an equivalent solute concentration. However, the results of Chirife et al. (1994) showed exactly the opposite; all molds (*A. niger, A. flavus,* and

FIGURE 14. Mold spoilage in an IM dog food (a_w = 0.89) formulated with glucose/glycerol or fructose/propylene glycol after 20 d of incubation at 34°C.

Eurotium herbariorum) germinated readily in glycerol (1 to 2 d) but none of the molds were able to germinate in propylene glycol after 70 d of incubation at 28°C. This is simply because the behavior of propylene glycol cannot be explained solely on the basis of mobility or a_w effects alone, because this molecule possesses specific antimicrobial effects largely known in the literature (Haas and Herman, 1978; Ballesteros et al., 1993). Gervais et al. (1992) also showed that *Penicillum roquefortii* and *Trichoderma viride* did not germinate in propylene glycol solutions of similar a_w. These results are a dramatic demonstration that mobility effects per se are not able to explain the biological response of a microorganism to changes in its aqueous environment.

It may be concluded that the claims of Slade and Levine that water dynamics may be used to predict microbial stability of concentrated and intermediate moisture food systems are not substantiated by sound experimental evidence.

VIII. a_w AS A DETERMINANT OF MICROBIOLOGICAL GROWTH

The definition of a moisture condition at which pathogenic or spoilage microorganism cannot grow is of paramount importance in food preservation and it has been widely recognized that the concept of a_w has been a valuable tool in this aspect. Thanks to this concept, many processes have been adapted successfully and new products designed (van den Berg, 1991). It is accepted that measured values of a_w generally correlate well with the potential for growth and metabolic activity of microorganisms (Gould, 1985; 1988; Gould and Christian, 1988). Its measurement has been very valuable for predicting the microbial stability (and safety) of foods (Leistner and Rodel, 1975; Troller and Christian, 1978; Leistner et al., 1981; Silverman et al., 1983; Dodds, 1989; Glass and Doyle, 1991). It has been shown repeatedly in the literature that each microorganism has a critical a_w below which growth cannot occur (Brown, 1974; Troller and Christian, 1978; Gould and Measures, 1977; Troller, 1971; Leistner et al., 1981; Beuchat, 1983). For example, pathogenic bacteria cannot grow below an a_w of 0.85 to 0.86; yeasts and molds are more tolerant to reduced a_w, but usually no growth exists below a_w of about 0.62 (Scott, 1953; Hocking and Pitt, 1979; Beauchat, 1993; Silverman et al., 1983; Leistner, 1987; Jermini and Schmidt-Lorenz, 1987; Ballesteros et al., 1993). Table 1 shows some critical a_w values regarding the safety of foods (from various literature sources). Table 2 shows the minimal a_w for growth of food-borne bacterial pathogens at optimum pH, temperature, and source of nutrients. Many workers concluded that a_w is a main determinant of growth because it determines the osmotic stress and because the ability to grow is determined by the degree of that stress and the osmoregulatory capacity of a particular microbial cell. A fundamental requirement for growth of microorganisms on substrates of high osmolality is the intracellular accumulation of solutes, either by transport or synthesis, to concentrations that counterbalance the osmolality of the external medium (Prior et al., 1987; Hocking, 1988; Chirife

TABLE 1
Some Critical a_w Values Regarding the Safety of Foods

Minimal a_w	
0.94	Growth and toxin production by all types (A, B, C, E, and G) of *Clostridium botulinum*
0.91	Anaerobic growth of *Staphylococcus aureus*
0.85–0.86	Aerobic growth of *Staphylococcus aureus*
0.80	Production of micotoxins

TABLE 2
Minimal a_w[a] for Growth of Food-Borne Pathogens in Laboratory Media (at Optimum pH and Temperature)

Pathogen	Minimal a_w
Campylobacter jejuni	0.990
Aeromonas hydrophilia	0.970
Clostridium botulinum E	0.965
Clostridium botulinum G	0.965
Shigella spp.	0.960
Yersinia enterocolitica	0.960
Clostridium perfringens	0.945
Clostridium botulinum A and B	0.940
Salmonella spp.	0.940
Escherichia coli	0.935
Vibrio parahaemolyticus	0.936
Bacillus cereus	0.930
Listeria monocytogenes	0.920
Staphylococcus aureus (anaerobic)	0.910
Staphylococcus aureus (aerobic)	0.860

[a] Usually adjusted by the addition of sodium chloride.

From Chirife, J., 1993. *Food Control*, 4:210–215.

et al., 1981b); this process is often referred to as osmoregulation. Chirife et al. (1981b) calculated intracellular a_w from the solute composition of various bacterial cells (halophilic and nonhalophilic) grown in media of a_w between 0.85 and 0.993 and found that the intracellular a_w was generally equal or slightly lower than that of the growth medium. Their results are shown in Figure 15. The biochemical basis of osmoregulation has been the subject of numerous studies in the last decade (Anderson and Witter, 1982; Csonka, 1989; Landfald and Strom, 1986; Larsen et al., 1987; Gervais et al., 1992).

Franks (1991a) and Slade and Levine (1991a) have questioned the usefulness of specifications of values of a_w required for microbial safety and stability of semimoist foods on the basis of the so-called specific solute effect. Since the early reports by Scott (1953), it has been known that the microbial response may differ at a particular a_w when the latter is obtained with different solutes; and it has been established that the a_w of the medium is not the only determining factor regulating the biological response, but that the nature of the a_w-controlling solute also plays a role (Christian, 1981; Gould, 1988; Ballesteros et al., 1993). Gould (1985) acknowledged that in some instances solute effects may depend on the ability of the solute to permeate the cell membrane. Glycerol, for example, readily permeates the membrane of many bacteria and so does not initiate the same osmoregulatory response as nonpermeant solutes such as sodium chloride and sucrose, and therefore has a different (usually lower) inhibitory a_w. Table 3 shows this effect for many pathogenic bacteria; with the exception of *Staphylococcus aureus,* for which the reverse is true, bacteria are somewhat more tolerant to glycerol (lower minimal a_w) than to sodium chloride. The situation of *S. aureus* is discussed later in more detail. However, when the solutes most often present in reduced a_w-preserved foods (e.g., sodium chloride, sucrose, glucose, and potassium chloride) are used to control a_w, specific solute effects are less evident, as shown in Table 4. Christian and Scott (1953) studied the growth of *Salmonella oranienburg* in B.H. medium, using three different methods for controlling a_w: (1) adjusting the

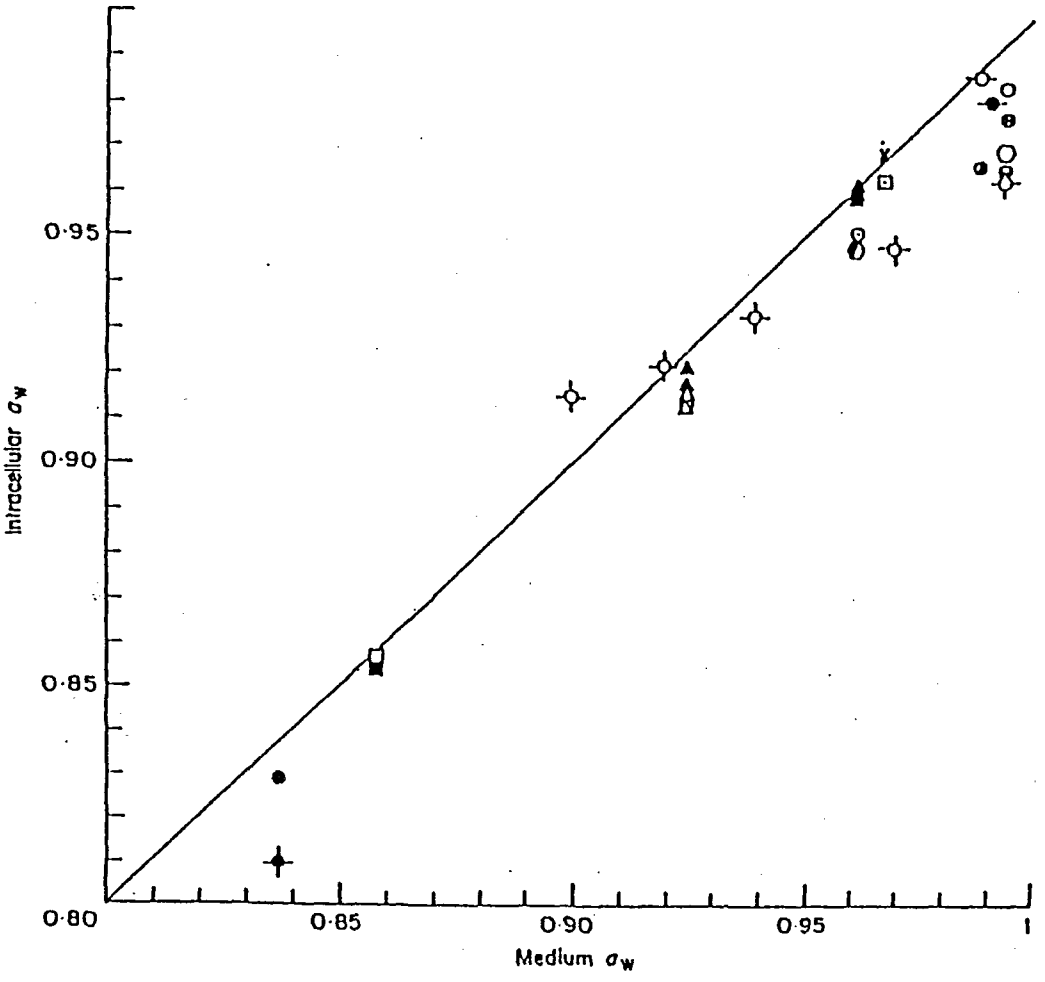

FIGURE 15. Comparison of intracellular and growth medium a_w for various halophilic and nonhalophilic bacteria. (From Chirife, J., et al., 1981. *J. Appl. Bacteriol.*, 50:475–479.)

water contents to the desired a_w; (2) adding the appropriate amounts of an NaCl:KCL:Na_2SO_4 (5:3:2) mixture to a basal B.H. medium; and (3) adding sucrose to the basal medium. Growth was inhibited in all three media at a_w values between 0.95 and 0.94. These data are relevant to food preservation because the solutes for which the specific effects are more evident, such as glycerol, propylene glycol, butylene glycols, polyethylene glycols, and the like, have at present little chance to be used for a_w control in human foods, either because of regulatory or of consumer demands for the so-called green label foods. Franks (1991a) and Slade and Levine (1991a), suggested that because microbial growth is subject to specific molecular and/or ionic interactions whether there is some other parameter or set of parameters, it could form the basis of a criteria of cell activity as influenced by water and the aqueous environment. In other words, specific effects must be taken into account in practical situations where it is desired to relate microbial growth to the nature of the aqueous environment. Included among these factors are a parameter to measure the permeability of the membrane to solutes present and a factor that takes the specific toxic effects of the molecules into account (Gould and Christian, 1988). Unfortunately, at present there

TABLE 3
Solute Effect (Sodium Chloride vs. Glycerol) on the Minimal a_w Supporting Growth of Pathogenic Bacteria in Laboratory Media[a]

Bacteria	a_w adjusted with NaCl	a_w adjusted with Glycerol
Clostridium botulinum E	0.966	0.943
Clostridium botulinum G	0.966	
Escherichia coli	0.949	0.940
Clostridium perfringens	0.945	0.930
Salmonella spp.	0.941	
Clostridium botulinum A and B	0.940	0.930
Vibrio parahaemolyticus	0.932	0.911
Bacillus cereus	0.930	0.920
Listeria monocytogenes	0.920	0.900
Staphylococcus aureus	0.860	0.890

[a] The a_w values quoted by different authors were checked using theoretical models for the prediction of a_w in aqueous solutions.

TABLE 4
Minimal a_w for Growth of Pathogenic Bacteria in Laboratory Media of a_w Adjusted with Salts (NaCl, KCl) or Sugars (Sucrose, Glucose)

Bacteria	NaCl	KCl	Sucrose	Glucose
Listeria monocytogenes	0.92	—	0.92	—
Vibrio parahaemolyticus	0.936	0.936	0.940	—
Clostridium botulinum G	0.965	—	0.965	—
Clostridium botulinum E	0.972	0.972	0.972	0.975
Clostridium perfringens	0.945	—	—	0.945
Staphylococcus aureus	0.864	0.864	0.867	—

From Chirife, J., 1993. *Food Control,* 4:210–215.

is no such parameter or set of them to replace (or to improve) the usage of a_w.

The effect of the solute used to adjust the a_w on the minimal a_w for growth of *Staphylococcus aureus* has been reviewed recently by Chirife (1994). For some solutes such as sodium chloride and sucrose the minimal a_w supporting growth is in the vicinity of 0.86; however, the minimal a_w allowing growth was well above 0.86 when solutes such as alcohols, diols, or polyethylene glycols were used to control a_w. This effect is shown in Table 5. Values range from as high as 0.975 for ethanol to as low as 0.86 for sucrose and sodium chloride. For various substrates (sodium chloride, salts mixture, sucrose and salts, dried soup, and dried milk) in which the minimal a_w for growth is relatively close to 0.86, a_w is certainly a much better indicator than moisture content, because the latter shows a pronounced variation in the different systems, as also shown in Table 5.

Slade and Levine (1991a) strongly questioned the usefulness of specifications of values of a_w required for microbial safety of IMF, for example, $a_w = 0.85$ for the growth of pathogenic bacteria (*S. aureus*) on the basis of specific solute effects. Chirife (1994) also examined the growth behavior of *S. aureus* in solutions whose a_w values were controlled using 16 different solutes comprising

TABLE 5
Minimal a_w (a_{wm}) for Growth of *S. aureus* at 30 to 37°C

	a_{wm}	Moisture content for a_{wm} = 0.86 (g H_2O/100 g solids)
Ethanol	0.975	
1,3-Butylene glycol	0.97	
Propyleneglycol	0.96	
PEG-200	0.93	
PEG-400	0.93	
Glycerol	0.89	
Sodium acetate	0.88	
Dried meat	0.88	
Glucose + salts	0.88	
Sucrose	0.867	
Sucrose + salts	0.86	74.2
Salts mixture	0.86	312
Dried milk	0.86	15.1
Dried soup	0.86	62.2
NaCl	0.86	445.3

From Chirife, J., 1994 *J. Food Eng.*, 22:409–419.

inorganic and organic salts, sugars, polyols, etc., including solutes that are unlikely to be ever used in foods. He reported that although the minimal a_w for growth depended in various cases on the solute used to adjust it (as noted above; Table 5), *S. aureus* could not grow below the current widely accepted minimal $a_w = 0.86$; this is a result of the utmost importance regarding food safety and actual food regulations. Ballesteros et al. (1993) studied specific solute effects on *S. aureus* cells subjected to reduced a_w activity controlled by the addition of solutes such as sodium chloride, sucrose, propylene glycol, butylene glycol, and various polyethylene glycols. As a first approximation they considered other modifications brought about by dissolution of solutes (in the growth medium) in addition to lowering of a_w and specific interactions between cell and solute. They reported that there is not a clear relationship between the *S. aureus* response to solute dissolution and the modification of certain physical properties of the medium (viscosity, dielectric constant, oxygen solubility, and oxygen diffusivity). A transmission electron microscopic study revealed that the inhibitory effects of sucrose and sodium chloride (important food solutes) on *S. aureus* were primarily ascribed to their a_w lowering abilities, showing no significant specific solute effects. However, the other solutes examined showed specific antibacterial activity against *S. aureus*, which may be compatible with cell wall attack.

IX. USE OF a_w AS A PREDICTOR OF MICROBIAL STABILITY IN ACTUAL FOODS: CHALLENGE OF MINIMAL a_w LIMITS FOR GROWTH

Minimal a_w values for growth of different microorganisms have been mostly determined in liquid laboratory media, using sodium chloride to depress the a_w (Baird-Parker and Freame, 1967; Briozzo et al., 1986; Emodi and Lechowich, 1969). As discussed in other sections of this review, there is no doubt that measured RVP in liquid laboratory media are equilibrium ones and thus $a_w = p/p_o$, because the viscosity of NaCl solutions is very small compared with the values corresponding to the onset of rubbery, nonequilibrium behavior (Soesanto and Williams, 1981). However, if we accept the idea that in a solid semimoist food, equilibrium may not be established during the time of observation (Slade and Levine, 1991a), the recorded vapor pressure will be mistaken for equilibrium, and this will create some concerns

about the safety of measured a_w values in foods with regard to microbial growth inhibition. This situation may be explored using literature data.

It has been well established that the minimum a_w for the growth of *Clostridium botulinum* types A and B in liquid broth media adjusted with NaCl is 0.94 to 0.95 (Baird-Parker and Freame, 1967; Ohye and Christian, 1966). Glass and Doyle (1991) confirmed this minimum value of a_w for solid foods in a study of the relationship between a_w of fresh pasta (meat- or cheese-filled tortellini and flat noodle linguine or fettucini) and toxin production by *Clostridium botulinum.* Four types of fresh pasta were prepared with different a_w inoculated with *C. botulinum,* packaged under modified atmosphere, and stored at 30°C for 8 to 10 weeks. The pH of all samples was favorable to *C. botulinum* growth. No toxin was detected in tortellini with an a_w of 0.94 when held at 30°C for 10 weeks. Toxin was produced at 2 weeks in linguine at an a_w of 0.96 held at 30°C, whereas linguine or fettucini at an a_w of 0.93 or 0.95 and held at 30°C did not become toxic during 8 to 10 weeks incubation. Glass and Doyle (1991) concluded that the a_w of fresh pasta is a principal factor in preventing botulinal toxin production in temperature-abused products. These results are in good agreement with predictions made from the behavior of *C. botulinum* in liquid broth media of known a_w and suggest that, at least within the time frame of the experiments, nonequilibrium effects in the determination of a_w of pasta are not significant. Dodds (1989) reported a study of the effects of a_w on toxin production by *C. botulinum* in cooked, vacuum-packaged potatoes whose a_w was controlled by the addition of NaCl and which was incubated up to 60 d at 25°C. Toxin was produced at an a_w of 0.96, but no toxin was detected when the a_w was 0.955, in good agreement with predictions made from the behavior of the bacterium in liquid broth media. These results also suggest that in the time frame of the experiments (which in some cases may be coincident with the shelf life of the product) nonequilibrium effects do not significantly influence the determination of a_w in potatoes, and, consequently, the bacterial response may be predicted adequately. Valik and Gorner (1993) studied the growth of *S. aureus* in pasta dough in relation to its a_w and found that the bacterium appeared to multiply until the a_w was below 0.86, at which stage it decreased; this is in good agreement with the minimal water activity ($a_w = 0.86$) for growth of *S. aureus* determined in liquid broth media adjusted with NaCl (Vaamonde et al., 1982; Chirife, 1994). Giannuzzi and Parada (1991) studied the behavior of *S. aureus* (three strains) in dehydrated milk, beef, and pork equilibrated at a_w values of 0.84 and 0.90 and incubated at 30°C for up to 30 d. No growth was observed in any of the foods at $a_w = 0.84$; however, growth occurred in all systems at $a_w = 0.90$; this agrees with the known behavior of *S. aureus* in liquid broth media. Silverman et al. (1983) reported that a_w was the main parameter in controlling *S. aureus* growth in precooked bacon. They reported that the limiting a_w for growth of *S. aureus* A-100 in bacon sealed in cans and stored up to 28 d at 37°C was 0.87 in good agreement with known behavior in liquid broth media, as noted above. King et al. (1984) studied the effect of a_w on mold growth in stored almond nutmeat. The nutmeats were equilibrated at different a_w values by placing in closed jars over saturated salt solutions; equilibration time was 1 month. No mold growth was observed after 18 months on almonds stored at $a_w = 0.70$, which is in good agreement with predictions made from the behavior of molds in laboratory media of controlled a_w (Beuchat, 1983).

X. THE SAFETY OF a_w ADJUSTMENT/ MEASUREMENT FOR THE CONTROL OF BACTERIAL GROWTH IN FOODS

For foods in which the a_w is a main factor controlling development of microbial hazard or spoilage, one must be certain that samples do not exceed a specified a_w. In this regard it is important to examine some aspects concerning the safety of a_w adjustment/measurement for the control of microbial growth in foods. These include the effect of processing and storage conditions on subsequent a_w, the utilization of proper values for the limiting a_w (solute effect; already discussed), and the error in the measurement of a_w (Chirife, 1993). For years now, mechanical, electrolytic, capacitance, or dew-point hygrometers have been used mostly to measure the a_w of foods. Recently, a new instrument for determining a_w in meat products has been introduced (Rodel et al., 1990). It is based on the thermometric determination of the

initial freezing point and is marketed in Germany (NAGY a_w-Kryometer). Another instrument also introduced recently to the market is the Water Activity Meter manufactured by Ottawa Instrumentation Ltd. (Canada). It consists of a thermometric device that unequivocally shows whether samples complied with a standard (Sharp et al., 1991). It is claimed that neither devices (NAGY a_w-Kryometer and Ottawa a_w-Meter) are affected by nonaqueous volatiles from foods (Rodel et al., 1990; Sharpe et al., 1991). For most electrical hygrometers, the confidence interval (with three to four replicate measurements) is in the range of ±0.005 (Labuza et al., 1976; Troller, 1977; Favetto et al., 1983; Kitic et al., 1986). However, collaborative studies of a_w measurements in different food systems showed a larger discrepancy, for example, in the range ±0.01 to ±0.02 (Labuza et al., 1976; Aguilera et al., 1990). It was generally agreed that a_w results must be reported to only two decimal places (Troller and Scott, 1992). Several factors are responsible for this error in the measurements, including (1) sensor contamination with nonaqueous food volatiles such as propylene glycol, ethanol, acetic acid, etc. (Pollio et al., 1986); (2) lack of agreement between different laboratories on values to be assigned to certain reference standards of saturated salt solutions; and (3) sensor calibration problems. Resnik et al. (1984) performed a world survey of a_w at 25°C of selected saturated salt solutions used as standards in the range of microbial growth; their results showed that although good agreement was observed on the value assigned to certain salts, a significant discrepancy in the values assigned to others existed. Chirife et al. (1983) used a theoretical approach based on the thermodynamic properties of strong electrolyte aqueous solutions to predict the a_w of saturated solutions. Table 6 shows calculated values for some selected salt solutions. In an attempt to reach a worldwide agreement on the a_w of standards used for calibration, Resnik and Chirife (1988) compiled and proposed a set of theoretical a_w values for saturated salts, sulfuric acid, and sodium chloride solutions to be used as standards in the range of microbial growth. Some of their results are shown in Table 7.

Sensor contamination with nonaqueous food volatiles is also a problem in a_w measurements. Although some manufacturers provide filters to protect the sensor, the use of these filters usually increases the equilibrium time for a_w readings due to additional resistance to mass transfer in the gas space between the sample and the sensor.

In order to take into account the error involved in the measurement of a_w in foods, it is convenient to set a safety margin in the selection of the working a_w value, for example, ±0.01 to ±0.02.

XI. GLASS TRANSITION IN SOLID AMORPHOUS FOODS AND BIOPOLYMERS: DOES GLASSY STATE PREVENT THE GROWTH OF MICROORGANISMS?

The characteristic temperature, T_g, at which the glass-rubber transition occurs has been pro-

TABLE 6
The a_w (at 25°C) of Selected Saturated Salt Solutions Used as Standards in the Range of Microbial Growth

Salt	Robinson and Stokes, 1965	Most accepted[a]	Predicted[b]
NaBr	0.577	0.577	
NaCl	0.753	0.753	0.751
$(NH_4)_2SO_4$	0.801	0.801	0.803
KCl	0.843	0.843	0.843
$BaCl_2$	0.902	0.902	0.903
KNO_3	0.925	0.925	
K_2SO_4		0.972	0.975

[a] From Resnik et al., 1984.
[b] From Chirife et al., 1983.

TABLE 7
Predicted a_w Values of Saturated and Nonsaturated Solutions

Predicted a_W Values of NaCl Solutions between 15 and 50°C

Conc. (% w/w)	a_w	Conc. (% w/w)	a_w	Conc. (% w/w)	a_w	Conc. (% w/w)	a_w
0.5	0.997	7.0	0.957	13.5	0.906	20.0	0.839
1.0	0.994	7.5	0.954	14.0	0.902	20.5	0.833
1.5	0.991	8.0	0.950	14.5	0.897	21.0	0.827
2.0	0.989	8.5	0.946	15.0	0.892	21.5	0.821
2.5	0.986	9.0	0.943	15.5	0.888	22.0	0.815
3.0	0.983	9.5	0.939	16.0	0.883	22.5	0.808
3.5	0.980	10.0	0.935	16.5	0.878	23.0	0.802
4.0	0.977	10.5	0.931	17.0	0.873	23.5	0.795
4.5	0.973	11.0	0.927	17.5	0.867	24.0	0.788
5.0	0.970	11.5	0.923	18.0	0.862	24.5	0.781
5.5	0.967	12.0	0.919	18.5	0.857	25.0	0.774
6.0	0.964	12.5	0.915	19.0	0.851	25.5	0.766
6.5	0.960	13.0	0.911	19.5	0.845	26.0	0.759

Predicted a_w Values of Selected Saturated Salt Solutions between 10 and 37°C

Temp. (°C)	NaCl	$(NH_4)_2SO_4$	KCl	$BaCl_2$	K_2SO_4
10	0.754	0.809	0.867	0.913	0.980
15	0.753	0.808	0.859	0.910	0.979
17	0.753	0.806	0.856	0.909	0.978
19	0.752	0.805	0.852	0.907	0.977
21	0.752	0.804	0.849	0.906	0.977
23	0.751	0.803	0.846	0.905	0.976
25	0.751	0.803	0.842	0.903	0.975
27	0.750	0.802	0.840	0.902	0.975
29	0.750	0.801	0.836	0.900	0.974
31	0.750	0.800	0.833	0.899	0.973
33	0.749	0.799	0.830	0.898	0.973
35	0.749	0.798	0.827	0.895	0.972
37	0.748	0.797	0.823	0.894	0.971

From Resnik, S. L. and Chirife, J., 1988. *J. Food Protect.*, 51:419–423.

posed as a physicochemical parameter that can determine product properties, stability, and safety of foods (Slade and Levine, 1987; 1991a; Levine and Slade, 1992). In a glass (at $T < T_g$), the rates of all diffusion-limited processes are much lower than in the rubbery state (at $T > T_g$). van den Berg (1991) noted that according to Slade and Levine's arguments, a food taken to below T_g should be perfectly stable in terms of any ordinary food chain from producer to the consumer, and the use of the T_g-water content relationship for the considered food product could eliminate the necessity of using a_w to predict food stability. Gould and Christian (1988) stated, however, that although high-viscosity states would greatly interfere with the growth of microorganisms in foods (for instance, through the restriction of diffusion of nutrients), this is an area where much remains to be done in order to identify the potential for deliberate use of high-viscosity states to inhibit microbial growth. In recent years a considerable amount of data have been reported on the effect of moisture content on the T_g of food systems. These data offer the opportunity to challenge the role of the glassy state on microbial growth inhibition, because above T_g, mobility is greatly increased. The

difference between the storage temperature and T_g ($T-T_g$) has been used to correlate changes in mechanical properties above T_g (Soesanto and Williams, 1981). Moisture content profoundly affects T_g in foods (Slade and Levine, 1987), and the rates of diffusion-limited processes would be much more rapid in the rubbery liquid state (governed by WLF kinetics [Karel et al., 1993]) than in the glassy solid state (governed by Arrhenius kinetics [Slade and Levine, 1991a]). The behavior of T_g vs. a_w for some plant materials (carrots, strawberries, potatoes, onions, and apples) and biopolymers (wheat starch, wheat gluten, and gelatin) is shown in Figure 16. Figure 17 shows the effect of moisture content on T_g of apple and onion. It is noticed that values for biopolymers are much higher than for plants (Figure 16). Although some biopolymers such as starch have high T_g values even at high values of a_w, it is likely that in many real foods the presence of relatively small amounts of low-molecular-weight solutes (e.g., sugars) would depress the T_g. Figure 18 shows the effect of low-molecular-weight solutes on the T_g values of some biopolymers. The T_g of amylopectin and maltodextrin DE 10 are reduced by the addition of 9% fructose or 7.5% (total solid basis) of D-xylose and lysine. For example, the addition of 7.5% of lysine and D-xylose reduced the T_g of maltodextrin by about 15 to 23°C. The glass curves of potato and starch are compared in Figure 19; despite the fact that starch is the main component of its dry matter, the T_g of potato is much lower than that of starch. This may be attributed to low-molecular-weight soluble components (glucose, fructose, sucrose, and minerals) present in potato tissue. It is interesting to note that the behavior of sorption isotherms is not influenced in the same manner; as shown in Figure 20, the adsorption isotherms of potato starch and potato tissue are not very different.

Chirife and Buera (1994) combined literature data on sorption isotherms with the particular

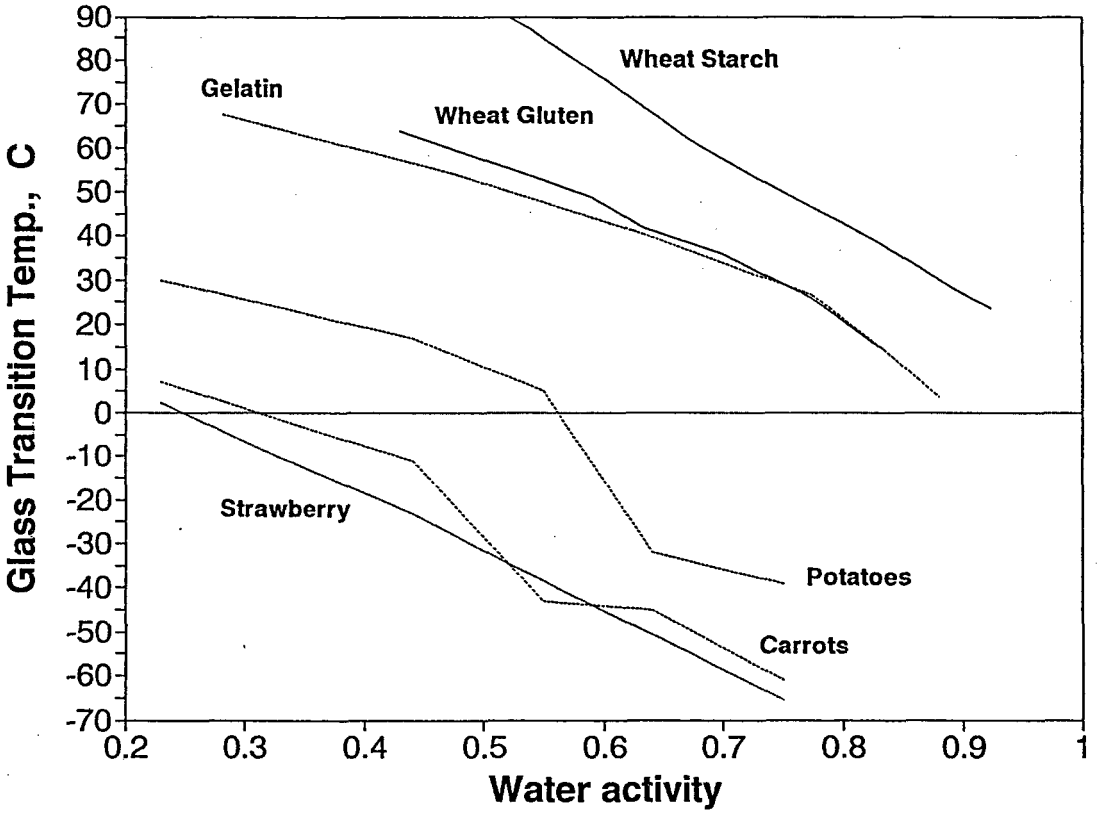

FIGURE 16. Relationship between a_w and T_g of various plant materials and biopolymers. (From Chirife, J. and Buera, M. P., 1995. *J. Food Eng.*)

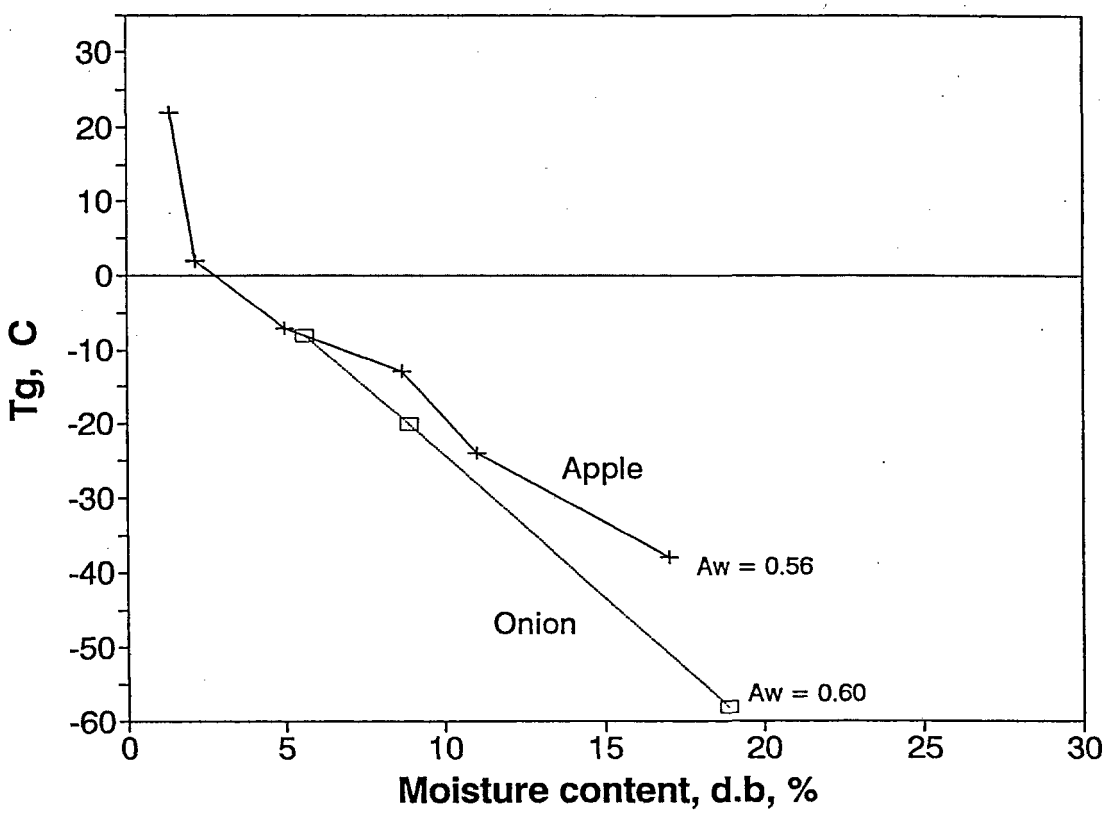

FIGURE 17. Effect of moisture content on T_g of apple and onion. (From data reported by Nelson, 1993 and Iglesias and Chirife, 1982).

