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REVIEW

Spectroscopic study of the physical properties making trehalose a stabilizing and shelf life extending compound in food industry

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Abstract

Introduction Trehalose, a glass-forming bioprotectant disaccharide, has been demonstrated to possess significant potential within the food industry. It does not interact with reactive molecules such as amino groups from peptides and proteins, preventing the degradation and aggregation due to Maillard reactions. *Objective* This paper aims to review at the molecular level the effects of trehalose on the structural and dynamical properties of water and on protein to highlight the stabilization and conservation properties on food products. *Results and Conclusions* The experimental findings presented show that water molecules are arranged in presence of trehalose in a particular configuration which avoids ice formation, so limiting damage due to freezing and cooling. On the other hand, homologous disaccharides, and trehalose to a greater extent, slow down the dynamics of water with a significant influence on the biological activity. These results imply that trehalose has a greater ability to bind volatile substances and deliver superior bioprotective effectiveness. