Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations

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Abstract

Sugars are important components of the amorphous regions of dehydrated foods, and their structure may define product performance. The objective of present work was to analyze the impact of sugar crystallization on product quality, and to discuss some strategies to avoid sugar crystallization. Chemical changes, (non-enzymatic browning, lipid oxidation and enzymatic activity) were accelerated upon crystallization of the sugar matrix. Oxidation of encapsulated pigments was not prevented in the glassy state but it was delayed by crystallization inhibition of the encapsulating sugar. At a kinetic level, sugars promote the formation of glassy matrices delaying deteriorative reactions. At a specific-interaction level, dehydroprotectants interact with proteins and membranes and stabilize them by hydrogen bonding. Both aspects are related, since specific interactions cannot be manifested if the sugar is in the crystalline state and vitrification is the property which ensures inhibition of crystallization. The study of delay/inhibition of sugar crystallization in supercooled liquids may increase the range of applications of sugars for specific purposes.

Keywords: Amorphous sugars; Dehydration; Crystallization; Dehydroprotectants; Structure

1. Introduction

The preservation of biomolecules in the dry state has acquired new interest in technological, clinical, biological and pharmaceutical fields. During drying, a certain degree of deterioration may occur, and the stabilization of biomaterials by their incorporation into carbohydrate and/ or polymer solutions before drying is a known preservation procedure (Colaço, Sen, Thangavelu, Pinder, & Roser, 1992; Crowe, Crowe, Carpenter, & Winstrom, 1987; Leslie, Israeli, Ligthart, Crowe, & Crowe, 1995; Rossi, Buera, Moreno, & Chirife, 1997). Many sugars are involved in preservation mechanisms of living organisms under extreme conditions. Among them, the non-reducing sugar trehalose is found at particular high concentrations in anhydrobiotic organisms, and resurrection plants (Salahas, Peslis, Georgiou, & Gavalas, 1997). Several seeds accumulate sucrose during development and β-fructofuranosyl oligosaccharides (raffinose, stachyose or verbascose) play a key role in the acquisition of seed desiccation tolerance (Koster & Leopold, 1988; Obendorf, 1997). These solutes have proved also to provide stabilization of dried or frozen labile biomaterials in vitro, which may be required for technological or research applications in different fields (Arakawa & Timasheff, 1982; Colaço et al., 1992; Crowe, Carpenter, & Crowe, 1998; Duddu & Dal Monte, 1997). Several mechanisms have been proposed to explain this protective effect. In the water replacement hypotheses, the stabilization was attributed to the formation of hydrogen bonds between sensitive components and disaccharide molecules when water is removed. The structural integrity of

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membranes and proteins is thus maintained (Crowe et al., 1987; Womersley & Smith, 1981). A second hypothesis is related to the ability of carbohydrates and polymers to form a glassy structure during drying under suitable conditions (Crowe, Crowe, & Carpenter, 1993; Franks, 1993; Slade & Levine, 1991) where the sensitive components are embedded. Amorphous glassy solids are meta-stable materials in a non-equilibrium state, since it is located below the saturation curve in the temperature-composition state diagram (Fig. 1), where the stable form is the crystal. However, crystallization of the amorphous solid is kinetically delayed if the sample remains in the glassy state, below the glass transition temperature ($T_g$). The $T_g$ of a material is a function of the relative proportion of its glass-forming components and of the water content. In the glassy state, most structural changes occur very slowly and only small motions of molecules, mainly rotational motions of side chains and vibrations, may occur (Levine & Slade, 1986; Slade & Levine, 1991). Molecular mobility in the amorphous region of the solid is important in determining its physicochemical stability (Hancock & Zografi, 1997). Crystallization of sugars may occur as the material undergoes the glass transition and above the transition with a rate dependent on the temperature difference, $T - T_g$ (Roos & Karel, 1991). Most physical changes in amorphous materials (including stickiness and structural collapse, or shrinkage) result from the sharp decrease of viscosity which occurs above their $T_g$ (Levine & Slade, 1986; Roos & Karel, 1991; Slade & Levine, 1991). There is evidence, however, that the maintenance of a glassy structure is not the only factor controlling enzyme stability, or chemical reactions like non-enzymatic browning and lipid oxidation (Cardona, Schebor, Buera, Karel, & Chirife, 1997; Crowe et al., 1993; Crowe et al., 1998; Mazzobre, Buera, & Chirife, 1997).