T_g-moisture content curve of different food systems and the corresponding $T–T_g$ was calculated for a typical incubation temperature (30°C) and for selected ranges of a_w. Their results are shown in Table 8; additional data for celery (Karel et al., 1993), bread (Le Meste et al., 1992), casein (Kalichevsky et al., 1993), onion, and apple (Nelson, 1993) are included. First of all, it should be indicated that it is not easy to separate the individual effects of a_w and physical state (glassy or rubbery) on microbial growth because in many foods the matrix is glassy at a m.c. at which the a_w is below the limiting value for growth. For moisture contents (or a_w) typical of the intermediate-moisture range, all foods examined (cabbage, potato, horseradish, strawberries, carrot, celery, onion, and apple), with the exception of bread, are in the rubbery state, as indicated by the corresponding values of $(T–T_g)$. In fact, the values of $T–T_g$ may well be approaching 90 to 100°C for a_w of about 0.8, a value at which many microorganisms cannot develop due to the osmotic stress. The exception is bread, which is in the glassy state even at a_w as high as 0.85. For the isolated biopolymers the behavior is diverse. Collagen and maltodextrin are in the rubbery state in practically the entire intermediate-moisture range, but for casein and starch, the glassy state covers most of the IM range. Other biopolymers are in the glassy state in the vicinity of $a_w = 0.65$, but they are rubbery above an a_w of about 0.8.

Figure 21 shows a comparison of the sorption isotherms of strawberries (Roos, 1987) and prunes (Tsami et al., 1990); some selected values of $T–T_g$ for strawberries at 25°C (calculated from T_g values measured by Roos [1987]) are indicated on the sorption isotherm for strawberries. Both isotherms are similar and this is expected because strawberries and prunes have about the same sugar content (50 and 54% d.b., respectively). Dried

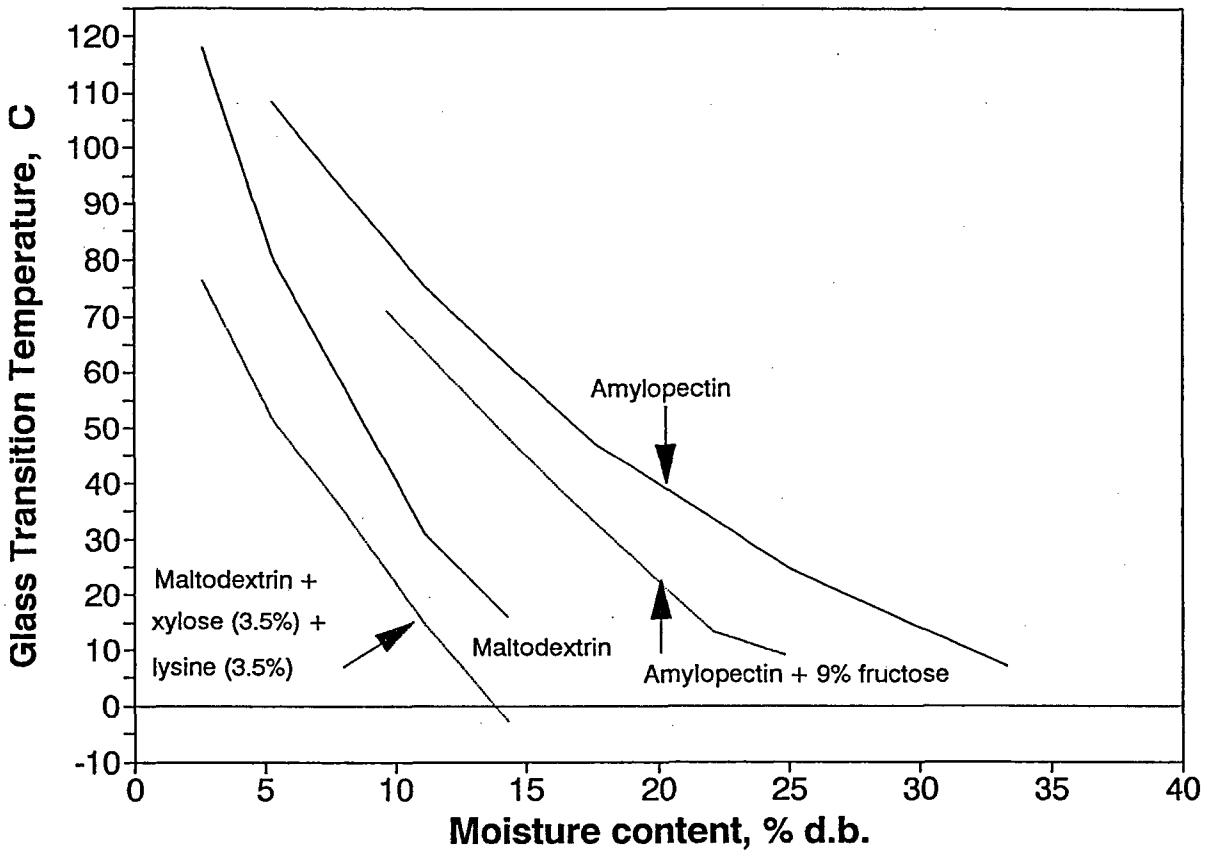

FIGURE 18. Effect of the addition of low-molecular-weight compounds on the glass curves of amylopectin-water and maltodextrin DE 10-water. (From Chirife, J. and Buera, M. P., 1994. *J. Food Eng.*)

fruits are nonequilibrium systems because the sugars are in an amorphous metastable state that is very sensitive to changes in moisture content and temperature; however, they are usually maintained in a state of pseudostability because it may last longer than the product's lifetime. Crystallization of the amorphous sugars are probably prevented by the nonsugar biopolymer matrix (Iglesias and Chirife, 1978). It may be assumed that the T_g–moisture content relationship for prunes and strawberries are similar; thus one may expect that the value of $T–T_g$ for prunes at, for example, 20% d.b., which corresponds to $a_w = 0.62$ (see dotted line in Figure 21), should be higher than 59°C. At this value the food matrix is highly plasticized by water and mobility effects (e.g., diffusion) are greatly enhanced. Nevertheless, at this moisture content prunes are resistant to microbial growth (Pitt and Christian, 1968) so it is to be concluded either that (1) if molecular mobility is determined by $T–T_g$, this effect plays little role on microbial growth inhibition (a_w seems to be the controlling parameter) or (2) molecular mobility is still reduced at high values of $T–T_g$, so growth does not occur, in which case $T–T_g$ does not determine mobility. This situation is illustrated in Figure 22 in which "transformed" (Slade and Levine, 1991a) selected water sorption data for prunes and the glass curve for strawberries (which is likely to be similar for prunes [Karmas et al., 1992]) are plotted; it can be seen that a combination of moisture-temperature at which microbial growth in prunes was inhibited is located well above the glass curve, that is, in the rubbery state.

The glass transition behavior of dried milk as a function of moisture content (and a_w) has been reported recently by Jouppila and Roos (1994)

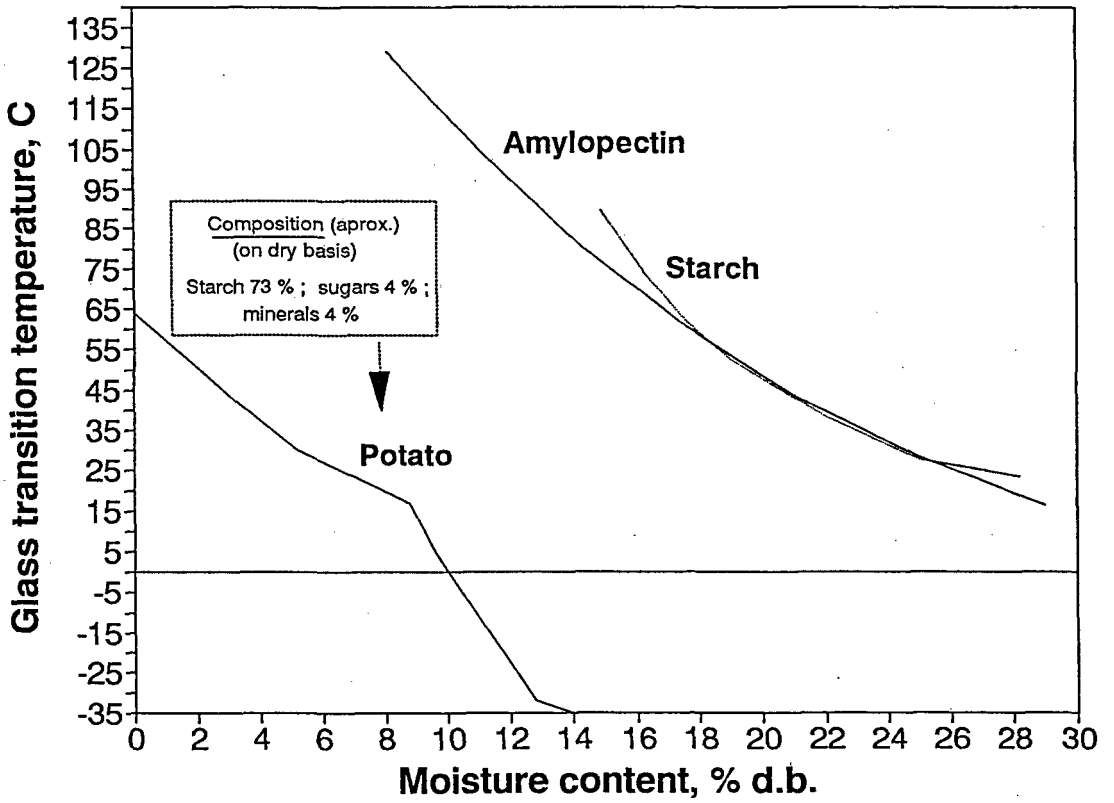

FIGURE 19. Comparison of T_g temperatures of potato (from data reported by Karmas et al., 1992) to that of starch (from data reported by Zeleznak and Hoseney, 1987) and amylopectin. (From data reported by Kalichevsky and Blanshard, 1993.)

and is shown in Figure 23. Giannuzzi and Parada (1991) studied the behavior of *Staphylococcus aureus* (three strains) in dehydrated milk at a_w values of 0.84 and 0.90 and incubated at 30°C for up to 30 d. Their results are shown in Figure 24. No growth is observed for any of the three strains at $a_w = 0.84$. Nevertheless, at this a_w, dried milk should have passed through the rubbery state (estimated value of $T-T_g$ above 82°C), and lactose probably should have recrystallized due to the increased mobility (Jouppila and Roos, 1994). In any case, mobility effects cannot be considered the sole determinants of bacterial growth inhibition, and it is likely to be controlled by a_w. It is noteworthy that in the *S. aureus* growth experiments performed by Giannuzzi and Parada (1991), a_w should have remained constant despite some water loss due to lactose crystallization, because dry milk was kept in a closed desiccator over a saturated salt solution (KCl), $a_w = 0.84$).

In his pioneering work on water activity, Scott (1953) also found that *S. aureus* cannot grow in dried milk at $a_w < 0.86$ (e.g., moisture content 16% d.b.), and this indicates inhibition of growth in a highly plasticized dried milk matrix (Jouppila and Roos, 1994).

Bothast et al. (1981) performed a study of the effects of moisture content and temperature on microbiological stability of wheat flour during storage. They reported that the mold count (*Aspergillus glaucus* and *A. candidus* were predominant) increased in flour with 17.6% moisture (d.b.) during storage at 25 or 34°C; the CO_2 in the headspace paralleled the increase in mold counts. Figure 25 shows the adsorption isotherms of wheat flour and wheat starch (Bushuk and Winkler, 1957), which are very similar because starch is by far the main component of the dry matter in flour. At a moisture content of 17.6% d.b. the a_w of flour is about 0.76, and this value will permit the growth

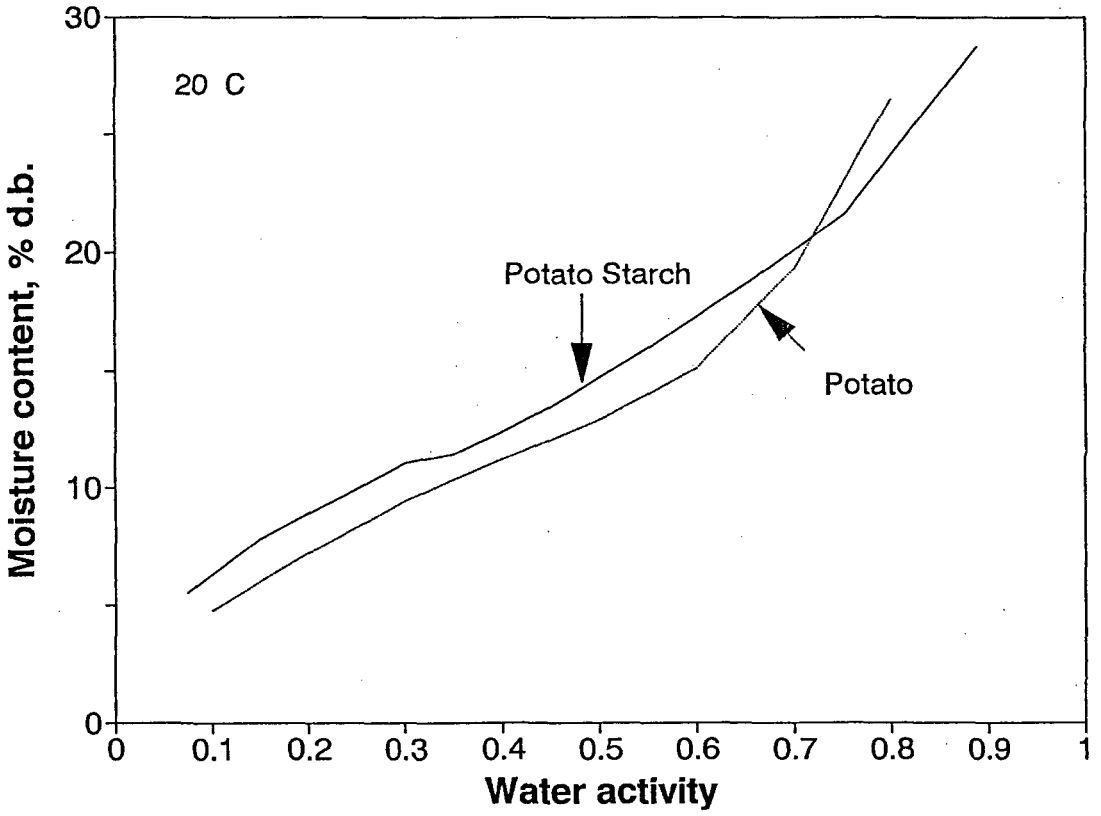

FIGURE 20. Comparison of water adsorption isotherms of potato starch (from data reported by van den Berg, 1981) and potato. (From data reported by Crapiste and Rotstein, 1982.)

