

# WATER PROPERTIES

IN FOOD, HEALTH,  
PHARMACEUTICAL AND  
BIOLOGICAL SYSTEMS:

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## **PART 1**

### **Invited Speakers and Oral Presentations**



## **Session 1**

# **Water Mobility/Dynamics and Its Application in Food and Pharmaceutical Systems**



## *Invited Speakers*



# 1 Complementary Aspects of Thermodynamics, Nonequilibrium Criteria, and Water Dynamics in the Development of Foods and Ingredients

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## Abstract

Temperature and water content of the systems have been the variables most widely employed to define and predict the kinetic coefficients of desirable and undesirable changes in foods. Supplemented temperature vs composition phase diagrams have been demonstrated to be helpful in determining the feasibility of occurrence of phase or state transitions. These diagrams include the glass transition temperature ( $T_g$ ) curve and the equilibrium liquidus curves. The inclusion of the nonequilibrium curves enables relationships with the time coordinate and, thus, with the dynamic behavior of the systems to be established and helps to predict whether the systems are under thermodynamic or kinetic control for given composition vs temperature conditions, provided that the thermal history of the samples is known. The present work analyzed how complementary aspects of thermodynamics and nonequilibrium criteria and water dynamics can be assembled in order to demonstrate the formulation and processing strategies to optimize the stability of food products and ingredients, especially in dry systems.

A wide variety of kinetic data from several chemical reactions (in vegetable and animal tissues, dairy products, ingredients, and pharmaceutical formulations or model systems) from the published literature on the results from specifically designed experiments were distributed in supplemented phase diagrams. The results indicated that both solid-water interactions and structural characteristics of the systems governed the dependence of reaction rates on relative humidity. In addition to the supplemented phase diagrams, structural aspects of the matrices where the reaction takes place, water-sorption properties, and water mobility itself were key aspects for a complete interpretation to describe the dynamics of the chemical reactions.

## Introduction

The kinetic control of desirable and undesirable aspects of chemical reactions represents a challenge in basic and applied areas of chemistry and food sciences. Therefore, the impact of the variables and mechanisms that determine reaction rates has been given much attention. Temperature and water content of the systems have been the variables most widely employed to define and predict the kinetic coefficients. The significance of state and phase transitions in the stability of amorphous food materials

and also their impact on chemical and enzymatic reactions have been evaluated since the 1980s (Slade and others 1989; Roos and Karel 1991; Karmas and others 1992; Levine and Slade 1992; Bell and Hageman 1994; Bell 1995; Buera and Karel 1995; Lievonon and others 1998; Bell and White 2000; Kouasi and Roos 2000). Since an equilibrium state does not exist in these systems, the conservation of desirable properties in foods and ingredients is governed by conditions of metastability, often based on the maintenance of the systems in an amorphous state (Levine and Slade 1992): although the system components are not thermodynamically stable, they are kinetically stabilized.

Supplemented temperature composition phase diagrams have proven helpful in determining the potential of phase or state transitions (Slade and others 1989; Levine and Slade 1992). These diagrams include the nonequilibrium glass transition temperature ( $T_g$ ) curve and the equilibrium liquidus curves. The inclusion of the nonequilibrium curves enables the relationships with the time coordinate and, thus, with the dynamic behavior of the systems to be established and helps to predict whether the systems are under thermodynamic or kinetic control for given temperature/composition conditions, providing that the thermal history of the samples is known. Structural aspects of the matrices where the reactions occur, sorption, and water mobility itself were also detected as key aspects in describing the dynamic of this reaction. The present work analyzed how complementary aspects of thermodynamics and nonequilibrium criteria and water dynamics can be assembled in order to point out formulations or processing strategies to optimize the stability of food products and ingredients, especially in dry systems.

## Reactions, Materials, and Methods

The stability of selected dehydrated systems toward the Maillard reaction, the loss of enzymatic activity, or carotene degradation was analyzed. Data obtained in freeze-dried vegetable tissues, dairy products, ingredients, and pharmaceutical formulations or model systems as the result of specifically designed experiments (Mazzobre and others 2001; Longinotti and others 2002; Prado and others 2006; Acevedo and others 2006, 2008b; Sutter and others 2007) were plotted in supplemented phase diagrams. Additional information on water dynamics and water-sorption properties was obtained.

Differential scanning calorimetry (DSC) was helpful in analyzing thermal transitions and generating temperature- vs composition-supplemented state diagrams. Time-resolved proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) was used to complement DSC with the aim of obtaining a better understanding of the mobility of water and food solids in the systems (Schmidt and Lai 1991; Kou and others 2000; Tang and others 2000; Chatakanonda and others 2003). X-ray diffraction and microscopy provided information on molecular and microscopic structural changes.

## Maillard Reaction

The Maillard reaction may have a positive or a negative contribution to the quality of food products, and its complexity necessitates a deep kinetic analysis of reaction

media and the variables involved to direct the reaction with the desirable outcome. In dehydrated foods, the reaction is the cause of off-flavors, off-colors, and loss of nutritional value. The kinetics of the Maillard reaction must be controlled also as a requirement of new food technologies: in the development of natural flavors, pigments, emulsifiers, antimicrobials, and antioxidants; in the formulation of protective media for biological systems and ingredients; and for controlled modification of biomolecular functionality or structure.

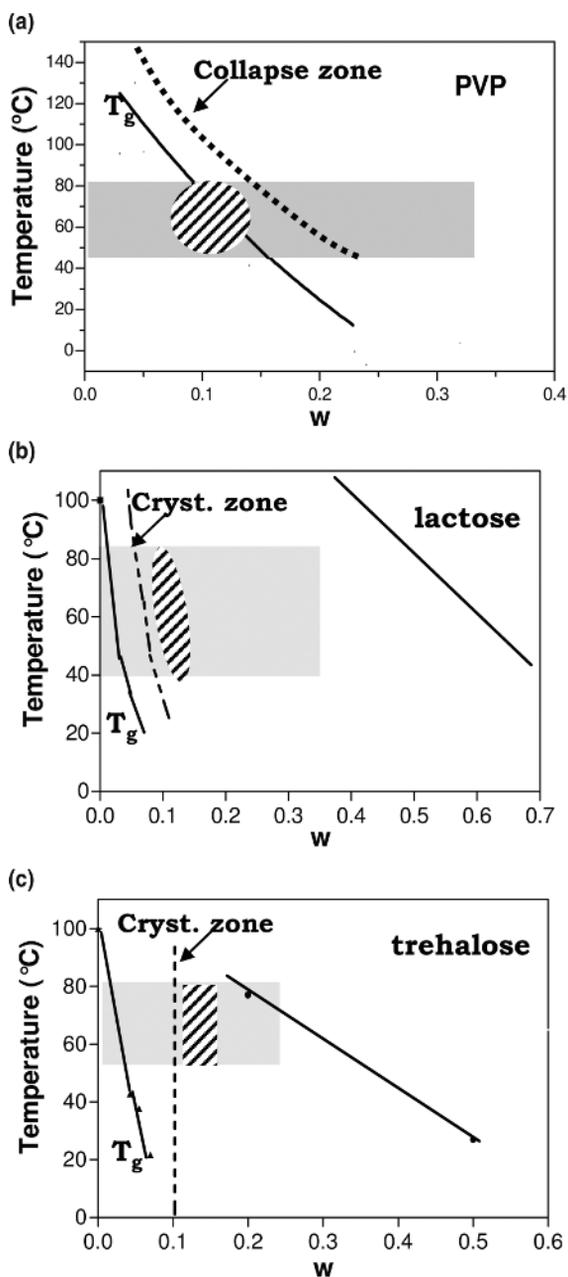
The reaction rate is strongly dependent on the concentration, ratio, and chemical nature of reactants, temperature, water content, pH, and water activity ( $a_w$ ) (Labuza and Baisier 1992), but it is also influenced by the physical properties of the media.

In liquid systems, the Maillard reaction rate diminishes continuously as relative humidity (RH) increases, mainly because water is a product of the reaction (Hodge 1953; Eichner and Karel 1972; Labuza and Saltmarch 1981). However, in solid or quasi-solid systems, in which reactants are constrained by mobility restrictions, a maximum rate of nonenzymatic browning (NEB) is observed at a given intermediate RH value. Thus, the presence of a maximum in the plot of rate versus water content (or  $a_w$ ) is a consequence of the low reaction rates due to mobility limitations of the reactants (at low water content) and inhibition by the product (at high water content) (Buera and Karel 1995; Van Boekel 2001). Systems with different structural characteristics were analyzed to elucidate the relationship among browning rate, water-solid interaction, and water mobility and the incidence of structural changes accompanying phase or state transitions: highly collapsible polymeric (polyvinylpyrrolidone [PVP-40]) matrices, crystallizing lactose and milk systems, and vegetable tissues exhibiting an intermediate degree of collapse because of the presence of water-insoluble polymers capable of providing residual structure. The shaded areas in Figures 1.1 and 1.2 represent the temperature vs composition conditions at which the Maillard reaction was analyzed, and the circled regions show the conditions at which the maximum rates were observed.

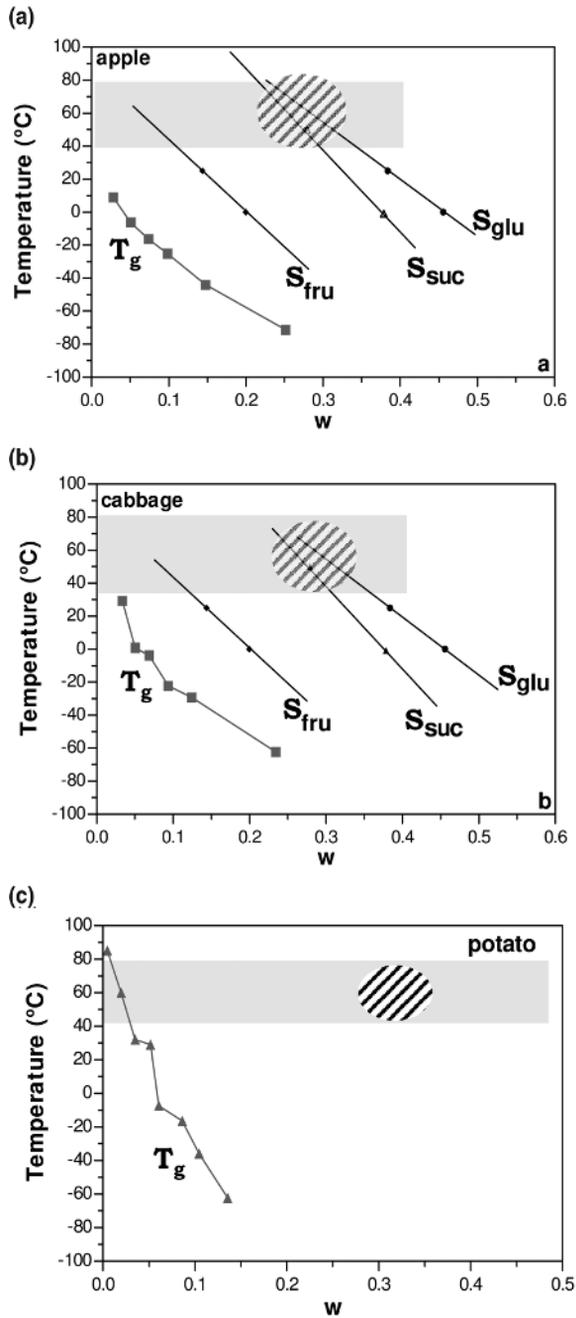
The corresponding  $T_g$  curves are shown in Figures 1.1 and 1.2 also as a function of water content. Since sugars are the main soluble components determining  $T_g$  values in vegetable and dairy systems, solubility curves for the main sugars present were included as a reference.