The action of cryo and dehydroprotectants can thus be ascribed to both kinetic and specific effects. At the kinetic level, cryo and dehydroprotectants promote the formation of amorphous, glassy systems, inhibit crystallization and influence the kinetics of deteriorative reactions upon storage. At the specific-interaction level, protectants are believed to interact with biological structures and stabilize them during both, freezing or drying, although by different mechanisms. Crowe et al. (1998) reported that vitrification of the structure is necessary to improve enzyme and liposome stability, but specific hydrogen-bond interactions between sugars and the biomaterial are also needed.

The objective of the present work was to analyze the relationship between several specific properties of amorphous sugars and the impact of their crystallization on the maintenance of product quality, and to discuss several strategies to avoid sugar crystallization.

2. Modification of physico-chemical aspects due to sugar crystallization

The stabilizing effect of sugars in the amorphous state can be related to decreasing the rates of: (a) chemical reactions (non-enzymic browning, enzymic activity, acid hydrolysis of sucrose), (b) protein denaturation or enzyme inactivation, (c) volatiles release, and (d) membrane disruption. The mechanisms by which amorphous sugars manifest these different protective effects are, however, of very different nature on each case. Amorphous sugars are highly hygroscopic and they may sorb large amounts of water from the surroundings, resulting in crystallization during storage above a critical, temperature-dependent relative humidity (Levine & Slade, 1986). Once crystallization is initiated, sugar molecules become tightly packed, and the amount of water that can be held therefore decreases, and, in closed containers, water remains in the amorphous phase and causes a significant depression of the $T_g$. Therefore, crystallization may also increase the rate of diffusion-controlled reactions (Roos, Jouppila, & Zielasko, 1996). Sugars that form anhydrous crystals (sucrose, lactose) may crystallize at room temperature after adsorbing the amount of water needed to decrease their $T_g$ to below room temperature (water contents about 3–4% for sucrose and 5% for lactose, in dry basis). Sugars that form hydrated crystals (trehalose dihydrate and raffinose tri, tetra or penta hydrates), retain higher amounts of water (about 10–12% in dry basis) without crystallization, since they need not only the water content necessary to get a $T_g$ value below room temperature, but they also need to have enough water to form the hydrated crystals (Crowe, Reid, & Crowe, 1996; Iglesias, Buera, & Chirife, 1997; Iglesias, Schebor, Buera, & Chirife, 2000; Kajiwara & Franks, 1997; Saleki-Gerhardt, Stowell, Byrn, & Zografi, 1995).

Table 1 lists the results of several experiments which showed that crystallization of the amorphous sugar matrices markedly accelerated some deteriorative reactions in foods, biological and model systems.

![Fig. 1. Phase diagram indicating the equilibrium solubility curve, the glass transition curve, and the time-dependent crystallization zone for sugars.](image-url)
2.1. Chemical reactions

The increased rates of chemical reactions upon sugar crystallization may be explained through the scheme depicted in Fig. 2a. When the matrix is amorphous, chemical reactions are delayed because the reactants remain diluted in a highly viscous medium. When sugar crystallizes, they are concentrated in the matrix, excluded from the crystals, and the reaction rate increases. It is well recognized that non-enzymatic browning reaction is a major deteriorative factor in the storage of dehydrated food products (Labuza & Saltmarch, 1981; Roos et al., 1996). Karmas and Karel (1994) showed how browning development was delayed by the presence of maltodextrin in a trehalose matrix containing browning reactants, in comparison with a matrix of trehalose alone. At the water activity at which trehalose crystallizes at 25 °C ($a_w = 0.43$), browning reactions are accelerated, and this was not observed in the maltodextrin system, in which trehalose crystallization was delayed. Kouassi and Roos (2000, 2001) observed that in a lactose matrix the enzymatic activity of $\beta$-fructofuranosidase was detected concomitantly with sugar crystallization, and that the inhibition of crystallization by the incorporation of maltodextrin retarded the enzyme activity by about one order of magnitude. In another kind of chemical reaction, acid hydrolysis of sucrose occurred faster in crystallizing lactose matrices than in systems in which lactose crystallization was delayed by the presence of carboxymethyl cellulose and trehalose (Schebor, Mazzobre, Burin, Buera, & Chirife, 1994).