of various xerophilic molds (Beuchat, 1983) as experimentally observed by Bothast et al. (1981). The T_g-moisture content curve for wheat flour is not available to us, but those of starch (main component) and gluten are reported in the literature. Figure 26 shows the effect of moisture content on T_g for wheat starch (Zeleznak and Hoseney, 1987; Roos and Karel, 1991b), gluten (Hoseney et al., 1986), and also lignin (Slade and Levine, 1991a), which is a minor component of dietary fiber in flour. At 17.6% moisture (d.b.) the T_g of starch and gluten are 63 and 21°C, respectively, but because starch is by far the main constituent of flour (starch, 80 to 82%; gluten, 12 to 14% d.b.), it may be assumed that wheat flour at this moisture content and stored at 25°C is likely to be glassy. However, mold growth (and CO_2 generation) was observed (Bothast et al., 1981). King et al. (1984) demonstrated that *Xeromyces* sp. and *Chrysosporium* sp. were able to grow in wheat flours during storage (25°C) at a_w values ranging from 0.66 to 0.68. Applying the same arguments as above, one may also conclude that wheat flour at the corresponding moisture contents (Bushuk and Winkler, 1957) is likely to be glassy. These situations are better illustrated in Figure 27, which shows "transformed" water sorption data in wheat flour and the relative location of the selected values at which growth occurred (King et al., 1984) and the glass curve for wheat flour based on data for starch. Cahagnier et al. (1993) studied the effects of different a_w values on growth of storage molds (*A. candidus* and *P. implicatum*) of maize. They reported mold growth, ergosterol accumulation, and development of fat acidity in maize of $a_w = 0.82$ stored at 30°C. Moisture content of maize at this a_w is about 18 to 19% d.b. (Iglesias and Chirife, 1982). At this moisture content the

TABLE 8
Approximate Values of $T-T_g$ at a_w Values in the Range of Microbial Growth for Various Food Systems at 30°C

	a_w	$T-T_g$ (°C)
Foods		
Apple	0.55	68
Bread, white	0.75	–20
	0.85	–8
Cabbage	0.64	84
	0.75	99
Carrot	0.64	75
	0.75	91
Celery	0.63	84
Horseradish	0.75	74
Onion	0.60	88
Potato	0.64	62
Strawberry	0.65	80
	0.75	95
Biopolymers		
Casein	0.75	–52
	0.85	–60
Collagen	0.62	42
Elastin	0.65	–223
	0.93	19
Gelatin	0.65	–16
	0.80	5
Gluten	0.65	–10
	0.81	14
Maltodextrin DE 36	0.75	65
	0.85	82
DE 5	0.75	–14
	0.84	7
Starch, wheat Native	0.65	–32
	0.89	9
Gelatinized	0.63	–14
	7	

Sources of data for the effect of moisture content on T_g: Karmas et al., 1992; Roos, 1987; Paakonen and Roos, 1990; Hoseney et al., 1986; Kakivaya and Hoeve, 1985; Batzar and Krelbich, 1981; Slade et al., 1989; Zeleznak and Hoseney, 1987; Roos and Karel, 1991b; Karel et al., 1993; Le Meste et al, 1992; Nelson, 1993; Kalichevsky et al., 1993.
Sources of data for sorption isotherms: Karmas et al., 1992; Roos, 1987; Paakonen and Roos, 1990; Bushuk and Winkler, 1957; Bull, 1944; van den Berg, 1981; Roos and Karel, 1991b; Iglesias and Chirife, 1982.

T_g of starch (main component of maize) is about 50 to 55°C (Zeleznack and Hoseney, 1987) so one might assume that maize stored at 30°C may be in the glassy state or in its proximity. Mobility of food components may be a key factor governing microbial growth in reduced moisture foods, if transport of nutrients from the food matrix is required. However, based on previous observations, we may conclude either that (1) the glassy state (based on T_g data for the main component of flour) does not preclude mold growth, or (2) although the "average" food matrix could be glassy

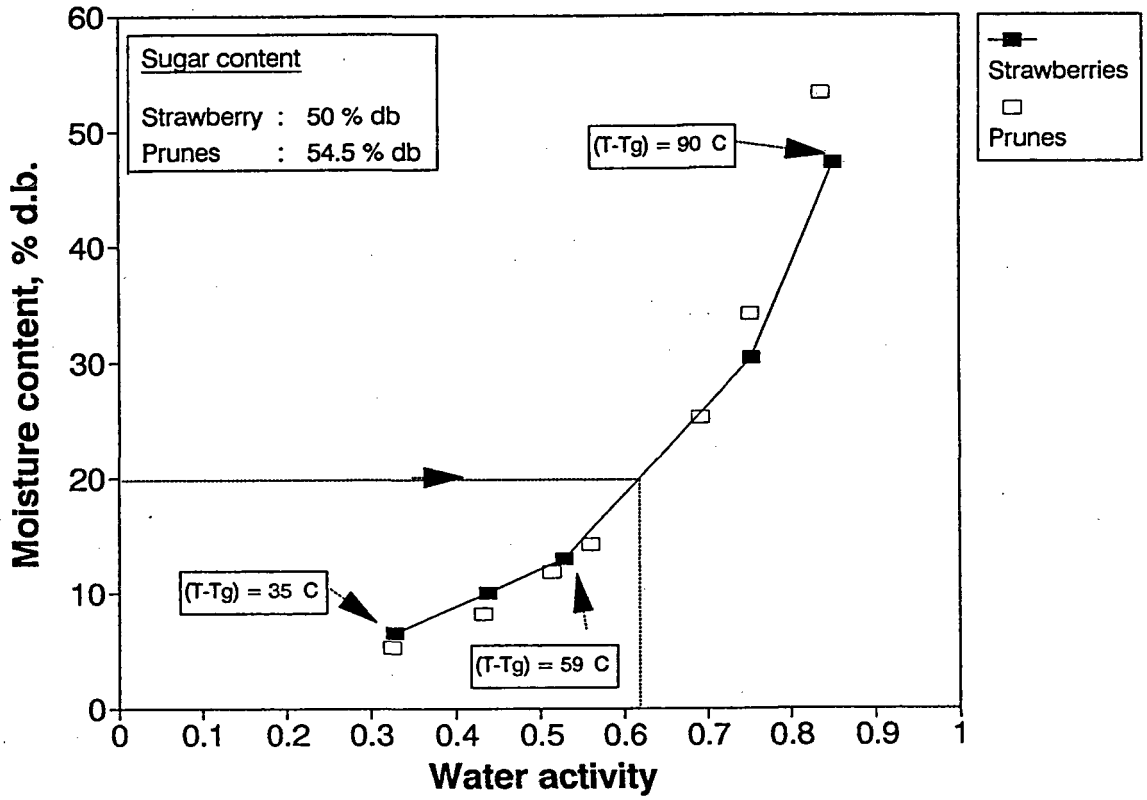

FIGURE 21. Sorption isotherms of strawberry (25°C; Roos, 1987) and prunes (30°C; Tsami et al., 1990) and selected T_g values for strawberries (Roos, 1987). Line represents moisture content-a_w at which microbial stability was observed.

(as determined by DSC), molds may grow in some non-glassy micro-regions of the substrate. The T_g for the gluten fraction is lower than that for starch, and, in wheat flour-based foods, for instance, a two phase system could be formed in which one fracture (gluten) could be rubbery and the other (starch) could be glassy. If small amounts of sugars are present, they could plasticize one of the phases selectively, or provide a separate phase in which molds may grow. If phase separations occur, each one could have its own T_g value. Even assuming that they could be detected (which is not easy if the component(s) is/are) in very low concentrations when compared with the main matrix), the T_g value that would serve as a reference to predict microbial growth would remain undetermined.

In any case, T_g data currently available for complex foods does not allow forcasting microbial stability, on the basis of mobility considerations, with confidence. Examples are given that show microbial growth in foods at glassy (or nearly glassy) conditions, and microbial inhibition of foods in the rubbery state.

XII. COMMENTS ON SOME INAPPROPRIATE GENERALIZATIONS OF FOOD APPLICATIONS OF GLASS TRANSITION-RELATED PHENOMENA

In trying to present the food polymer science approach as an all-embracing one, Slade and Levine (1987, 1991a,b, 1992) made some inappropriate generalizations of glass transition theory in areas that, although not strictly related to microbiology, also deserve some comments.

Slade and Levine (1992) stated:

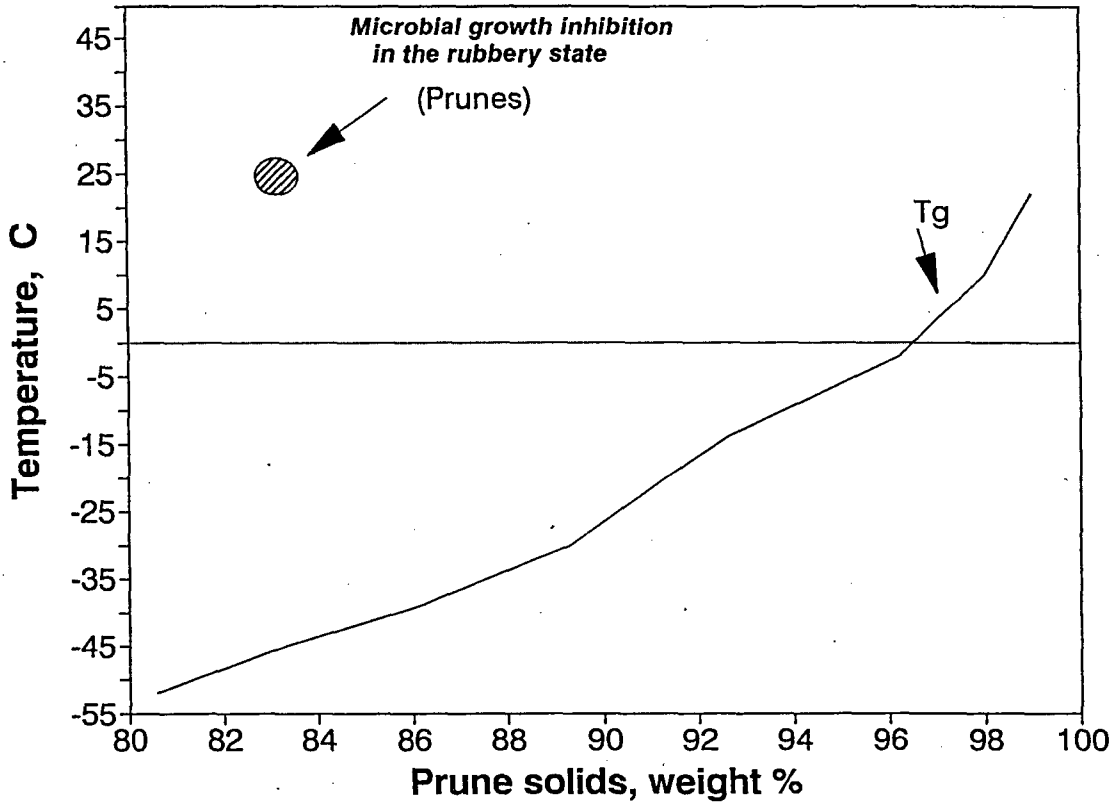

FIGURE 22. "Transformed" selected sorption data in prunes and the glass curve for strawberries (Roos, 1987), which is expected to be similar to that for prunes, to illustrate on microbial growth inhibition in the rubbery state.

Since starch and gluten are the major storage polymers of wheat endosperm, and they are synthesized, stored in the mature seed, and hydrolyzed by enzymes of germination in the same temperature and moisture environment, it appears biochemically and physiologically logical that their aqueous glass curves should be similar, if not identical.

They added that the results and conclusions of many literature studies seem to support this suggestion. As shown in Figure 28, the glass curves of wheat starch (Zeleznak and Hoseney, 1987) and wheat gluten (Hoseney et al., 1986) are far from being identical; for example, at 25% moisture (d.b.) there is a difference of about 35°C between their T_g values!!

Slade and Levine (1991a) proposed that conventional water sorption isotherms may be transformed into a water/glass dynamics map. They took combinations of moisture content and temperature that give the same value of observed RVP and replotted these data (as a series of iso-RVP contours) in the two dimensions of temperature and weight percent of solids compared to the corresponding temperature-moisture content location of a hypothetical glass curve for the solid. They performed this procedure for milled hard wheat sorption isotherms at various temperatures and compared the replotted sorption data to the corresponding temperature-moisture content of a hypothetical glass curve for wheat flour. Because they did not have the glass curve for wheat, they said that the curve shown was based on data for starch and gluten. They added that the results revealed that combinations of temperature and moisture content that fall below the glass curve give observed RVPs approaching zero. The problem is that the glass curve shown in their data does not correspond either to starch, gluten, or

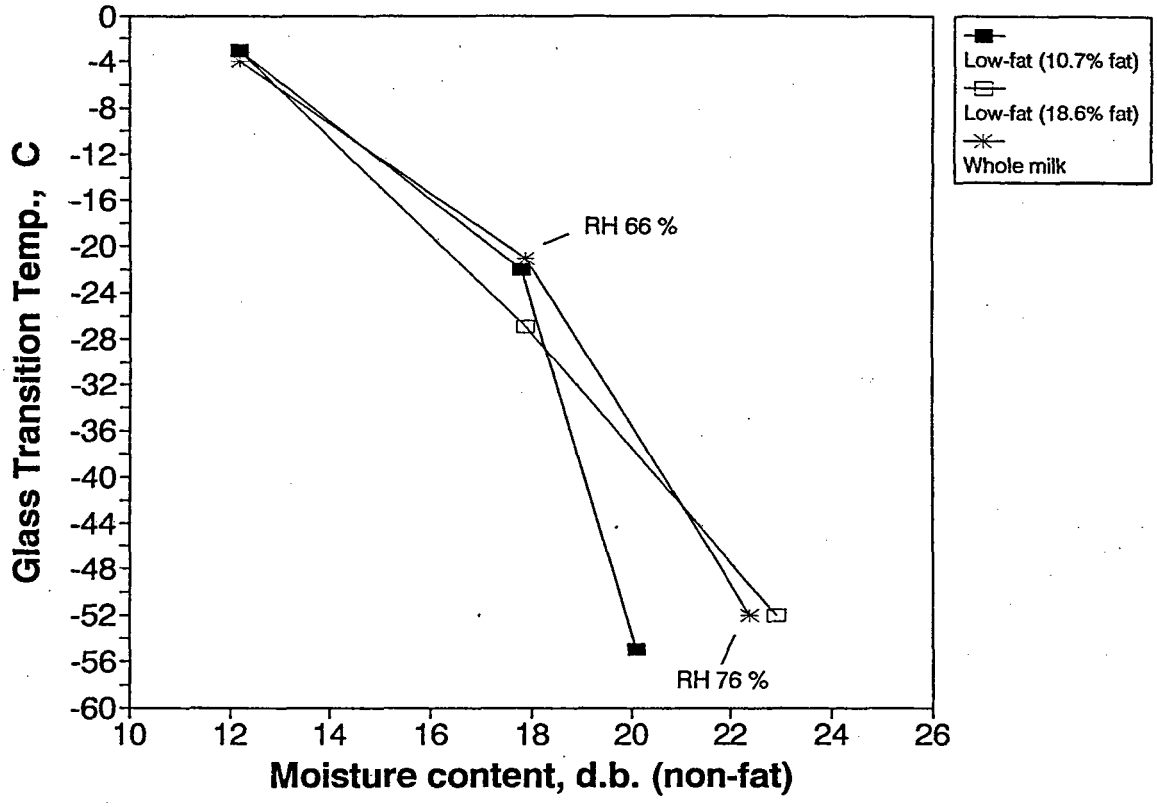

FIGURE 23. Effect of moisture content on T_g of dehydrated milk powder. (From data reported by Jouppila and Roos, 1994.)

amylopectin. The actual curve of native wheat starch (and also of amylopectin) is completely different from the glass curve they used, invalidating their conclusions. Their approach was further tested using published literature water sorption (at various temperatures) and glass transition data for wheat starch and wheat gluten. The results, shown in Figures 29 and 30, do not indicate that combinations of moisture content and temperature that fall below the glass curve give RVPs (or a_w values) approaching zero as claimed by Slade and Levine (1991a).