In PVP systems (Figure 1.1) at a given temperature, the Maillard reaction rate increased as water content increased, and the maximum rate occurred at a water content at which  $T_g$  was close to the storage temperature. Above this point, the samples presented a fully collapsed fluid aspect, and the Maillard rate decreased when water content increased (behavior similar to that of liquid systems). In the crystallizable lactose- or trehalose-containing systems (Figure 1.1), the maximum rate occurred at conditions in which a considerable degree of sugar crystallization had occurred, but when the samples were totally crystalline the rate decreased. Figure 1.3 shows the rate of the Maillard reaction as a function of water content for lactose systems compared with the rates with milk and lactose-starch.

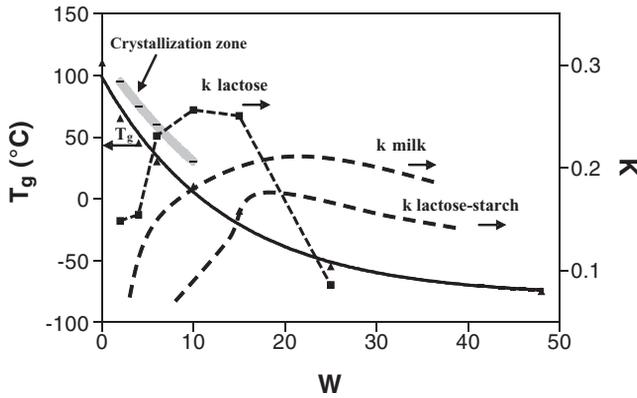
It should be noted that, in milk or lactose-starch systems, the presence of proteins or biopolymers retarded lactose crystallization. Consequently, the maximum rate of



**Figure 1.1.** State diagrams showing the glass transition temperatures ( $T_g$ ) as a function of the mass fraction of water ( $w$ ) for (a) polymeric (PVP), (b) lactose, and (c) trehalose matrices in which the Maillard reaction was developed. Shaded areas represent the regions in which the experiments were performed. The conditions at which fast-collapse or crystallization phenomena were observed are indicated by dotted lines. Circled regions indicate the conditions at which the maximum browning rate was observed.



**Figure 1.2.** State diagrams showing the glass transition temperatures ( $T_g$ ) as a function of the mass fraction of water ( $w$ ) for (a) apple, (b) cabbage, and (c) potato in which the Maillard reaction was developed. Shaded areas represent the regions in which the experiments were performed. The solubility curves ( $S$ ) for the sugars fructose (fru), glucose (glu), and sucrose (suc) are indicated as a reference. Circled regions indicate the conditions at which the maximum browning rate was observed.



**Figure 1.3.** State diagrams showing the lactose glass transition temperature ( $T_g$ ) as a function of the mass fraction of water ( $W$ ). Browning rate constants ( $k$ ) for the lactose, milk, and lactose-starch systems are also shown as a function of  $W$ .

browning occurred with a higher water content, and the maximum rate was maintained in higher water contents than in the sugar-only matrices (complete crystallization occurred at higher water content values in those samples). In vegetable tissues containing structure-maintaining water-insoluble biopolymers and presenting an intermediate degree of collapse, the maximum rate of NEB occurred at RHs in a range of 50%–80% when the samples were well above  $T_g$  at the storage temperature (Figure 1.2).

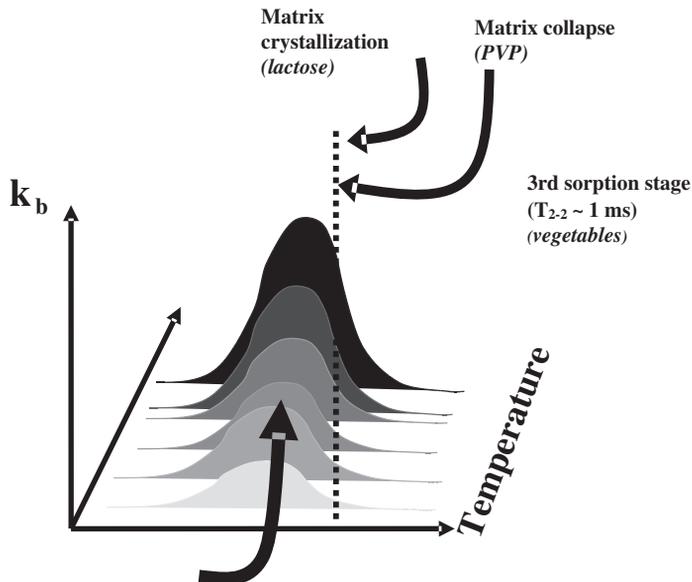
In all cases analyzed, the Maillard reaction occurred below the  $T_g$  and required a minimum of water content ( $w$  in Figure 1.2) to mobilize the reactants, but the reaction was immediately inhibited by water in excess of this requirement. The minimum water content was directly related neither to the monolayer Guggenheim-Anderson-de Boer (GAB) value nor to the  $T_g$  value of the systems. The browning rate of the vegetables and food models analyzed was very low in the glassy state, but at temperatures above the  $T_g$ , in addition to the decreasing viscosity and increasing rate, other changes such as crystallization and collapse affected the browning rate.