2.2. Protein denaturation

The effect of crystallization on protein denaturation is schematically represented in Fig. 2b. In this case, the hydroxyl groups of sugars have to interact with the hydrophilic sites of proteins in dehydrated systems in order to avoid protein denaturation. When sugar crystallizes, this effect cannot be performed anymore, and the protein is excluded from the sugar crystals, where, besides lacking the stabilizing effect of hydroxyl groups of the sugar, the changes in pH, concentration of reactive groups and ionic strength may also negatively affect their stability. This behavior was observed for $\beta$-galactosidase and $\beta$-fructofuranosidase in dehydrated sugar matrices. In a crystallizing matrix of trehalose the degree

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Table 1
Reactions that were observed to be accelerated upon crystallization of sugar matrices

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-enzymatic browning</td>
<td>Karmas and Karel (1994) and Burin et al. (2000)</td>
</tr>
<tr>
<td>Enzymatic activity</td>
<td>Kouassi and Roos (2000, 2001)</td>
</tr>
<tr>
<td>Acid hydrolysis of sucrose</td>
<td>Schebor et al. (1994)</td>
</tr>
<tr>
<td>Enzyme activity loss</td>
<td>Cardona et al. (1997), Mazzobre et al. (1997), Suzuki et al. (1997), and Miller et al. (1998)</td>
</tr>
<tr>
<td>Dry liposome rupture</td>
<td>Sun et al. (1996)</td>
</tr>
<tr>
<td>Release and oxidation of encapsulated lipids</td>
<td>Labrousse et al. (1992)</td>
</tr>
<tr>
<td>Diacetyl loss</td>
<td>Senoussi et al. (1995)</td>
</tr>
</tbody>
</table>

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Fig. 2. Schematic interpretation of the effect of sugar crystallization on: the acceleration of chemical reactions (a), protein denaturation (b), membrane integrity (c) and release of encapsulated compounds (d).
of enzyme inactivation sharply increased in the $a_w$ region at which trehalose crystallizes (Cardona et al., 1997; Mazzobre et al., 1997).

2.3. Membrane and cell damage

Certain solutes added to protect cells during drying and rehydration may act on membrane integrity and/or on protein structure of the cells. The hydrogen bonding capacity of compounds added as protective agents of phospholipid head groups and proteins of membranes could be the critical factor which determines the survival of cells submitted to different treatments (Sun, Leopold, Crowe, & Crowe, 1996; Sun & Leopold, 1997). In the case of membrane stabilization, sugars may act to prevent the fusion of phospholipids heads. Fig. 2c schematizes the action of sugars preventing damage to membranes, and how this effect is lost when the sugar crystallizes.

Crowe et al. (1987) found that maltose and trehalose gave the best stability to freeze-dried unilamellar vesicles and biological membranes, compared with other mono, di or tri saccharides. They also showed that some polymers (such as dextran and hydroxyethyl starch) did not have direct interactions with the head group phosphates, nor lowered the melting transition phase of the lipids and did not protect liposomes during drying (Crowe et al., 1996). The combination of trehalose to improve membrane and protein thermal resistance and a high molecular weight compound, such as maltodextrin, to improve physical stability (delaying sugar crystallization) was particularly analyzed in dehydrated yeast cells (Lodato, Huergo, & Buera, 1999). The analysis of these systems illustrated both the lack of correlation between the physical stability of the external matrix and the remaining stability of yeasts, and the importance of trehalose concentration in the external matrix. Mazzobre, Hough, and Buera (2003) made a distinction between the different requirements to protect yeast cells or enzymes in sugar matrices: while incorporation of 50% maltodextrin was detrimental for yeast stability, it improved enzyme stability, avoiding sugar crystallization.