Slade and Levine (1991a) suggested that using the food polymer science approach to interpret results from analysis of conventional drying processes may lead to increased understanding and insight. Ease of drying can be viewed as a functional manifestation of mobility in a food system. It is known that a critical moisture content separates the period of constant rate drying (where the rate of removal as a function of time is constant) from the period of falling rate drying (where the rate of water removal as a function of time decreases dramatically and is no longer constant). They claimed that this critical point for rice and other starch-based products is about 27 to 30% water and is related to W'g (maximally freeze-concentrated glass), but do not give any serious references to corroborate this statement. Moreover, all drying literature demonstrates that the critical moisture content is not solely a function of the physical properties of the solid, but also depends on the drying conditions at the surface (e.g., air velocity, temperature, and humidity; Jason, 1958; Fornell et al., 1980). Results from Leung and Steinberg (1979) and Bakshi and Singh (1980), for example, clearly contradict

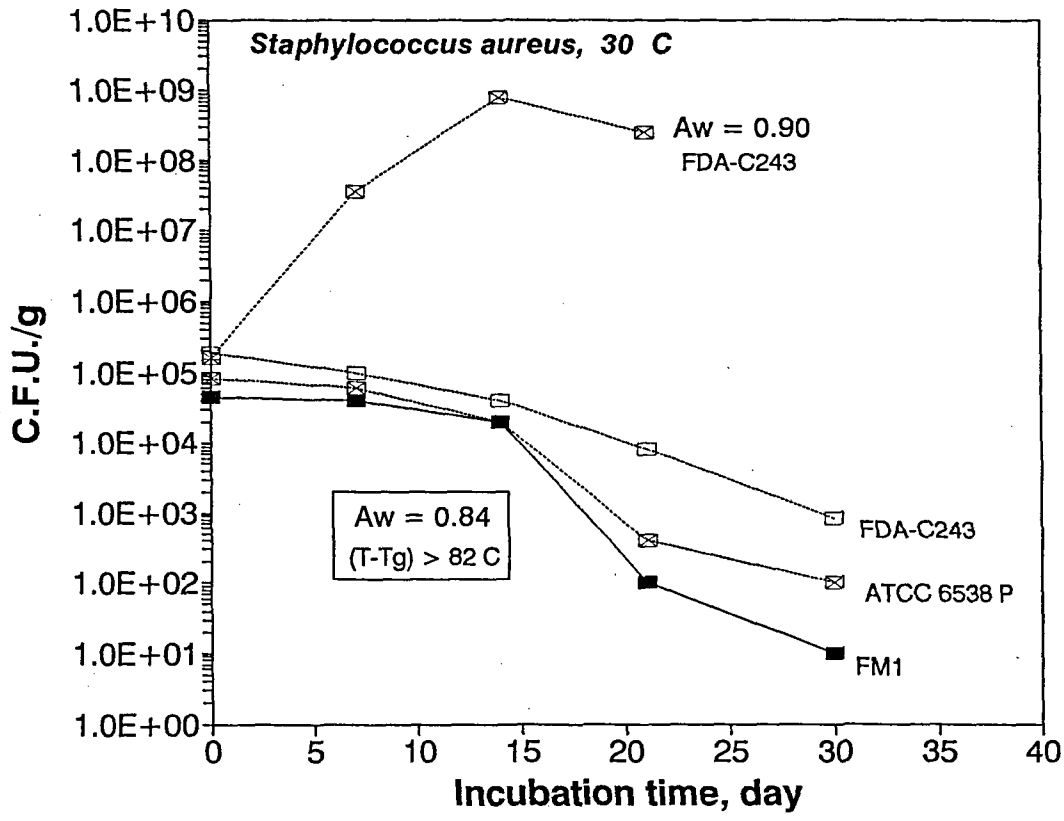

FIGURE 24. Inhibition of growth of *Staphylococcus aureus* (at 30°C) in rubbery dry milk. (From data reported by Giannuzzi and Parada, 1991.)

the statements of Slade and Levine (1991a) that for starch-based products the critical moisture content (which separates the periods of constant rate and falling rate) is 27 to 30% water.

In their criticisms to the a_w concept, Slade and Levine (1991a) discouraged the usage of a_w to define safe limits for microbial growth inhibition in foods. They stated:

> The possibility that a community of food scientists could believe that specifying a maximum a_w value of 0.85 (limiting value for growth of *S. aureus* which is the most a_w-tolerant pathogen) for a cheese cake filling can guarantee product safety, without any consideration of the nature of the mixture of water-compatible solids used to produce a particular a_w value, is both disheartening and potentially deadly.

For Slade and Levine (1991a), the choice of this example (cake filling) to substantiate their "apocalyptical" statements was unfortunate because it has been well established that a_w does play a key role on the growth behavior of *S. aureus* in cream fillings. Cream pie fillings are a perishable product that can support rapid microbiological growth of this bacterium because of its high nutritional value, and the possibility for cream to be contaminated with *staphylococci* is high (Cathcart et al., 1947; Hirooka et al., 1987; Preonas et al., 1969). Hirooka et al., (1987) inoculated a cream filling of known composition (milk, sucrose, corn flour, yellow dye, and egg yolk) with *S. aureus* and incubated at 37°C. The a_w of this cream was calculated from its compositional data and found to be 0.97; its pH was 6.0 to 6.7. Schmidt and Gould (1969) also inoculated a cream filling of known composition, but in this case dextrose was the soluble solid responsible for a_w lowering; the a_w was calculated to be also 0.97.

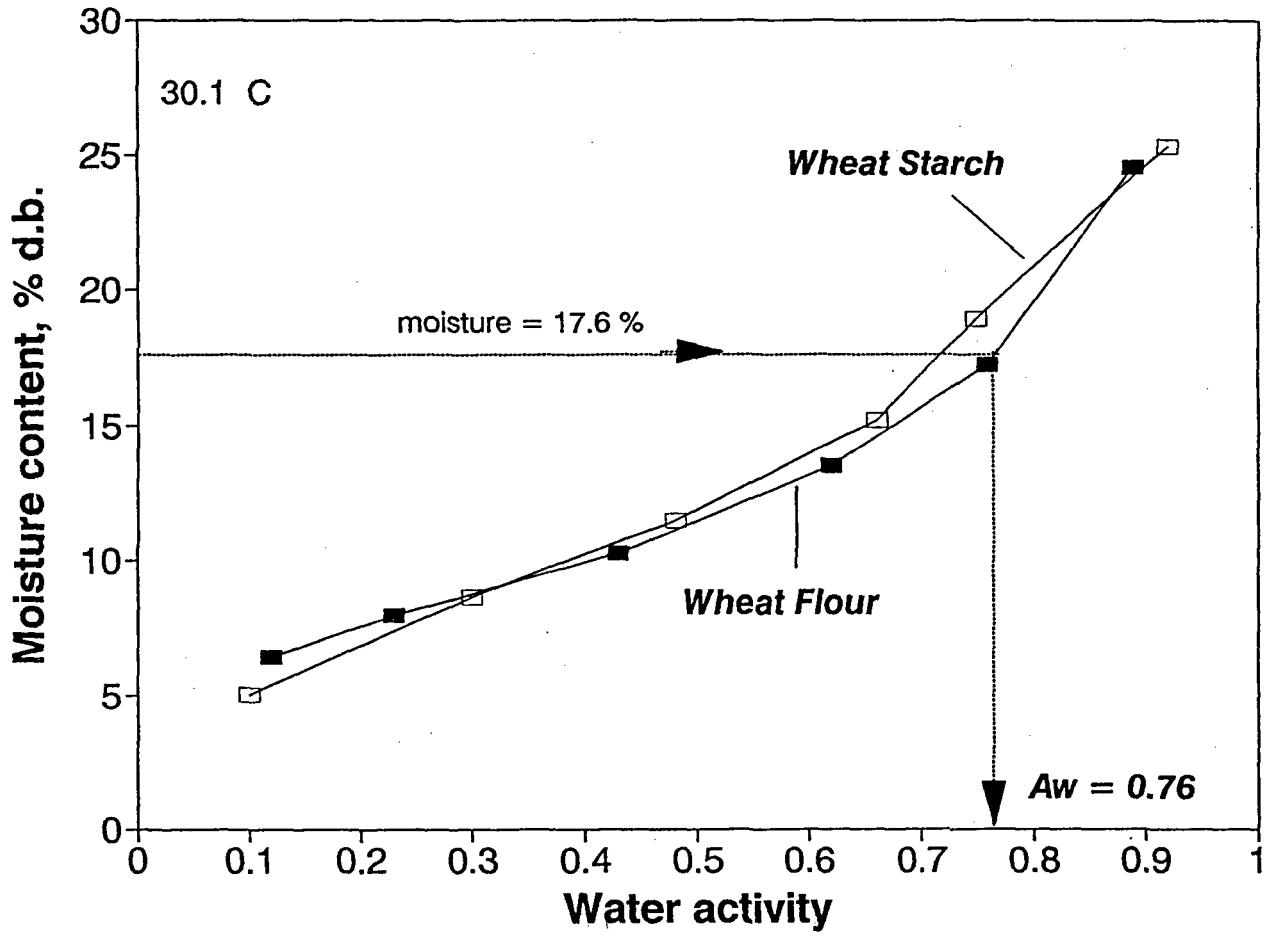

FIGURE 25. Comparison of water adsorption isotherms of wheat starch and wheat flour at 30.1°C. (From data reported by Bushuk and Winkler, 1957.)

Because the limiting a_w for growth of *S. aureus* is about 0.86 (Chirife, 1994), one may anticipate that, other factors being adequate (pH, nutrients, and temperature), the cells may grow easily in these cream fillings, as experimentally observed by Hirooka et al. (1987) and Schmidt and Gould (1969) and shown in Figure 31. Preonas et al. (1969) inoculated pie fillings (flour, shortening, sucrose, nutmeg, vanilla, salt, whole egg, skim milk, corn syrup, and water) of a_w 0.96 to 0.97 with *S. aureus*, and growth was observed when incubated at 30°C. However, addition of dextrose to further lower the a_w prevented growth. Silliker and McHugh (1967) studied the factors influencing microbial stability of butter-cream type fillings and found that a_w of the fillings (adjusted either with sucrose, dextrose, or invert sugar) played a key role in the inhibition of growth of inoculated *S. aureus*. They reported that fillings (sugar, shortening, margarine, nonfat dry milk, salt, vanilla, water, and gelatin) containing 2.1 to 3.0 parts sucrose to 1 of water (a_w = 0.85 and lower) were inherently bactericidal to *staphylococci,* as shown in Figure 32. In fillings containing equivalent concentrations (in osmotic terms) of dextrose and invert sugar, the results were similar.

XIII. CONCLUDING REMARKS

Slade and Levine (1991a) harshly critiqued the use of a_w in the food industry, saying:

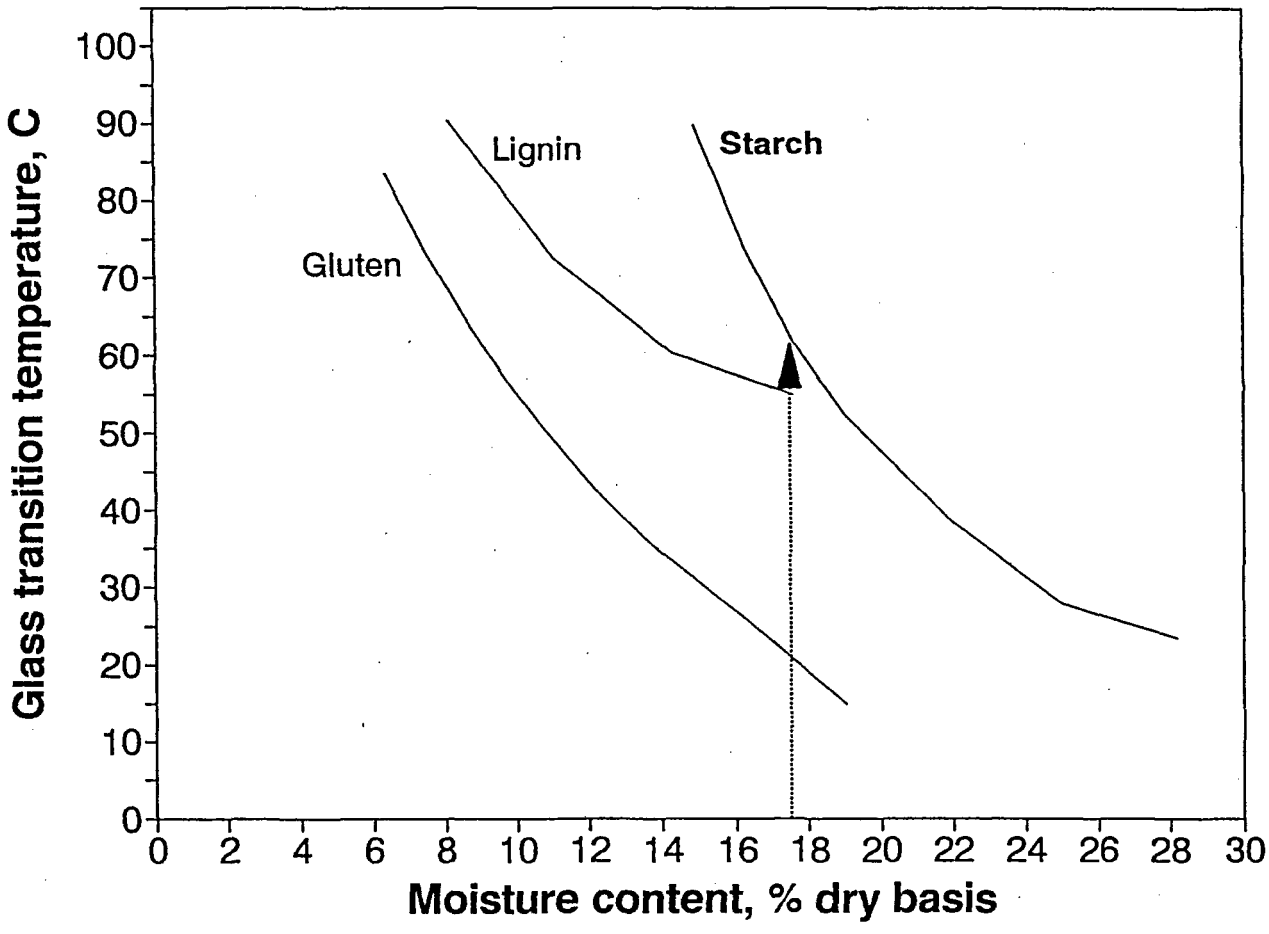

FIGURE 26. Effect of moisture content on T_g transition of wheat starch, gluten, and lignin. (From T_g–moisture content data reported by Zeleznak and Hoseney, 1987; Hoseney et al., 1986; Kalichevsky and Blanshard, 1993; and Slade and Levine, 1991a.)

> The real danger of this careless and oversimplified usage (of a_w) relates to government defined and imposed specifications for values of a_w required by law for microbial safety of IMF products for human consumption. The potential for disaster inherent in naive compliance with such a rigid quantitative approach is frightening.

As the only example, they mentioned that very similar compounds such as glucose and fructose may have very different degrees of growth inhibition at the same measured RH. As mentioned before, van den Berg (1985) and Franks (1991a) have also joined Slade and Levine (1991a) in mentioning "difficulties produced by the use of a_w in food preservation." However, their claims were not supported by referenced experimental evidence. Moreover, the present review details the shortcomings of the supposed experimental evidences reported by Slade and Levine (1987; 1991a) as proof of their claims.