As already discussed, the maximum reaction rates were reached either close to or well above the  $T_g$ , depending on the system structure. The observation of the maximum rate as a function of water content indicated that the reaction rate decreased at a point at which the matrix was unable to adsorb water either because of crystallization (as in the case of lactose-containing systems), saturation of the most active sites of the water-adsorbing matrix, or capillary condensation. I thus analyzed the location of the beginning of the third water-sorption stage, the transverse spin-spin  $^1\text{H-NMR}$  relaxation times by spin echo after the Hahn sequence pulse, and the detection of frozen water by DSC. The location of the beginning of the third sorption stage was analyzed by the inverse plot of the GAB model, as proposed by Timmermann and Chirife (1991). The analysis of proton spin-spin relaxation times by the Hahn spin-echo pulse sequence in the samples at different water contents showed two  $T_2$  components. A fast-decaying component, with  $T_{2\text{-Hahn-1}}$  values on the order of 30–40 ms was present

at all the RHs analyzed and corresponds to both protons of solids and protons of water molecules that are strongly influenced by their proximity to the solid components. (It could also be detected by free-induction decay [FID] measured after a single  $90^\circ$  pulse.) Above a given water content (in the proximity of  $a_w = 0.22$ ), a slow-relaxing component ( $T_{2\text{-Hahn-}2}$ ) in the range of 400–950 ms was also observed, the value of which increased linearly up to the beginning of the third sorption stage, as previously defined, and then became constant. The inhibitory water concentration was associated with the second inflection point of the water-sorption isotherm up to the appearance of freezable water as determined by DSC and up to the saturation of the second sorption stage as determined by proton spin-spin transverse relaxation times ( $T_2$ ) analyzed by  $^1\text{H-NMR}$  with the Hahn pulse sequence (Acevedo and others 2008b).

Upon the appearance of freezable water and highly mobile water (determined by the  $T_{2\text{-Hahn-}2}$  value), the NEB rate decreased as the water molecules inhibited the reaction and/or diluted the reactants.

As the scheme in Figure 1.4 shows, the water content at a maximum Maillard reaction rate results from the compromise between water plasticization and its inhibitory effect. These conditions can be predicted on the basis of sorption and structural properties and of thermal transitions of the matrices where the reaction takes place and through the analysis of water mobility.



**Figure 1.4.** Schematic tridimensional plot showing the browning rate ( $k_b$ , in arbitrary units) as a function of the temperature and mass fraction of water ( $w$ ). The conditions for the decreasing rate constant after the maximum were different for each type of system and are indicated by the *arrows*. PVP, polymeric matrix.

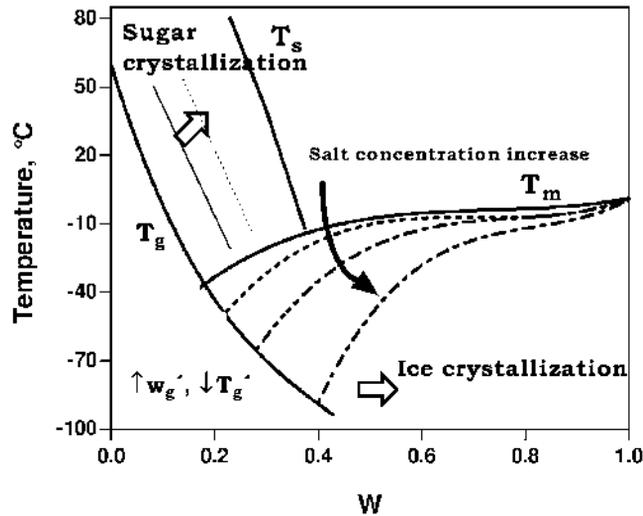
## Enzyme Stability

Due to chemical and physical changes, most proteins lose their activity when stored for extended periods in aqueous solution, and they are generally freeze-dried to achieve a stable product. The dry state slows chemical degradation and alleviates physical problems, such as protein unfolding and aggregation. However, protein stability in the solid state can be worse than that in liquid state if adequate components are not present during the process to form suitable matrices (Crowe and others 1998). Sugars, and particularly trehalose, have been found to be optimal in protecting enzymes during drying and later storage (Leslie and others 1995; Suzuki and others 1997; Crowe and others 1998). Much knowledge about protein stability is derived from understanding how organisms survive thermal and hydric stresses (Sun and Leopold 1997). Vitrification of protective sugars is possibly the main strategy in nature to avoid the crystallization of these sugars in anhydrobiotic organisms.

Mazzobre and others (2003, 2008) analyzed the stability of freeze-dried enzymes ( $\beta$ -galactosidase, invertase, honey amylase, soy urease, and soy transaminase) over a wide range of temperature and water-content conditions. Materials capable of forming amorphous matrices, but with different physicochemical characteristics, were chosen to compare their efficiency in protecting the enzymes. In the polymeric glassy matrices, the enzyme stability was diminished either by increasing water content at a certain temperature or by increasing the storage temperature at fixed water content. The good glass-former polymers maltodextrin and PVP were not effective in protecting the enzyme during heat treatment, the enzyme stability being affected mainly by effects of temperature. Crowe and others (1998) reported that although vitrification of the structure is necessary for improved enzyme stability, it is not the only condition required for the protection of molecules, since specific hydrogen-bond interactions between the matrix and the protein are also needed. According to this, sugars, and especially trehalose, were more effective protectants despite their lower  $T_g$  values, even at relatively high temperatures. The enzymes retained quite good activity in the trehalose supercooled region, but their activity decreased drastically when the sugar crystallized at a mass fraction of water greater than 0.1. Starch protected the enzymes adequately at high water content and low temperature where stability was completely lost in other systems. Enzyme interactions with the surface of starch granules and the high  $T_g$  value of this matrix may have played a role in enzyme stability.

When sugar crystallizes, the protein is excluded from the sugar crystals, and is exposed to a matrix where, besides lacking the stabilizing effect of hydroxyl groups, the changes in pH, concentration of reactive groups, and ionic strength may also negatively affect its stability. However, it has been observed that, for many enzymes, if sugar crystallization is inhibited or conveniently delayed, the protective action of sugars may be extended to the supercooled-liquid state (Suzuki and others 1997; Buera and others 2005; Mazzobre and others 2008).

The addition of polymers, other sugars, or salts extended the protective effect of sugars to the supercooled region by delaying crystallization (Mazzobre and others 1997; Gabarra and Hartel 1998). In dairy systems, compared with pure-lactose systems



**Figure 1.5.** Supplemented phase diagram showing the sucrose glass transition temperature ( $T_g$ ) as a function of the mass fraction of water ( $W$ ):  $T_s$ , sucrose solubility curve; and  $T_m$ , water-melting curve. The effect of magnesium chloride on the sucrose curves is indicated by arrows. The dotted lines indicate the freezing-point depression for increasing sugar/salt proportions ( $R$ ), from  $R_1$  to  $R_3$ :  $w_g'$ , the mass fraction of water for the maximum cryoconcentrated matrix; and  $T_g'$ , the glass transition temperature of the maximally concentrated matrix. Adapted from Mazzobre and others (2001).