2.4. Release of encapsulated volatiles and lipids

For encapsulated compounds there are even more complex interactions than for soluble components. The effect of amorphous sugars on aroma retention during storage has been extensively investigated (Chirife, Karel, & Flink, 1973; Flink & Karel, 1972). Senoussi, Dumoulin, and Berk (1995) showed that diacetyl retention decreased sharply in a lactose matrix, when about 60% of lactose crystallized. In dehydrated skim milk, however, lactose crystallization was delayed (probably by the presence of proteins and salts), and the sharp increase of diacetyl loss observed in the lactose matrix did not occur. In systems containing encapsulated lipids, the protective action of the solid matrix is lost when crystallization occurs. In non-crystalline sugar systems, the stability of encapsulated lipids may be increased upon collapse of the samples above the glass transition temperature (Labrousse, Karel, & Roos, 1992). A compromise between structural collapse (favorable for retention of encapsulated compounds) and matrix crystallinity (promoting the release of encapsulated compounds) was always observed. It is interesting to note that the loss of β-carotene was related to the crystallization of the sugar matrix, but the moisture content of the amorphous phase was determinant of the extent of carotene remaining encapsulated (Elizalde, Herrera, & Buera, 2002; Prado, Elizalde, & Buera, 2002; Sutter, Elizalde, & Buera, 2002).

The effect of crystallization on the release of volatiles and lipids is schematically represented in Fig. 2d. Sugar crystals can exclude the solute and it is released. However, depending on crystallization conditions the crystals can encapsulate the solutes.

3. Modifying the kinetics of sugar crystallization

On the basis of previous studies, several strategies can be proposed to avoid sugar crystallization in a formulated system:

3.1. Vitrification

As previously discussed, regarding the phase diagram shown in Fig. 1, when a solid is maintained below $T_g$, in the glassy state, crystallization is virtually nonexistent. Vitrification can then be considered as an important strategy to avoid sugar crystallization. However, Crowe et al. (1998) proposed that vitrification was necessary but not the only requirement for stabilization of liposomes at low moisture contents. The good glass-former polymers maltodextrin and PVP, were not effective in protecting the yeasts during heat treatment (Lodato et al., 1999). On the contrary, maltose and trehalose matrices protected cell viability very well even in the liquid-supercooled state, but a critical concentration of disaccharides was necessary for the retention of yeast viability during heating. The protection provided by trehalose and maltose on yeast cells could be due to a specific effect, not directly associated with their capacity to form an external glassy matrix. Suzuki, Imamura, Yamamoto, Satoh, and Okazaki (1997) enhanced the importance of the non-crystalline nature of the sugar matrix on the stability of enzymes and Rossi et al. (1997) confirmed that the stabilization of a restriction enzyme could be achieved in liquid supercooled matrices. Tan et al. (1995) demonstrated that not only the physical stability (as determined by $T_g$ values), but also...
the hydrogen bonding capacity of the saccharides was important to determine the number of germinating mold spores after storage at 30 °C.

It can thus be generalized that if the adequate polihydroxylic compounds (namely sugars) are maintained vitrified; their protective action will be preserved. However, if sugar crystallization is inhibited or conveniently delayed, the protective action may be extended to the supercooled-liquid state.

3.2. Combination of sugars and polymers

Several authors have indicated that the presence of a polymer promoted a delay in the crystallization process of sugars: sucrose crystallization was inhibited by starch (Roos & Karel, 1991) and by corn syrup polymers (Gabarra & Hartel, 1998) and trehalose crystallization was delayed by maltodextrins (Mazzobre et al., 1997). In skim milk powder (Jouppila & Roos, 1994; Senoussi et al., 1995) and modified-whey powders (Burin, Jouppila, Roos, Kansikas, & Buera, 2004) the presence of proteins caused a delay of lactose crystallization, in comparison with pure lactose systems. Fig. 3 shows thermograms of mixtures of sugars and proteins. It can be seen that the presence of gelatin inhibits the crystallization of the sugar raffinose (Fig. 3a) and that in the presence of BSA crystallization of both sucrose and raffinose is inhibited (Fig. 3b), even at very high relative humidities (84% RH) (Schebor, Moreno, Chirife, & Buera, 2002).

3.3. Combination of various sugars

In the case of seeds, embryos and plant tissues several oligosaccharides occur, this fact was related to seed longevity due to protection of proteins and membranes, and to the prevention of sucrose crystallization (Obendorf, 1997). The effect of the mixture of several sugars was analyzed in freeze-dried model systems. In trehalose–lactose systems the time to crystallization of lactose increased when trehalose was added (Mazzobre, Soto, Aguilera, & Buera, 2001). The addition of raffinose had also a retarding effect on sucrose crystallization (Fig. 4a and b) (Mazzobre et al., 2001). It can be observed that in the dynamics runs (Fig. 4a) the onset temperature for sugar crystallization increased when raffinose was added, and was even avoided during the run if enough amount raffinose was added. In the isothermal run (Fig. 4b), both the crystallization time and the rate of sucrose crystallization increased in the systems containing raffinose.