Slade and Levine (1991a) also pointed out that the growth behavior of microbial cells depends not just on the pH, but also on the chemical nature of the buffer. Thus, pH and a_w are only partially diagnostic of cell growth, and the "misuse of a_w" is comparable to the misuse of pH. It is well documented in the literature that the microbial response to lowered pH is determined by both the pH of the system and the acidulant used in acidification. For example, acetic acid is recognized as a better inhibitor (at similar pH) of bacterial growth than other acids used in food preser-

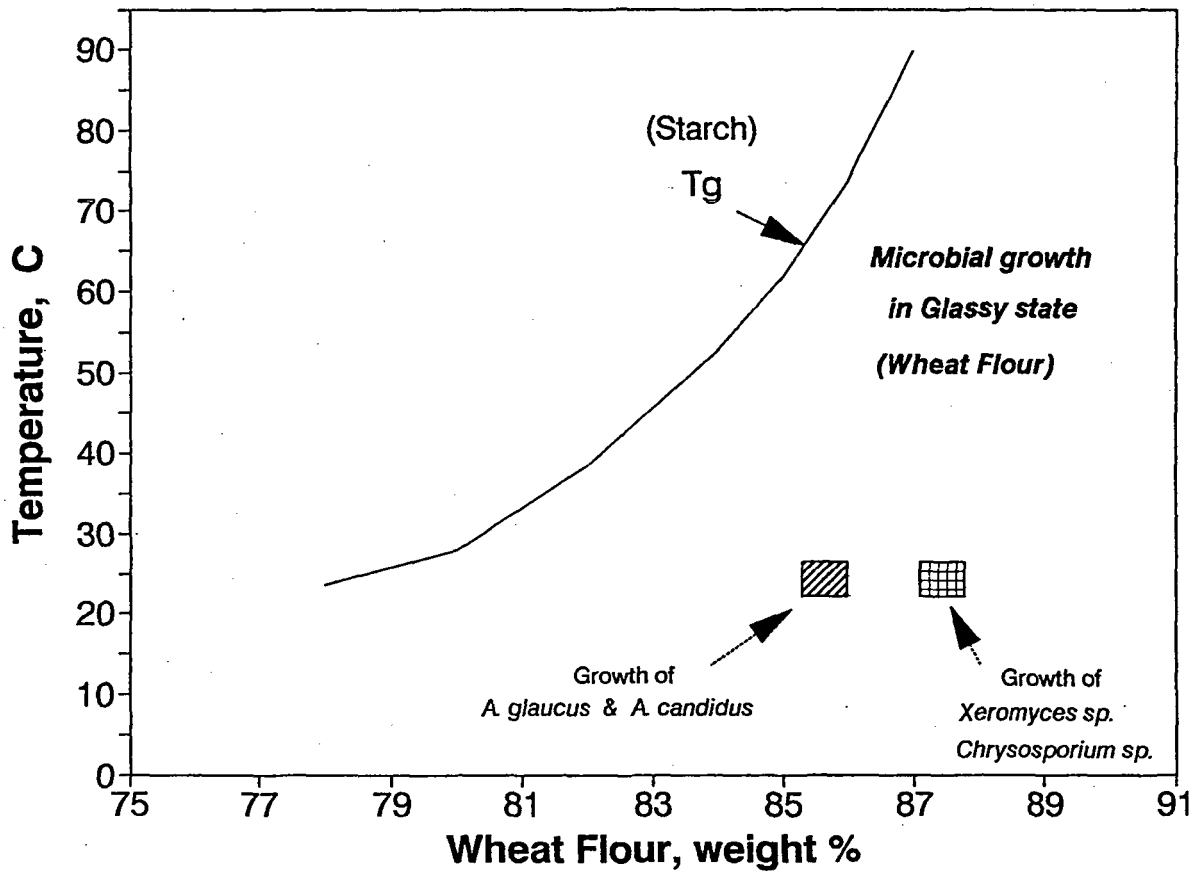

FIGURE 27. "Transformed" selected sorption data in wheat flour and the glass curve for wheat flour based on data for starch (Zeleznak and Hoseney, 1987) to illustrate microbial growth in the glassy state.

vation such as citric, lactic, or malic acid (Abdul-Raouf et al., 1993; Tsang et al., 1985; Minor and Marth, 1972). The minimum pH required to inhibit growth and toxin production by *Clostridium botulinum* depends on the acidulant used (Minor and Marth, 1972; Smelt et al., 1982). Nevertheless, it is generally accepted that the limiting pH of 4.6 provides a good margin of safety against the hazards of botulism in acidified foods, and such products are given only a mild treatment (Post et al., 1985). The well-documented existence of a solute effect for pH did not preclude its widespread usage in food preservation. And this is simply because any specified safe pH limit usually does not correspond to the most inhibitory acid, but to less inhibitory ones. Sorrels et al. (1989) studied the effect of pH and type of acid on growth of *Listeria monocytogenes*. They reported that the overall antimicrobial action (when based on equal pH) was acetic acid > lactic acid > citric acid > malic acid. A pH of 5.0 was sufficient to inhibit growth of *L. monocytogenes* when acetic acid was used; a lower pH was needed for the others. Nevertheless, usual literature tabulations indicate a pH lower than 4.6 to inhibit the growth of this pathogen in accordance with a safe practice (Brown and Booth, 1991). The same happens with the solute effect for a_w limits for microbial

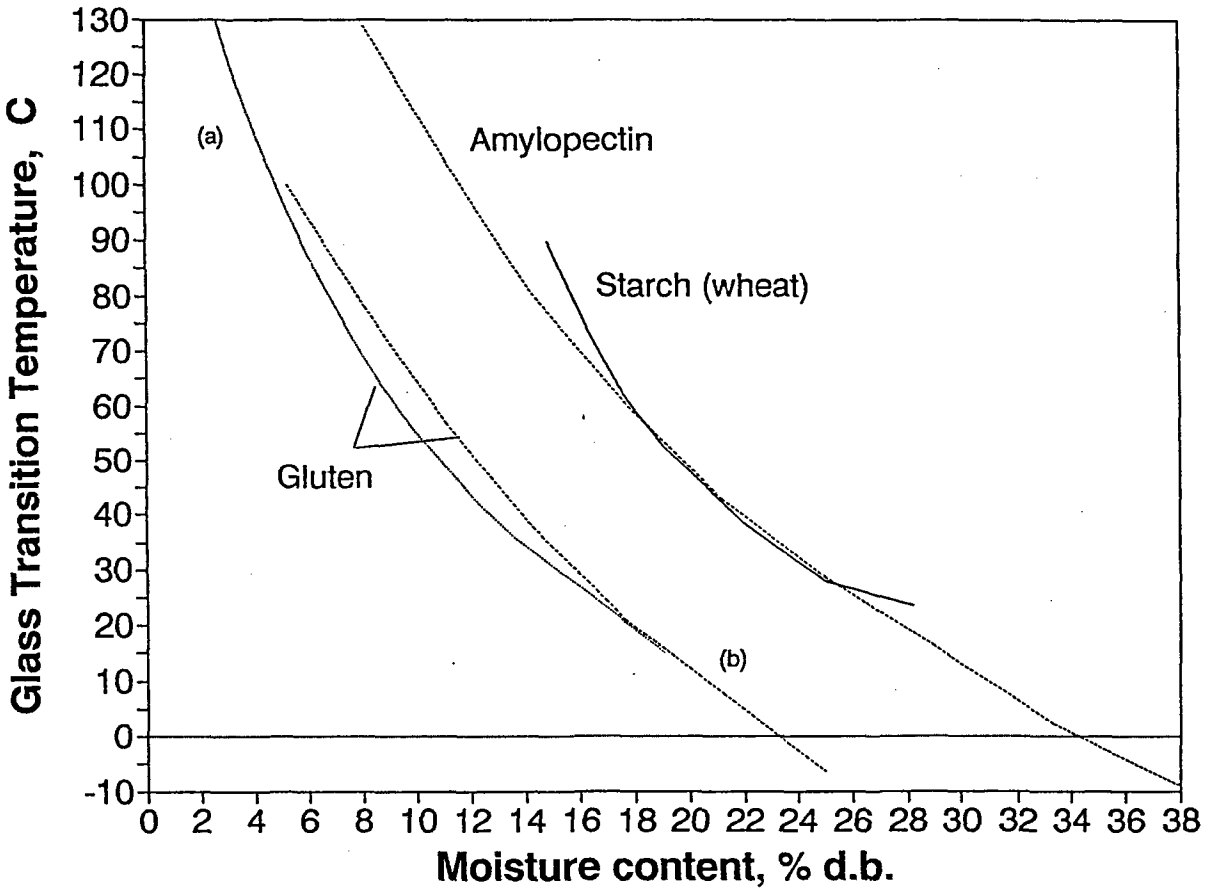

FIGURE 28. Comparison of the glass curves of wheat gluten (Hoseney et al., 1986; Kalichevsky et al., 1992) and wheat starch (Zeleznak and Hoseney, 1987; Kalichevsky and Blanshard, 1993).

growth. For example, literature tabulations of minimal a_w specify that the lowest a_w permitting growth of *S. aureus* is 0.86, even though this bacteria may be inhibited with much higher a_w values, using less conventional solutes (Chirife, 1994).

Despite the repeated claims of Slade and Levine (1987; 1991a,b, 1992), experimental evidence of mobility factors controlling the safety and microbial stability of IM foods is still to be provided. The analysis of experimental evidence does not substantiate their claims in the sense that the "food polymer science approach to understanding of aqueous sugar glasses and concentrated solutions may be used to predict the microbial stability of food systems." And, certainly, this approach does not presently offer a better alternative to the concept of a_w as a predictor of microbial growth in foods. The measurement of a_w which has been long used for predicting the safety and spoilage of foods, continues to prove empirically highly useful and should become exploited to its fullest potential with a greater appreciation of its theoretical (and sometimes practical) limitations (Duckworth, 1988). Gould (1988) indicated that the uncritical use of a_w as a determinant of cell activity had the effect of steering attention away from some more fundamental aspects of the water relations of microbial cells.

The authors are not saying here that mobility factors, in addition to a_w may not be useful for a better definition and prediction of microbial behavior in foods. However, much more research is needed in order to define the conditions under which those factors (viscosity/

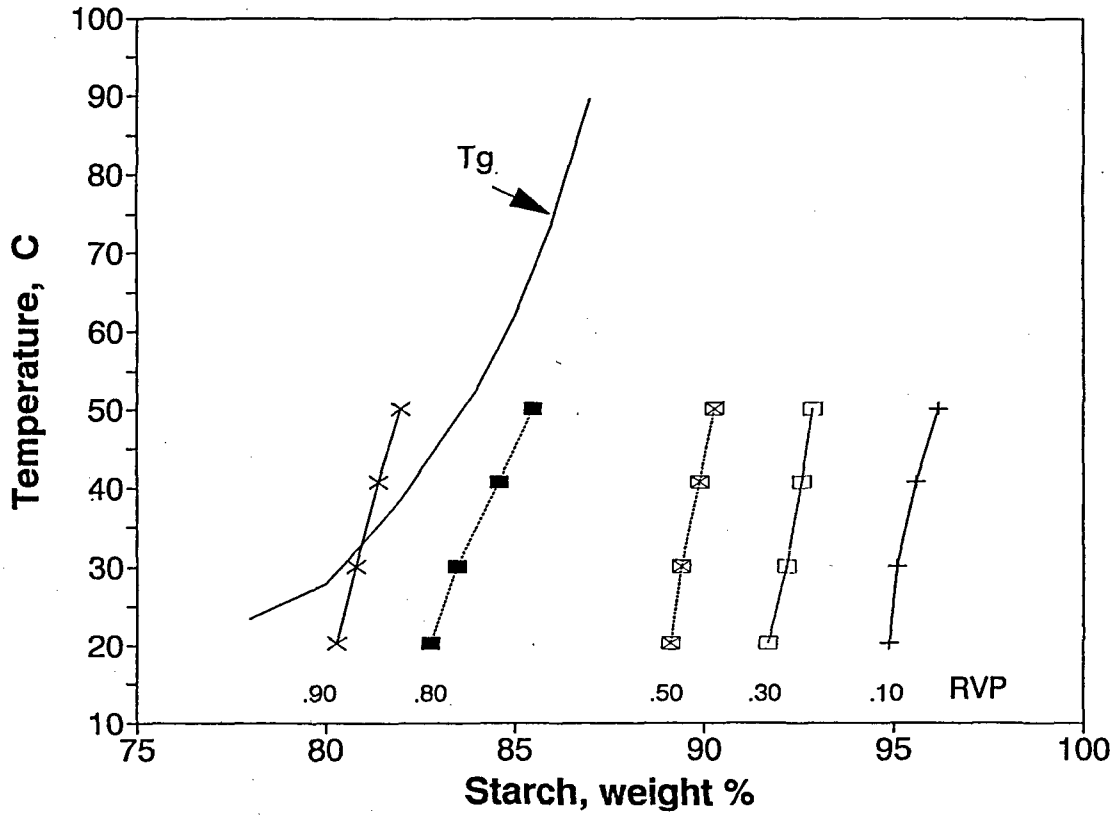

FIGURE 29. "Transformed" isotherms for water adsorption in wheat starch showing the relative locations of iso-a_w contours and the glass curve for starch. (Glass curve for wheat starch from data reported by Zeleznak and Hoseney, 1987; water adsorption data for wheat starch at various temperatures from data reported by Bushuk and Winkler, 1957.)

diffusivity) may effectively inhibit (or retard) microbial growth. Opportunities that have a kinetic rather than an equilibrium basis, such as those that may derive from better-directed control of viscosity/diffusivity, may play a role and deserve to be investigated, but this does not mean that the use of a_w should be disregarded. At this point it is useful to recall the following statements of van den Berg (1991):

> Water activity and glass transition are two different entities; the former being a solvent property and the latter a property related to the structure of the solid; they each relate rather complementary, to important aspects and knowledge of both is needed for understanding the food-water relationships of the system under consideration.

The nonequilibrium effects discussed in the present review (e.g., inability of water to diffuse in semimoist foods) appear to be, in many cases, slow within the time frame (food's shelf life) of the experiments and/or so small that they do not affect seriously the application of the a_w concept as a predictor of microbial stability. This is regardless of the rigorous theoretical meaning of any measured a_w, for example, a thermodynamic activity, a nonequilibrium RPV, or an empirical pseudo-a_w. This does not mean that, because changes in nonequilibrium food systems might be slow and/or small, considerations of nonequilibrium effects, glassy and rubbery states, etc. are unnecessary or that the a_w concept is all that one needs to ensure the safety of semimoist foods.

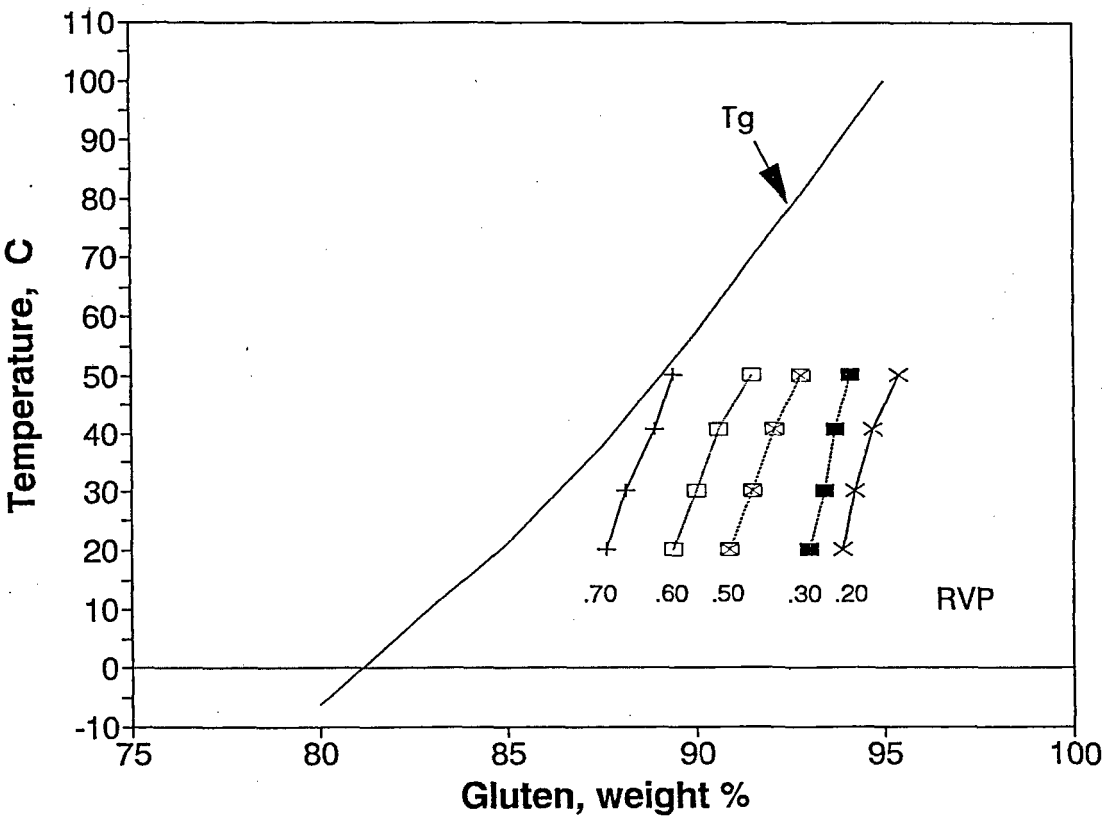

FIGURE 30. *"Transformed" isotherms* for water adsorption in wheat gluten showing the relative locations of iso-a_w contours and the glass curve for gluten. (Glass curve for gluten from data reported by Hoseney et al., 1986; water adsorption data for gluten at various temperatures from data reported by Bushuk and Winkler, 1957.)

This is not so. The a_w concept has several limitations and should always be used carefully; this must include considerations (and precautions) regarding the possible influence of nonequilibrium situations, such as those pointed out by Slade and Levine (1987, 1991a). These warnings, however, are not new. Almost 20 years ago they were clearly stated by Reid (1976) at an international symposium entirely devoted to intermediate-moisture foods, held in Reading, England. He pointed out that because a_w is a thermodynamic concept, it refers only to equilibrium; thus, if equilibrium has not been attained, the definition does not apply. Reid (1976) also discussed the implications of a solution capable of supersaturation (pseudoequilibrium). The vapor pressure of the supersaturated solution is lower than that of the saturated solution; thus the a_w is also lower. Whether this observation is meaningful depends on the stability of the supersaturated solution. His concepts were summarized as follows: "Since activity is a thermodynamic concept anyone who is going to employ the term must be aware of the requirements of the definition."