(Jouppila and Roos 1994), the presence of proteins delayed lactose crystallization. Gelatin inhibited crystallization of raffinose and, in the presence of bovine serum albumin, of sucrose, and raffinose crystallization was inhibited even at high RH (84%) (Espinosa and others 2006). Additives like polymers that raise the overall  $T_g$  of pure sugars reduced molecular mobility and inhibited crystallization, although delayed crystallization in many studies was related to the modification of the molecular environment of the crystallizing sugar in those mixtures, affecting thermodynamic and geometric factors that control nucleation without changing  $T_g$  significantly (Mazzobre and others 2001; Longinotti and others 2002).

A special set of studies was dedicated to the analysis of the effect of salts on the phase diagram of sugars (Mazzobre and others 2001, 2008; Longinotti and others 2002), which are presented in Figure 1.5. Water sorption and water or sugar crystallization behavior indicated that the effect of salts on the sugar phase diagram was directly related to the charge/mass ratio of the cations present ( $Mg > Ca > Na > K$ ).

Measurements of electrical conductivity in concentrated sugar-salt-water systems revealed a high population of local inhomogeneities, which were induced by preferential solvation of the ions as a consequence of the larger ion-water interactions as compared with the ion-disaccharide interactions. Therefore, whereas the ion mobility is enhanced by a low-viscosity local environment, sugar molecular mobility should

be depressed by a high local viscosity. Ediger (2000) described spatially heterogeneous dynamics in supercooled liquids, as resulted from analysis by different techniques. The dynamics in regions separated by a few nanometers could be different by several orders of magnitude. The short-range dynamics of sugar-water systems could change dramatically without modifying the  $T_g$  of the system, which is a result of suprastructural relaxation. Thus, the presence of salts could retard the sugar crystallization even when  $T_g$  remains unchanged. This effect should be more pronounced for ions with stronger interactions with water. In frozen systems, on the other hand, the salts have a colligative effect (Figure 1.5) and promote an increase of the water associated with the maximally concentrated nonfrozen phase ( $w$  in Figure 1.5). Consequently, the  $T_g$  of this phase decreases. Decreased enzyme activity was observed in these salt-containing systems.

Mazzobre and others (2008) observed that the effect of salts, with regard to sugar crystallization kinetics and enzyme inactivation, seems also to be associated with the magnitude of their effect on disrupting the tetrahedral hydrogen-bond network of water. Water structure-maker ions (citrate > acetate;  $Mg^{+2}$ ) enhance the tetrahedral coordinated hydrogen-bond structure of water, and water structure-breaker ions ( $K^+$ ) disrupt the tetrahedral coordination of water. Some ions, such as  $Na^+$  or  $Cl^-$ , are considered neutral (Calligaris and Nicoli 2006). Trehalose acts as a structure breaker but provides enzyme stabilization by strong hydrogen-bonding interactions (Patist and Zoerb 2005). In restricted water environments, such as the dehydrated (or frozen) systems analyzed in the present work, the amount of water determines the kinetics of phase changes and enzyme inactivation. Thus, the type of water-ion interactions are manifested in those dynamic changes, and the use of the so-called Hofmeister series could offer great help in their description.

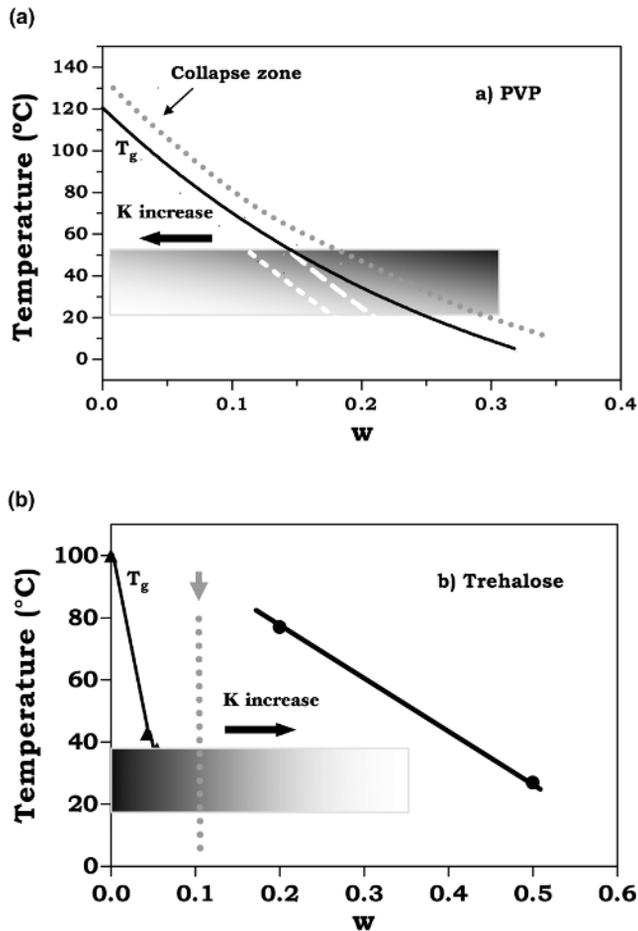
To optimize the efficiency of biomolecular dehydroprotectant agents, the development of state diagrams is a good starting point for the analysis of the dynamics of quality changes, but the diagrams must be complemented by knowledge of the intermolecular interactions that may occur. Besides supramolecular aspects, like  $T_g$  and crystallinity, the density of the molecular packing of the matrices, reducing power, and hydrogen-bonding capacity determined the effectiveness of agents as biomolecular protectants. Electrolytes commonly present in biological media or food and pharmaceutical formulations modified metastable systems, affecting the kinetics of water and sugar crystallization and enzyme inactivation, by both molecular and supramolecular interactions.