Fig. 3. DSC thermograms of sugar/protein samples showing the crystallization of the sugar and/or the denaturation of the protein. Raffinose/gelatin samples (a), BSA/sucrose and BSA/raffinose (b) (Schebor et al., 2002).
In the food industry, the addition of a polymeric material to avoid crystallization, as previously discussed, cannot always be performed, due to quality and sensorial considerations. It would be thus necessary to use a material of similar characteristics to the one it is meant to stabilize, and the employment of a second sugar may serve to this purpose (Mazzobre et al., 2001).

### 3.4. Combination of sugars and salts

Evidence exists on the modification of some physical properties of sugars by salts (Miller, de Pablo, & Corti, 1997; Miller, Anderson, & de Pablo, 1998). The effect of electrolytes is of special interest because of their universal presence in biological systems, their major influence on water structure and their possible interactions with biomolecules. The synergistic effects of sugars and divalent cations on protein stabilization have been reported (Carpenter, Crowe, & Arakawa, 1986) but they are yet to be explained. Several physical methods have provided evidence for the existence of sugar–metal complexes in solution and many complexes of sugars and sugar derivatives with inorganic salts have been isolated in solid, and often in crystalline form (Angyal, 1973; Morel-Desrosier, Lhermet, & Morel, 1991; Rongere, Morel-Desrosiers, & Morel, 1995). Mazzobre and Buera (1999) reported that magnesium chloride could be a synergistic compound to extend the range of trehalose activity. It is interesting to note that the inhibition/delay of trehalose crystallization was afforded by the presence of salts, and especially MgCl₂ and KCl, with parallel retention of the enzymatic activity. It is interesting to note that the $T_g$ values of the mixtures trehalose-salts had the same values than the pure sugar systems (Mazzobre & Buera, 1999). Recent experiments employing sucrose confirmed that the presence of salts affected the kinetics of sucrose crystallization, besides modifying the sorption behavior (increasing the water uptake) and the kinetics of ice crystallization in solutions of higher water content. The degree at which the salts modified the properties of the sugar systems was in the order Mg > K > Na > Cs, which is the same as the charge/radius ratios of those cations. It is proposed that the salt effect on sugar crystallization occurs at a molecular level, but in such a dynamic way that the cooperative relaxations leading to glass transitions are not affected (Longinotti, Mazzobre, Buera, & Corti, 2002). It is interesting to note that by the addition of salts the protective effects of the sugars can be extended to a liquid-supercooled region of the phase diagram, at which sugar crystallization occurs fast in the absence of salts.

### 4. Evidences of delayed sugar crystallization in biological and food systems

The several examples analyzed in the previous sections indicated that the quality of the product may be maintained when crystallization is delayed/inhibited. As mentioned before, in those matrices formed mainly by sugars, crystallization has a direct impact on quality loss. Since biological and food materials are frequently very complex systems, it can be expected that sugar crystallization is naturally delayed in them, in comparison with that of pure sugars, by the presence of polymers, other sugars and/or salts. Indeed, Table 2 lists some literature references which show that sugar crystallization is naturally delayed in complex foods and biological systems. Fig. 6 shows thermograms of yeast components mixed with trehalose. It can be seen that trehalose crystallization was not possible in the presence of yeast cytoplasmatic solutes. However, in the presence of yeast proteins trehalose crystallization was detected, which indicates that other solutes present in the cytoplasm are responsible for the delay/inhibition of sugar crystallization (Espinosa, Schebor, Buera, Moreno, & Chirife, 2002).