The field of food science and technology has recently enjoyed an exponential growth of interest in glasses and glass transition in foods and the plasticizing effect of water. Slade and Levine were pioneers in this aspect and their efforts should be fully appreciated and commended. However, this approach — which is certainly original and stimulating — should not be presented as an all-embracing one without adequate experimental evi-

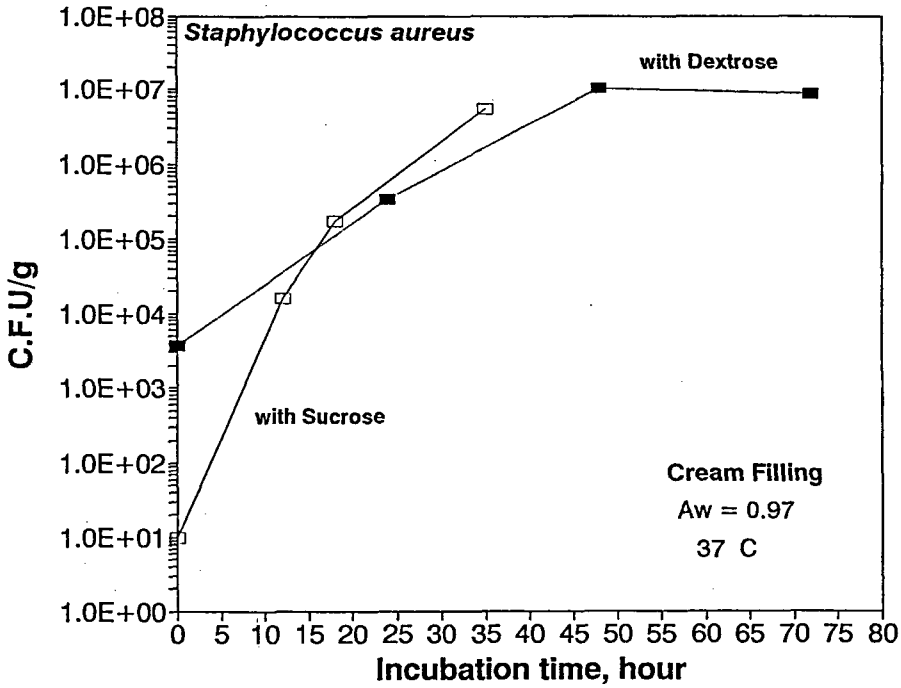

FIGURE 31. Growth of *Staphylococcus aureus* in cream fillings of a_w = 0.97. (From data reported by Hirooka, et al., 1987 and Schmidt and Gould, 1969.)

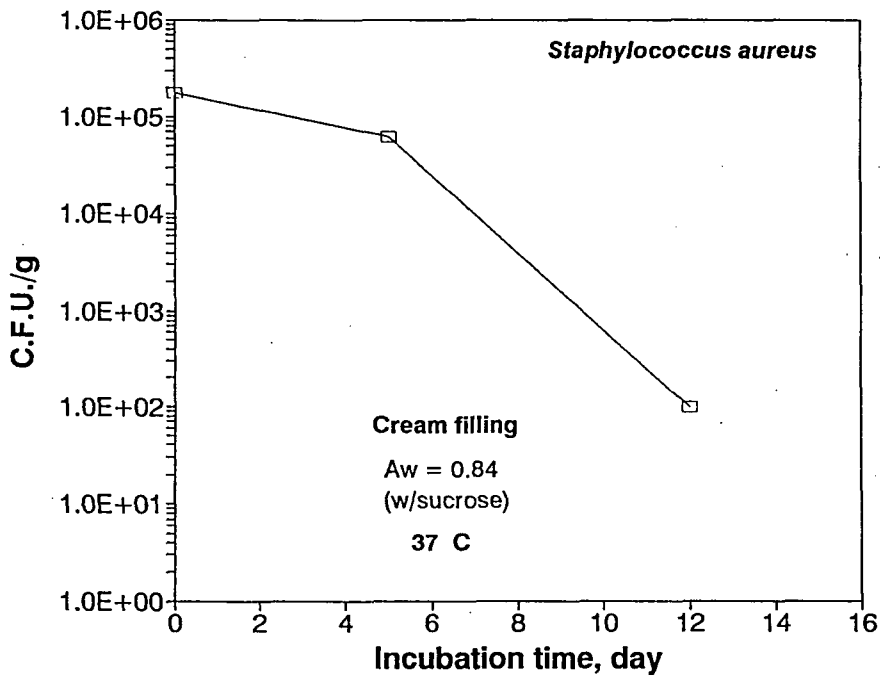

FIGURE 32. Inhibition of growth of *S. aureus* in a cream filling of a_w adjusted to about 0.84 by the addition of sucrose. (From data reported by Silliker and McHugh, 1967.)

dence. This will help the development of an area of research that offers many challenging questions and promises many opportunities for technological development.

ACKNOWLEDGMENTS

The authors acknowledge financial support from Universidad de Buenos Aires and Consejo Nacional de Investigaciones Científicas y Técnicas de la Argentina.

REFERENCES

Abdul-Raouf, U. M., Beauchat, L. R., and Ammar, M. S., 1993. Survival and growth of *Escherichia coli* 0157:H7 in ground roasted beef as affected by pH, acidulants, and temperature. *Appl. Environ. Microbiol.*, **59**:2364–2368.

Aguilera, J. M., Chirife, J., Tapia de Daza, M. S., and Welti Chanes, J., 1990. Inventario de Alimentos de Humedad Intermedia Tradicionales de Iberoamerica. Ed. Parada Arias, Instituto Politécnico Nacional, Mexico, D.F., Mexico.

Anderson, C. B. and Witter, L. D., 1982. Glutamine and proline accumulation by *Staphylococcus aureus* with reduction in water activity. *Appl. Environ. Microbiol.*, **43**:1501–1503.

Andrieu, J., Stamatopoulos, A., and Zafiropoulos, M., 1985. Equation for fitting desorption isotherms of durum wheat pasta. *J. Food Technol.*, **20**:651–657.

Ayranci, E., Ayranci, G., and Dogantan, Z., 1990. Moisture sorption isotherms of dried apricot, fig and raisin at 20°C and 36°C. *J. Food Sci.*, **55**:1591–1593, 1625.

Baird-Parker, A. C. and Freame, B., 1967. Combined effects of water activity, pH and temperature on the growth of *Clostridium botulinum* from spore and vegetative cell inocula. *J. Appl. Bacteriol.*, **30**:420–429.

Bakshi, A. A. and Singh, P. R., 1980. Drying characteristics of parboiled rice, in *Drying 80,* Vol. 2, A. S. Mujumdar, Ed., Hemisphere, Washington D.C., pp. 282–286.

Ballesteros, S. A., Chirife, J., and Bozzini, J. P., 1993. Specific solute effects on *Staphylococcus aureus* cells subjected to reduced water activity. *Int. J. Food Microbiol.*, **20**:51–66.

Beuchat, L. R., 1983. Influence of water activity on growth, metabolic activities and survival of yeasts and molds. *J. Food Protect.*, **46**:135–141.

Batzer, H. and Kreibich, U., 1981. Influence of water on thermal transitions in natural polymers and synthetic polyamides. *Polym. Bull.*, **5**:585–590.

Bizot, H., Buleon, A., Mouhous-Riou, N., and Multon, J. L., 1985. Some factors concerning water vapour sorption hysteresis in potato starch, in *Properties of Water in Foods in Relation to Quality and Stability,* D. Simatos and J. L. Multon, Eds., Martinus Nijhoff, Dordrecht, Netherlands, pp. 83–93.

Bolin, H. R., 1980. Relation of moisture to water activity in prunes and raisins. *J. Food Sci.*, **45**:1190–1191.

Bothast, J., Anderson, R. A., Warner, K., and Kwolek, W. F., 1981. Effects of moisture content and temperature on microbiological and sensory properties of wheat flour and corn meal during storage. *Cereal Chem.*, **58**:309–311.

Boylan, S. L., Acott, K. A. S., and Labuza, T. P., 1976. *Staphylococcus aureus* challenge study in an intermediate moisture food. *J. Food Sci.*, **41**:918–921.

Briozzo, J., de Lagarde, E. A., Chirife, J., and Parada, J. L., 1986. Effect of water activity and pH on growth and toxin production by *Clostridium botulinum* type G. *Appl. Environ. Microbiol.*, **51**:844–848.

Brown, A. D., 1974. Microbial water relations: features of the intracellular composition of sugar-tolerant yeasts. *J. Bacteriol.*, **118**:769–777.

Brown, M. H. and Booth, I. R., 1991. Acidulants and low pH, in *Food Preservatives,* N. J. Russell and G. W. Gould, Eds., Glasgow, pp. 22–43.

Buera, M. P., Chirife, J., Resnik, S. L., and Wetzler, G., 1987. Nonenzymatic browning in liquid model systems of high water activity. 2. Kinetics of color changes due to Maillard reactions between different single sugars and glycine and comparison with caramelization browning. *J. Food Sci.*, **52**:1063–1070.

Bull, H. B., 1944. Adsorption of water vapor by proteins. *J. Am. Chem. Soc.*, **66**:1499–1504.

Bushuk, W. and Winkler, C. A., 1957. Sorption of water vapor on wheat flour, starch and gluten. *Cereal Chem.*, **34**:73–86.

Cahagnier, B., Lesage, L., and Richard-Molard, D., 1993. Mould growth and conidiation in cereal grains as af-

fected by water activity and temperature. *Lett. Appl. Microbiol.*, **17**:7–13.

Cañellas, J., Rosello, C., Simal, S., Soler, L., and Mulet, A., 1993. Storage conditions affect quality of raisins. *J. Food Sci.*, **58**:805–809.

Cathcart, W. H., Godkin, W. J., and Barnett, G., 1947. Growth of *Staphylococcus aureus* in various pastry fillings. *Food Res.*, **12**:142–150.

Chen, C. S., 1987. Relationship between water activity and freezing point depression of food systems. *J. Food Sci.*, **52**:433–435.

Chen, C. S., 1990. Predicting water activity in solutions of mixed solutes. *J. Food Sci.*, **55**:494–497, 515.

Chhinnan, M. S. and Beuchat, L. R., 1985. Sorption isotherms of whole cowpeas and flours. *Lebensm. Wiss. Technol.*, **18**:83–87.

Chirife, J., 1993. Physicochemical aspects of food preservation by combined factors. *Food Control*, **4**:210–215.

Chirife, J., 1994. Specific solute effects with special reference to *Staphylococcus aureus*. *J. Food Eng.*, **22**:409–419.

Chirife, J. and Buera, M. P., 1994a. Water activity, glass transition and microbial stability in concentrated and semimoist food systems. *J. Food Sci.* (in press).

Chirife, J. and Buera, M. P., 1995. A critical review of the effect of some non-equilibrium situations and glass transitions on water activity values of foods in the microbiological growth range. *J. Food Eng.*, **25**:531–552.

Chirife, J., Favetto, G. J., Ferro Fontán, C., and Resnik, S. L., 1983. The water activity of standard saturated salt solutions in the range of IM foods. *Lebensm. Wiss. Technol.*, **16**:36–38.

Chirife, J., Ferro Fontán, C., and Favetto, G. J., 1981a. The water activity of fructose solutions in the intermediate moisture range. *Lebensm. Wiss. Technol.*, **15**:159–160.

Chirife, J., Ferro Fontán, C., and Scorza, C. O., 1981b. The intracellular water activity of bacteria in relation to the water activity of the growth medium. *J. Appl. Bacteriol.*, **50**:475–479.

Chirife, J., González, H. H. L., and Resnik, S. L., 1994. On water dynamics and germination time of mold spores in concentrated sugars and polyiol solutions. *Food Res. Int.* (in press).

Christian, J. H. B., 1981. Specific solute effects on microbial water relations, in Water Activity: *Influences on Food Quality*, L. B. Rockland and G. F. Stewart, Eds., Academic Press, New York, pp. 825–854.

Chvoj, Z. and Kozisek, Z., 1991. Metastable phase diagrams, in *Kinetic Phase Diagrams*, Chvoj, Sestak, and Triska, Eds., Elsevier, New York.

Crapiste, G. H. and Rotstein, E., 1982. Prediction of sorptional equilibrium data for starch-containing foodstuffs. *J. Food Sci.*, **47**:1501–1507.

Csonka, L. N., 1989. Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Rev.*, **53**:121–147.

Dodds, K. L., 1989. Combined effects of water activity and pH on inhibition of toxin production by *Clostridium botulinum* in cooked, vacuum-packaged potatoes. *Appl. Environ. Microbiol.*, **55**:656–660.

Duckworth, R. B., 1988. Foreword, in *Food Preservation by Moisture Control*, C. C. Seow, T. T. Teng, and C. H. Quah, Eds., Elsevier, London.

Emodi, A. S. and Lechowich, R. V., 1969. Low-temperature growth of type E *Clostridium botulinum* spores. II. Effect of solutes and temperature. *J. Food Sci.*, **34**:82–87.

Engels, C., Hendrickx, A., and Tobback, P., 1987. Limited multilayer desorption of brown, parboiled rice. *Int. J. Food Sci. Technol.*, **22**:219–223.

Favetto, G. J., Resnick, S. L., Chirife, J., and Ferro Fontán, C., 1983. Statistical evaluation of water activity measurements obtained with the Vaisala Humicap humidity meter. *J. Food Sci.*, **48**:534–538.

Fornell, A., Bimbenet, J. J., and Almin, Y., 1980. Experimental study modelization for air drying of vegetable products. *Lebensm. Wiss. Technol.*, **14**:96–100.

Franks, F., 1991a. Water activity: a credible measure of food safety and quality? *Trends Food Sci. Technol.*, **March**, 68–72.

Franks, F., 1991b. Hydration phenomena: an update and implications for the food processing industry, in *Water Relationships in Foods*, H. Levine and L. Slade, Eds., Plenum Press, New York, pp. 1–19.

Gal S., 1981. Recent developments in techniques for obtaining complete sorption isotherms, in *Water Activity: Influences on Food Quality*, L. B. Rockland and G. F. Stewart, Eds., Academic Press, New York, pp. 89–102.

Gervais, P., Perrier-Cornet, J. M., and Berner, J. L., 1992. Osmoregulation mechanisms of the yeast *Sporidiobolus salmonicolor*. *J. Agric. Food Chem.*, **40**:1717–1721.

Giannuzzi, L. and Parada, J. L., 1991. Sobre el crecimiento de *Staphylococcus aureus* en medio sólidos de actividad acuosa inferior a 0.86. *Rev. Argen. Microbiol.*, **23**:79–85.

Glass, K. and Doyle, M. P., 1991. Relationship between water activity of fresh pasta and toxin production by proteolitic *Clostridium botulinum*. *J. Food Protect.*, **54**:162–165.

Gould, G. W., 1985. Osmoregulation: is the cell just a simple osmometer? The microbiological experience, in A Discussion Conference: Water Activity: A Credible Measure of Technological Performance and Physiological Viability? Faraday Div., R. Soc. Chem., Girton College, Cambridge, England, July 1–3.

Gould, G. W., 1988. Interference with homeostasis-food, in *Homeostatic Mechanisms in Microorganisms*, J. G. Banks, R. T. G., Board, G. W. Gould, and R. W. Mittenbury, Eds., Bath University Press, England.

Gould, G. W. and Measures, J. C., 1977. Water relations in single cells. *Philos. Trans. R. Soc. London*, **278**:151.

Gould, G. W. and Christian, J. H. B., 1988. Characterization of the state of water in foods — biological aspects, in *Food Preservation by Moisture Control*, C. C. Seow, T. T. Teng, and C. H. Quah, Eds., Elsevier, New York, pp. 43–56.

Haas, G. J. and Herman, E. B., 1978. Bacterial growth in intermediate moisture food systems. *Lebensm. Wiss. Technol.*, **11**:74–78.

Hansen, J. R., 1976. Hydration of soybean protein. *J. Agric. Food Chem.*, **24**:1136–1141.

Hayakawa, K. I., Matas, J., and Hwang, M. P., 1978. Moisture sorption isotherms of coffee products. *J. Food Sci.*, **43**:1026–1027.

Hendel, C. E., Silveira, V. G., and Harrington, W. O., 1955. Rates of nonenzymatic browning of white potato during dehydration. *Food Technol.*, **9**:433–438.

Hill, P. E. and Rizvi, S. S. H., 1982. Thermodynamic parameters and storage stability of drum dried peanut flakes. *Lebensm. Wiss. Technol.*, **15**:185–190.

Hirooka, E. Y., Presa de Salzberg, S. C., and Bergdoll, M. S., 1987. Production of *staphylococcal* enterotoxin A and thermonuclease in cream pies. *J. Food Protect.*, **50**:952–955.

Hocking, A. D., 1988. Strategies for microbial growth at reduced water activities. *Microbiol. Sci.*, **5**:280–284.

Hocking, A. D. and Pitt, J. I., 1979. Water relations of some *Penicillium* species at 25°C. *Trans. Br. Mycol. Soc.*, **73**:141–145.

Hoseney, R. C., Zeleznak, K., and Lai, C. S., 1986. Wheat gluten: a glassy polymer. *Cereal Chem.*, **63**:285–286.

Hutchinson, D. H. and Otten, L., 1984. Equilibrium moisture content of white beans. *Cereal Chem.*, **63**:155–158.

Iglesias, H. A. and Chirife, J., 1978. Delayed crystallization of amorphous sucrose in humidified freeze fried model systems. *J. Food Technol.*, **13**:137–144.