## Degradation of Carotenes

Stability and retention of labile biomolecules during drying and later storage often depend on encapsulation of the biomolecules in the amorphous matrix formed during dehydration processes (Constantino and others 1998; Pyne and others 2003). The high degree of unsaturation in the structure of carotenes renders them extremely susceptible to oxidation. Amorphous sugars are effective encapsulating agents. However, sugar crystallization as a consequence of storage above the  $T_g$  promotes not only the loss of the stabilizing effect on biomolecules such as enzymes, as previously discussed, but

also the release of encapsulated lipids (Shimada and others 1991; Labrousse and others 1992). The changes in the physical structure of the matrix may also lead to increased permeability and diffusivity of gases (water and oxygen) that affect reaction rates and decrease stability of encapsulated active materials (Karel 1991; Karel and Saguy 1991).

Figure 1.6 presents the phase diagram of PVP (a) and crystallizable trehalose (b) matrices. The shaded area indicates the magnitude of the kinetic constants of carotene degradation.



**Figure 1.6.** State diagram showing the glass transition temperatures ( $T_g$ ) as a function of the mass fraction of water ( $w$ ) for (a) polymeric (PVP) and (b) trehalose matrices in which  $\beta$ -carotene was encapsulated. The conditions at which fast-collapse or crystallization phenomena were observed are indicated by dotted lines. Shaded areas represent the regions in which the experiments were performed; within those areas, the darker regions indicate a high  $\beta$ -carotene loss rate. Arrows show the direction of the kinetic constant increase for  $\beta$ -carotene loss.

Elizalde and others (2002) showed that the rate of encapsulated  $\beta$ -carotene loss in a trehalose matrix was affected mainly by the moisture in excess of that necessary for trehalose dihydrate crystallization. In fact, when water content was low but higher than 10%, the available water crystallized with trehalose, forming the dihydrate, and less water was free to liberate the encapsulated  $\beta$ -carotene. Once crystallization was completed, the kinetics of  $\beta$ -carotene loss were strongly accelerated (Figure 1.6a). Prado and others (2006) demonstrated that  $\beta$ -carotene loss in a freeze-dried polymeric (PVP-40) matrix was observed mainly in the glassy state (below  $T_g$ ), where the high-porosity matrix allowed oxygen diffusion and then fast degradation of  $\beta$ -carotene. To the contrary, the lower degradation rate constants were observed under conditions in which the structural collapse caused the disappearance or dramatic decrease of matrix micropores (Figure 1.6b). Although maltodextrins improved the shelf life of  $\beta$ -carotene in spray-dried carrot juice (Desobry and others 1998), and the release of encapsulated material has been qualitatively related to structural collapse or shrinkage caused by storage above the matrix  $T_g$  (Omatete and King 1978; Levi and Karel 1995; Selim and others 2000; Serris and Biliaderis 2001), in the case of oxidizable compounds the sample porosity in freeze-dried amorphous systems may negatively affect the stability of encapsulated compounds.

Mannitol is a popular excipient used in freeze-dried formulations to stabilize biomolecules (proteins, enzymes, hormones, and vitamins). Obtaining amorphous pure mannitol is difficult because of the great tendency of this polyol to crystallize. Various solutes that remain amorphous in frozen solutions and during freeze drying (sodium chloride, dipotassium hydrogen phosphate [ $K_2HPO_3$ ], and glycine) were reported to inhibit mannitol crystallization (Pikal and others 1991; Constantino and others 1998; Pyne and others 2003; Yoshinari and others 2003). As in the case of sugars, discussed previously with regard to enzyme stability, the effect of salts on carotene encapsulation was studied in mannitol systems (Sutter and others 2007). Phosphate salts significantly delayed mannitol crystallization during freeze drying, and consequently the degree of  $\beta$ -carotene encapsulation increased (Sutter and others 2007). This effect was maintained for quite a long time during storage of the freeze-dried samples at 25°C. The divalent cations showed a synergistic effect and also modified  $\beta$ -carotene degradation kinetics during storage, increasing carotene's stability. The mechanism of crystallization inhibition likely includes a change in the hydrogen-bond network and/or a change in molecular mobility in the presence of divalent cations and phosphate anions. The degradation rate of  $\beta$ -carotene in a mannitol-potassium dihydrogen phosphate matrix increased as the %RH increased until reaching a value at which the samples collapsed (75% RH) and then the degradation rate decreased.

## Structural Effects

In systems with very low water content, water released during the Maillard reaction or sugar crystallization accelerated enzyme inactivation and browning (Kim and others 1981; Burin and others 2000), and the magnitude of the effect depended on the degree of collapse or porosity, which affected the retention of water in the systems (Buera

and Karel 1995; Burin and others 2004). The water-content increase resulting from the Maillard reaction was also reflected in a  $T_g$  depression (Roos and others 1996; Burin and others 2004).

Acevedo and others (2008a) showed that the different structure generated during drying of apple discs, depending on the type of drying method used (air convection or the use of different freezing rates before freeze drying) affected sorption properties of the dried material and, consequently, the rate of browning development, which in turn were also different from those of dried-apple powdered samples.

An inverse correlation was observed between degradation rate constants for  $\beta$ -carotene and degree of collapse. Thus, matrix collapse under controlled conditions during product processing may improve the stability of encapsulated biomolecules.

These observations demonstrated that factors such as microstructure and matrix porosity may be important modifiers of reaction kinetics, and the sample structure is another variable that has to be considered when the kinetics of deteriorative reactions are analyzed.

## **Concluding Remarks**

The rates of three different chemical reactions were analyzed from the perspective of phase diagrams of the matrices where the reactions occurred. Both solid-water interactions and structural characteristics of the systems governed the dependence of reaction rates on RH.

In addition to affecting chemical reactions via  $a_w$  and by plasticizing amorphous systems, water mobility itself was demonstrated to have a direct impact on chemical reactivity in low-moisture and intermediate-moisture systems.