Crystallization in the cytoplasm was proposed as a cause of viability loss in living organisms, however, no studies exist in which sugar crystallization was detected in vivo (Buitink, Hoekstra, & Hemminga, 2000a; Buitink, van den Dries, Hoekstra, Alberda, & Hemminga, 2000b; Schebor, Galvagno, Buera, & Chirife, 2000; Sun & Leopold, 1997). The only components with detectable crystallization in seeds and embryos (with deleterious consequences) are water and lipids, although at subzero temperatures (Vertucci, 1989; Williams, 1994). Water crystallization occurs at higher moisture levels than those of dehydrated media (the moisture contents need to observe water crystallization were from 25% (in dry basis) for yeast, recalcitrant seeds, cereal leaves and strawberries to about 50% (in dry basis), for silver maple seed) (Vertucci, 1989).

![Fig. 5. Effect of salts on the degree of trehalose crystallization, and parallel β-galactosidase activity retention in trehalose or trehalose-salt matrices of mass fraction of water $w = 0.1$, after storage at 45 °C.](image-url)
5. Concluding remarks

Table 3 summarizes some of the explanations for the stabilizing properties of the amorphous sugars, and the additional requirements, besides their amorphous condition, that are needed to perform the protective effect.

Several discussions have arisen during the last decade about the real impact of the glass transition temperature on food stability. The conclusion of the above observations, may establish that in matrices composed mainly by sugars, vitrification is the property which ensures the amorphous state in the matrix, and avoiding sugar crystallization is the first step to define product stability. This is the case of some pharmaceutical preparations, ingredients and model systems. In these systems the first limiting factor of their stability is the crystallization of the amorphous matrix. The glass transition of the systems is indirectly involved, since it is the factor that allows crystallization to be observed. Therefore, the glass transition temperature of the systems is one of the main factors to take into account to predict stability. However, chemical reactions are not completely inhibited in them, since below the macroscopic $T_g$ there may be local heterogeneities that allow diffusion and reaction. If the reaction starts in local zones below the macroscopic $T_g$ (as determined by DSC), the water content around the reaction site may increase, allowing further reaction to occur through further plasticization. Deteriorative reactions were also related to the physical structure of the matrix, either porous or mechanically compressed (Buera & Karel, 1995; Burin, Jouppila, Roos, Kansikas, & Buera, 2000).

In complex natural biological systems and foods, in which the presence of polymers, other sugars and salts naturally inhibit or delay sugar crystallization, the stability of the system will be limited by the degree at which physicochemical reactions (mainly non-enzymatic browning, lipid oxidation or protein denaturation) can occur. In fact, amino-carbonyl condensations (or Maillard reaction), decreased enzyme activities and lipid oxidation are known as important factors limiting the viability of seeds, pollen and dehydrated yeasts. These reactions are on turn affected by several factors, such as temperature, water activity, $T_g$. The heterogeneous nature of biological materials makes it difficult to establish a clear relationship between an overall physical property (such as glass transition temperature) and the kinetics of a chemical reaction. Local viscosity (at a molecular level) instead of bulk viscosity (related to the glass/supercooled liquid) seems to determine the mobility of small molecules (Chinachotti, 1997). Supramolecular relaxations, such as those occurring at $T_g$, do not necessarily have a direct impact on changes

### Table 3

<table>
<thead>
<tr>
<th>Stabilizing property</th>
<th>Explained by</th>
<th>Additional structural requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of browning and enzymatic reactions</td>
<td>Formation of high viscosity media</td>
<td>Non-reducing stable sugars in glassy state is the best combination, avoiding collapse or compression</td>
</tr>
<tr>
<td>Protective effect on proteins</td>
<td>Interaction of sugar hydroxyls with hydrophilic protein sites</td>
<td>Not crystalline (either glassy or supercooled)</td>
</tr>
<tr>
<td>Protective effect on membranes</td>
<td>Interaction with phospholipid heads preventing melting; decreasing membrane phase transitions; protection to protein membranes</td>
<td>Not crystalline (either glassy or supercooled)</td>
</tr>
<tr>
<td>Lipid encapsulation</td>
<td>Surface properties</td>
<td>Not crystalline, collapsed, compressed</td>
</tr>
</tbody>
</table>
occurring at a molecular level, such as those above-mentioned reactions. On the basis of previous work of other researchers and of our group it could be hypothesized that kinetics and specific interactions of sugars and biomolecules are important and are somehow related, since the specific interactions leading to biomolecule protection can only be manifested if the sugar is in the amorphous state (Suzuki et al., 1997), and vitrification is the property which ensures inhibition of crystallization.

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