Iglesias, H. A. and Chirife, J., 1982. *Handbook of Food Isotherms*, Academic Press, New York.

Jayas, D. S. and Mazza, G., 1991. Equilibrium moisture characteristics of safflower seeds. *Trans. ASAE*, **34**:2099–2103.

Jason, A. C., 1958. A study of evaporation and diffusion processes in the drying of fish muscle, in *Fundamental Aspects of the Dehydration of Foodstuffs*, Society of the Chemical Industry, London, pp. 103–135.

Jermini, M. F. G. and Schmidt-Lorenz, W., 1987. Growth of osmotolerant yeasts at different water activity values. *J. Food Protect.*, **50**:404–410.

Jouppila, K. and Roos, Y. H., 1994. Glass transitions and crystallization in milk powders. Submitted to *J. Dairy Sci.*

Kakivaya, S. R. and Hoeve, C. A. J., 1985. The glass transition of elastin. *Proc. Natl. Acad. Sci. U.S.A.*, **72**:3505–3507.

Kalichevsky, M. T., Jaroskiewicz, E. M., and Blanshard, J. M. V., 1992. Glass transition of gluten 1: gluten and gluten-sugar mixtures. *Int. J. Biol. Macromol.*, **14**:257–266.

Kalichevsky, M. T. and Blanshard, J. M. V., 1993. The effect of fructose and water on the glass transition of amylopectin. *Carbohydr. Polym.*, **20**:107–113.

Kalichevsky, M. T., Blanshard, J. M. V., and Tokarczuk, P. F., 1993. Effect of water content and sugars on the glass transition of casein and sodium caseinate. *Int. J. Food Sci. Technol.*, **28**:139–151.

Karel, M., Roos, Y., and Buera, M. P., 1993. Effects of glass transitions on processing and storage, in *Glassy State in Food*, J. M. V. Blanshard and P. J. Lillford, Eds., Nottingham University Press, Loughborough (in press).

Karel, M. and Roos, Y., 1993. Effects of glass transitions on dynamic phenomena in sugar containing food systems, in *Glassy State in Food,* J. M. V. Blanshard and P. J. Lillford, Eds., Nottingham University Press, Loughborough (in press).

Karmas, R., Buera, M. P., and Karel, M. 1992. Effect of glass transition on rates of nonenzymatic browning in food systems. *J. Agric. Food Chem.,* **40**:873–879.

King, C. J., 1968. Rates of moisture sorption and desorption in porous, dried foodstuffs. *Food Technol.,* **22**:509–515.

King, D., Jr., Pitt, J. I., Beuchat, L. R., and Corry, E. L., 1984. Comparison of enumeration methods for molds growing in flour stored in equilibrium with relative humidities between 66 and 68%, in *Methods for the Mycological Examination of Food,* Plenum Press, New York, pp. 143–145.

Kitic, D., Favetto, G. J., Chirife, J., and Resnik, S. L., 1986. Measurement of water activity in the intermediate moisture range with the Novasina Thermoconstanter humidity meter. *Lebensm. Wiss. Technol.,* **19**:297–301.

Kumar, M., 1974. Water vapour adsorption on whole corn flour, degermed corn flour and germ flour. *J. Food Technol.,* **9**:433–444.

Labuza, T. P., Acott, K., Tatini, S. R., Lee, R. Y., Flink, J. M., and McCall, W., 1976. Water activity determination: a collaborative study of different methods. *J. Food Sci.,* **41**:910.

Lagoudaki, M., Demertzis, P. G., and Kontominas, M. G., 1993. Moisture adsorption behavior of pasta products. *Lebensm. Wiss. Technol.,* **26**:512–516.

Landfald, B. and Strom, A. R., 1986. Choline-glycine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli. J. Bacteriol.,* **165**:849–855.

Larsen, P. I., Sydnes, L. K., Landfald, B., and Strom, A. R., 1987. Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid and trehalose. *Arch. Microbiol.,* **147**:1–7.

Leistner, L. and Rodel, W., 1975. The significance of water activity for microorganisms in meats, in *Water Relations of Foods,* R. B. Duckworth, Ed., Academic Press, New York, pp. 309–323.

Leistner, L., Rodel, W., and Krispien, K., 1981. Microbiology of meat and meat products in high and intermediate moisture ranges, in *Water Activity: Influences on Food Quality,* L. B. Rockland and G. F. Stewart, Eds., Academic Press, New York, pp. 855–916.

Leistner, L., 1987. Shelf stable products and intermediate moisture foods based on meat, in *Water Activity: Theory and Applications to Food,* L. B. Rockland and L. R. Beuchat, Eds., Marcel Dekker, New York, pp. 295–327.

Le Meste, M., Huang, V. T., Panama, J., Anderson, G., and Lentz, R., 1992. Glass transition of bread. *Cereal Foods World,* **37**:264–267.

Leung, H. K. and Steinberg, M. P., 1979. Water binding of food constituents as determined by NMR, freezing, sorption and dehydration. *J. Food Sci.,* **44**:1212–1217.

Levine, H. and Slade, L., 1992. Glass transitions in foods, in *Physical Chemistry of Foods,* H. G. Schwartzberg and R. W. Hartel, Eds., Marcel Dekker, New York, pp. 83–220.

Lima, A. W. O. and Cal-Vidal, J., 1983. Hygroscopic behaviour of freeze-dried bananas. *J. Food Technol.,* **18**:687–696.

Lomauro, C. J., Bakshi, A. S., and Labuza, T. P., 1985. Moisture transfer properties of dry and semimoist foods. *J. Food Sci.,* **50**:397–400.

Lupin, H. M., Boeri, R. L., and Moschiar, S. M., 1981. Water activity and salt content relationship in moist salted fish products. *J. Food Technol.,* **16**:31–38.

Macchia, D. J. and Bettelheim, F. A., 1964. Sorption of water vapor by amylase "B". *Polym. Lett.,* **2**:1101–1104.

Mazza, G. and Jayas, D. S., 1991. Evaluation of four three-parameter equations for the description of the moisture sorption data of *Lathyrus* pea seeds. *Lebensm. Wiss. Technol.,* **24**:562–565.

Minor, T. E. and Marth, E. H., 1972. Growth of *Staphylococcus aureus* in acidified pasteurized milk. *J. Milk Food Technol.,* **33**:516–520.

Nelson, K., 1993. Reaction Kinetics of Food Stability: Comparison of Glass Transition and Classical Models for Temperature and Moisture Dependence, Ph.D. thesis, University of Minnesota.

Ohye, D. F. and Christian, J. H. B., 1966. Combined effects of temperature, pH and water activity on growth and toxin production by *Clostridium botulinum* types A,B

and E, in Proc. 5th Int. Symp. Food Microbiology, Moscow, p. 217.

Paakonen, K. and Roos, Y., 1990. Effects of drying conditions on water sorption and phase transitions of freeze dried horseradish roots. *J. Food Sci.*, **55**:206–209.

Piñaga, F. and Lafuente, B., 1965. Horchata en polvo. I. Humedades de equilibrio. *Rev. Agroq. Tecnol. Alimentos*, **5**:99–105.

Pitt, J. I. and Christian, J. H. B., 1968. Water relations of xerophilic fungi isolated from prunes. *Appl. Microbiol.*, **16**:1853–1858.

Pitt, J. I., 1975. Xerophilic fungi and the spoilage of foods of plant origin, in *Water Relations of Foods*, R. B. Duckworth, Ed., Academic Press, London, pp. 273–307.

Pollio, M. L., Kitic, D., Favetto, G. J., and Chirife, J., 1986. Effectiveness of available filters for an electric hydrometer for measurement of water activity in the food industry. *J. Food Sci.*, **51**:1358–1359.

Post, L. S., Amoroso, T. L., and Solberg, M., 1985. Inhibition of *Clostridium botulinum* type E in model acidified food systems.

Preonas, D. L., Nelson, A. I., Ordal, Z. J., Steinberg, M. P., and Wei, L. S., 1969. Growth of *Staphylococcus aureus* MF 31 on the top and cut surfaces of southern custard pies. *Appl. Microbiol.*, **18**:68–75.

Prior, B. A., Kenyon, C. P., van der Veen, M., and Mildenhall, J. P., 1987. Water relations of *Pseudomonas fluorescens*. *J. Appl. Bacteriol.*, **62**:119–128.

Rasekh, J. G., Stillings, B. R., and Dubrow, D. L., 1981. Moisture adsorption of fish protein concentrate at various relative humidities and temperatures. *J. Food Sci.*, **36**:705–707.

Reid, D. S., 1976. Water activity concepts in intermediate moisture foods, in *Intermediate Moisture Foods*, R. Davies, G. G. Birch, and K. J. Parker, Eds., Applied Science Publishers, London, pp. 54–65.

Resnik, S. L. and Chirife, J., 1979. Effect of moisture content and temperature on some aspects of nonenzymatic browning in dehydrated apple. *J. Food Sci.*, **44**:601–605.

Resnik, S. L., Favetto, G. J., Chirife, J., and Ferro Fontán, C., 1984. A world survey of water activity of selected saturated salt solutions used as standards at 25°C. *J. Food Sci.*, **49**:510–513.

Resnik, S. L. and Chirife, J., 1988. Proposed theoretical literature values at various temperatures for selected solutions to be used as reference sources in the range of microbial growth. *J. Food Protect.* **51**:419–423.

Rodel, W., Scheuer, R., and Wagner, R., 1990. A new method of determining water activity in meat products. *Fleischwirtschaft*, **70**:905–907.

Roos, Y., 1987. Effect of moisture on the thermal behavior of strawberries studied using differential scanning calorimetry. *J. Food Sci.*, **52**:146–149.

Roos, Y., 1993. Water activity and physical state effects on amorphous food stability. *J. Food Process. Preserv.*, **16**:433–447.

Roos, Y. and Karel, M., 1991a. Plasticizing effect of water on thermal behavior and crystalization of amorphous food models. *J. Food Sci.*, **56**:38–43.

Roos, Y. and Karel, M., 1991b. Phase transitions of mixtures of amorphous polysaccharides and sugars. *Biotechnol. Prog.*, **7**:49–53.

Ross, K. D., 1975. Estimation of water activity in intermediate moisture foods. *Food Technol.*, **29**:26–30.

Ruegg, M. and Blanc, B., 1981. The water activity of honey and related sugar solutions. *Lebensm. Wiss. Technol.*, **14**:1–5.

Saravacos, G. D., Tsiourvas, D. A., and Tsami, E., 1986. Effect of temperature on the water adsorption isotherms of sultana raisins. *J. Food Sci.*, **51**:381–383, 387.

Schmidt, E. W., Jr. and Gould, W. A., 1969. Chemical preservatives to inhibit the growth of *Staphylococcus aureus* in synthetic cream pies acidified to pH 4.5 to 5.0. *Food Technol.*, **23**:92–95.

Scott, W. J., 1953. Water relations of *Staphylococcus aureus* at 30°C. *Aust. J. Biol. Sci.*, **6**:549–556.

Sharpe, A. N., Diotte, M. P., and Dudas, I., 1991. Water activity tester suited to compliance and high A_w work. *J. Food Protect.*, **54**:277–282.

Silliker, J. H. and McHugh, S. A., 1967. Factors influencing microbial stability of butter-cream type fillings. *Cereal Sci. Today*, **12**:63–74.

Silverman, G. J., Munsey, D. T., Lee, C., and Ebert, E., 1983. Interrelationship between water activity, temperature and 5.5 percent oxygen on growth and enterotoxin A secretion by *Staphylococcus aureus* in precooked bacon. *J. Food Sci.*, **48**:1783–1786, 1795.

Slade, L. and Levine, H., 1987. Structural stability of intermediate moisture foods — a new understanding, in *Food Structure — Its Creation and Evaluation,* J. R. Mitchell and J. M. V. Blanshard, Eds., Butterworth, London, pp. 115–147.

Slade, L. and Levine, H., 1991a. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.,* **30:**115–360.

Slade, L. and Levine, H., 1991b. A food polymer science approach to structure-property relationships in aqueous food systems, in *Water Relationships in Foods,* H. Levine and L. Slade, Eds., Plenum Press, New York, pp. 29–101.

Slade, L., Levine, H., and Finey, J., 1989. Protein–water interactions: water as a platicizer of gluten and other protein polymers, in *Protein Quality and the Effects of Processing,* R. D. Phillips and J. W. Finlay, Eds., Marcel Dekker, New York, pp. 9–123.

Smelt, J. P. P. M., Raatjes, G. J. M., Crowther, J. S., and Verrips, C. T., 1982. Growth and toxin formation by *Clostridium botulinum* at low pH values. *J. Appl. Bacteriol.,* **52:**75–82.

Soesanto, T. and Williams, M. C., 1981. Volumetric interpretation of viscosity for concentrated and dilute sugar solutions. *J. Phys. Chem.,* **85:**3338–3341.

Sorrels, K. M., Enigl, D. C., and Hatfield, J. R., 1989. Effect of pH, acidulant, time and temperature on the growth and survival of *Listeria monocytogenes. J. Food Protect.,* **52:**571–578.

Spiess, W. E. L. and Wolf, W. R., 1985. The results of the COST 90 project on water activity, in *Physical Properties of Foods,* F. Escher, B. Halllstrom, H. S. Meffert, W. E. L. Spiess, and G. Voss, Eds., Applied Science Publishers, New York, pp. 65–87.

Teng, T. T. and Lenzi, F., 1974. Water activity data representation of aqueous solutions at 25°C. *Can. J. Chem. Eng.,* **52:**387–391.

Tokuoka, K. and Ishitani, T., 1991. Minimum water activities for the growth of yeasts isolated from high-sugar foods, *J. Gen. Appl. Microbiol.,* **37:**111–119.

Troller, J. A., 1981. Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus. Appl. Microbiol.,* **21:**435.

Troller, J. A., 1977. Statistical analysis of water activity measurement obtained with the Sina scope. *J. Food Sci.,* **42:**86.

Troller, J. A. and Christian, J. H. B., 1978. *Water Activity and Food,* Academic Press, New York.

Troller, J. A. and Scott, V. N., 1992. Measurement of water activity (Aw) and acidity, in *Compendium of Methods for the Microbiological Examination of Foods,* C. Vanderzant and D. F. D. F. Splittstoesser, Eds., American Public Health Association, Washington, D.C., pp. 135–151.

Tsami, E., Marinos-Kouris, D., and Maroulis, Z. B., 1990. Water sorption isotherms of raisins, currants, figs, prunes and apricots. *J. Food Sci.,* **55:**1594–1597, 1625; *J. Food Sci.,* **50:**961–965.

Tsang, N., Post, L. S., and Solberg, M., 1985. Growth and toxin production by *Clostridium botulinum* in model acidified systems. *J. Food Sci.,* **50:**961–965.

Vaamonde, G., Chirife, J., and Scorza, O. C., 1982. An examination of the minimal water activity for *Staphylococcus aureus* ATCC 6538 P growth in laboratory media adjusted with less conventional solutes. *J. Food Sci.,* **47:**1259–1262.

Valdez-Niebla, J. A., Paredes-López, O., Vargas-López, J. M., and Hernandez, López, D., 1993. Moisture sorption isotherms and other physicochemical properties of nixtamalized amaranth flour. *Food Chem.,* **46:**19–23.

Valik, L. and Gorner, F., 1993. Growth of *Staphylococcus aureus* in pasta in relation to its water activity. *Int. J. Food Microbiol.,* **20:**45–48.

van den Berg, C. and Bruin, 1981. Water activity and its estimation in food systems: theoretical aspects, in *Water Activity: Influences on Food Quality,* L. B. Rockland and G. F. Stewart, Eds., Academic Press, New York, pp. 1–61.

van den Berg, C., 1981. Vapour Sorption Equilibria and Other Water-Starch Interactions; A Physicochemical Approach. Doctoral thesis, Agricultural University, Wagening, The Netherlands.

van den Berg, C., 1985. Water activity, in *Concentration and Drying of Foods,* D. Mac Carthy, Ed., Elsevier, London, pp. 11–35.

van den Berg, C., 1991. Food-water relations: progress and integration, comments and thoughts, in *Water Relationships in Foods,* H. Levine and L. Slade, Eds., Plenum Press, New York.

Wolf, W., Spiess, W. E. L., Jung, G., Weisser, H., Bizot, H., and Duckworth, R. B., 1984. The water-vapour sorption isotherms of microcrystalline cellulose (MCC) and of

purified potato starch. Results of a collaborative study. *J. Food Eng.*, **3**:51–73.

Wedzicha, B. L. and Quine, E. C., 1983. Sorption of water vapour by wafer biscuits. The effects of components, ingredients and the baking process. *Lebensm. Wiss. Technol.*, **16**:115–118.

Zeleznak, K. J. and Hoseney, R. C., 1987. The glass transition in starch. *Cereal Chem.*, **64**:121–124.