In this way, besides the valuable information provided by localizing the plausible system conditions (compositions and temperatures) on supplemented phase diagrams, structural aspects of the matrices where the reaction occurs, water-sorption properties, and water mobility itself were detected also as key aspects that must be considered for a complete interpretation in describing the dynamics of the chemical reactions. Product formulation, process, and storage may be managed through knowledge of reaction kinetics, solid and water dynamic properties, transition temperatures, and process variables (mainly water content and temperature). Potential topics for further research include the study of macroscopic and molecular properties of the materials, such as the effect of sub- $T_g$  relaxations on the kinetics of chemical reactions, or local heterogeneities in water distribution at microscopic scales. Also, the quantification of structural effects such as collapse and compression would be valuable in the complete interpretation of deteriorative reaction kinetics.

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## References

- Acevedo NC, Briones V, Buera MP, Aguilera JM. 2008a. Microstructure affects the rate of chemical, physical and color changes during storage of dried apple discs. *J Food Eng* 85:222–31.
- Acevedo NC, Schebor C, Buera MP. 2006. Water-solids interactions, matrix structural properties and the rate of non-enzymatic browning. *J Food Eng* 77:1108–15.
- Acevedo NC, Schebor C, Buera MP. 2008b. Non-enzymatic browning kinetics analyzed through water-solids interactions and water mobility. *J Agric Food Chem* 108:900–6.
- Bell L. 1995. Kinetics of non-enzymatic browning in amorphous solid systems: distinguishing the effects of water activity and the glass transition. *Food Res Int* 28:591–7.
- Bell LN, Hageman MJ. 1994. Differentiating between the effects of water activity and glass transition dependent mobility on a solid state chemical reaction: aspartame degradation. *J Agric Food Chem* 42:2398–401.
- Bell LN, White KL. 2000. Thiamin stability in solids as affected by the glass transition. *J Food Sci* 65:498–501.
- Buera MP, Karel M. 1995. Effect of physical changes on the rates of nonenzymic browning and related reactions. *Food Chem* 52:167–73.
- Buera MP, Schebor C, Elizalde B. 2005. Carbohydrate crystallisation phenomena in dehydrated food and ingredient formulation: involved factors, consequences and prevention. *J Food Eng* 67:157–65.
- Burin L, Jouppila K, Roos Y, Kansikas J, Buera MP. 2000. Color formation in dehydrated modified whey powder systems as affected by compression and  $T_g$ . *J Agric Food Chem* 48:5263–8.
- Burin L, Jouppila K, Roos Y, Kansikas J, Buera MP. 2004 Retention of  $\beta$ -galactosidase activity as related to Maillard reaction, lactose crystallization collapse and glass transition in low moisture whey systems. *Int Dairy J* 14:517–25.
- Calligaris S, Nicoli MC. 2006. Effect of selected ions from lyotropic series on lipid oxidation rate. *Food Chem* 94:130–5.
- Chatakanonda P, Chinachoti P, Sriroth K, Piyachomkwan K, Chotineeranat S, Tang HR, Hills B. 2003. The influence of time and conditions of harvest on the functional behavior of cassava starch: a proton NMR relaxation study. *Carbohydr Polym* 53:233–40.
- Constantino HR, Andya JD, Nguyen PE, Dasovich N, Crowe JH, Carpenter JF, Crowe LM. 1998. The role of vitrification in anhydrobiosis. *Annu Rev Physiol* 60:73–103.
- Crowe JH, Carpenter JF, Crowe LM. 1998. The role of vitrification in anhydrobiosis. *Annu Rev Physiol* 60:73–103.
- Desobry SA, Netto FM, Labuza TP. 1998. Preservation of  $\beta$ -carotene from carrots. *Crit Rev Food Sci Nutr* 38:381–96.
- Ediger MD. 2000. Spatially heterogeneous dynamics in supercooled liquids. *Annu Rev Phys Chem* 51:99–128.
- Eichner K, Karel M. 1972. The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J Agric Food Chem* 20:218–23.
- Elizalde BE, Herrera L, Buera MP. 2002. Retention of  $\beta$ -carotene encapsulated in a trehalose matrix as affected by moisture content and sugar crystallization. *J Food Sci* 57:3039–45.
- Espinosa L, Schebor C, Buera MP, Moreno S, Chirife J. 2006. Inhibition of trehalose crystallization by cytoplasmic yeast components. *Cryobiology* 52:157–60.
- Gabarra P, Hartel W. 1998. Corn syrup solids and their saccharide fractions affect crystallization of amorphous sucrose. *J Food Sci* 63:523–8.
- Hodge JE. 1953. Chemistry of browning reactions in model systems. *J Agric Food Chem* 1:928–43.
- Jouppila K, Roos YH. 1994. Glass transitions and crystallization in milk powders. *J Dairy Sci* 77:2907–15.

- Karel M. 1991. Physical structure and quality of dehydrated foods. In: Mujumdar AS, Filkova I, editors. *Drying '91*. Amsterdam: Elsevier. p 26–35.
- Karel M, Saguy I. 1991. Effects of water on diffusion in food systems. In: Levine H, Slade L, editors. *Water relationships of foods*. New York: Plenum. p 157–73.
- Karmas R, Buera MP, Karel M. 1992. Effect of glass transition on rates of non-enzymatic browning in food systems. *J Agric Food Chem* 40:873–9.
- Kim M, Saltmarch M, Labuza TP. 1981. Non-enzymatic browning of hygroscopic whey powders in open versus sealed pouches. *J Food Proc Preserv* 5:49–57.
- Kou Y, Dickinson LC, Chinachoti P. 2000. Mobility characterization of waxy corn starch using wide-line <sup>1</sup>H nuclear magnetic resonance. *J Agric Food Chem* 48:5489–95.
- Kouasi K, Roos YH. 2000. Glass transition and water effects on sucrose inversion by invertase in a lactose-sucrose system. *J Agric Food Chem* 48:2461–6.
- Labrousse S, Roos Y, Karel M. 1992. Collapse and crystallization in amorphous matrices with encapsulated compounds. *Sci Aliments* 12:575–769.
- Labuza T, Baisier WM. 1992. The kinetics of nonenzymatic browning. In: Schwartzberg H, Hartel R, editors. *Physical chemistry of foods*. New York: Marcel Dekker. p 595–649.
- Labuza T, Saltmarch M. 1981. Kinetics of browning and protein quality loss in whey powders during steady state and nonsteady state storage conditions. *J Food Sci* 41:92–6.
- Leslie SB, Israeli E, Lighthart B, Crowe JH, Crowe LM. 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Appl Environ Microbiol* 61:3592–7.
- Levi G, Karel M. 1995. The effect of phase transitions on release of n-propanol entrapped in carbohydrate glasses. *J Food Eng* 24:1–13.
- Levine H, Slade L. 1992. Glass transitions in foods. In: Schwartzberg H, Hartel R, editors. *Physical chemistry of foods*. New York: Marcel Dekker. p 83–221.
- Lievonen SM, Laaksonen TJ, Roos YH. 1998. Glass transition and reaction rates: nonenzymatic browning in glassy and liquid systems. *J Agric Food Chem* 46:2778–84.
- Longinotti MP, Mazzobre MF, Buera MP, Corti HR. 2002. Effect of salts on the properties of aqueous sugar systems in relation to biomaterial stabilization. 2. Sugar crystallization rate and electrical conductivity behaviour. *Phys Chem Chem Phys* 4:533–40.
- Mazzobre MF, Buera MP, Chirife J. 1997. Protective role of trehalose on thermal stability of lactase in relation to its glass and crystal forming properties and effect of delaying crystallization. *Lebensm Wiss Technol* 30:324–9.
- Mazzobre MF, Hough G, Buera MP. 2003. Phase transitions and functionality of enzymes and yeasts in dehydrated matrices. *Food Sci Technol Int* 9:163–72.
- Mazzobre MF, Longinotti MP, Buera MP, Corti HR. 2001. Effect of salts on the properties of aqueous sugar systems in relation to biomaterial stabilization. 1. Water sorption behavior and ice crystallization/melting. *Cryobiology* 43:199–210.
- Mazzobre MF, Santagapita PR, Gutiérrez N, Buera MP. 2008. Consequences of matrix structural changes on functional stability of enzymes as affected by electrolytes. In: Gutiérrez-López GF, Barbosa-Cánovas GV, Welti-Chanes J, Parada-Arias E, editors. *Food engineering: integrated approaches*. New York: Springer. p 73–87.
- Omatete OO, Judson King C. 1978. Volatiles retention during rehumidification of freeze-dried food models. *J Food Technol* 13:265–80.
- Patist A, Zoerb H. 2005. Preservation mechanisms of trehalose in foods and biosystems. *Colloids Surf [B]* 40:107–13.
- Pikal MJ, Dellerman KM, Roy MI, Riggin RM. 1991. The effects of formulation variables on the stability of freeze-dried human growth hormone. *Pharm Res* 8:427–36.
- Prado SM, Buera MP, Elizalde BE. 2006. Structural collapse prevents  $\beta$ -carotene loss in a super-cooled polymeric matrix. *J Agric Food Chem* 54:79–85.
- Pyne A, Koustov CH, Suryanarayanan R. 2003. Solute crystallization in mannitol-glycine systems: implications on protein stabilization in freeze-dried formulations. *J Pharm Sci* 92:2272–83.
- Roos Y, Jouppila K, Zielasko B. 1996. Non-enzymatic browning-induced water plastification: glass transition temperature depression and reaction kinetics determination using DSC. *J Thermal Anal* 47:1437–50.

- Roos YH, Karel M. 1991. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *J Food Sci* 56:38–43.
- Schmidt SJ, Lai H. 1991. Use of NMR and MRI to study water relations in foods. In: Levine H, Slade L, editors. *Water relationships in foods*. New York: Plenum. p 405–52.
- Selim K, Tsimidou M, Biliaderis CG. 2000. Kinetic studies of saffron carotenoids encapsulated in amorphous polymer matrices. *Food Chem* 71:199–206.
- Serris GS, Biliaderis CG. 2001. Degradation of beetroot pigment encapsulated in polymeric matrices. *J Sci Food Agric* 81:691–700.
- Shimada Y, Roos Y, Karel M. 1991. Oxidation of methyl linoleate encapsulated in amorphous lactose-based food model. *J Agric Food Chem* 39:637–41.
- Slade L, Levine H, Finlay JW. 1989. In: Phillips R, Findlay LAW, editors. *Protein quality and the effects of processing*. Amsterdam: Elsevier Science. p 9–124.
- Sun WQ, Leopold AC. 1997. Cytoplasmatic vitrification and survival of anhydrobiotic organisms. *Plant Physiol* 89:767–72.
- Sutter SC, Buera MP, Elizalde BE. 2007.  $\beta$ -Carotene encapsulation in a mannitol matrix as affected by divalent cations and phosphate anion. *Int J Pharm* 332:45–54.
- Suzuki T, Imamura K, Yamamoto K, Satoh T, Okazaki M. 1997. Thermal stabilization of freeze-dried enzymes by sugars. *J Chem Eng Jpn* 30:609–13.
- Tang HR, Godward J, Hills B. 2000. The distribution of water in native starch granules: a multinuclear NMR study. *Carbohydr Polym* 43:375–87.
- Timmermann EO, Chirife J. 1991. The physical state of water sorbed at high activities in starch in terms of the GAB sorption equation. *J Food Eng* 13:171–9.
- Van Boekel MA. 2001. Kinetic aspects of the Maillard reaction: a critical review. *Nahrung/Food* 45:150–9.
- Yoshinari T, Forbes RT, York P, Karawahisma Y. 2003. Crystallization of amorphous mannitol is retarded using boric acid. *Int J Pharm* 258:109–20.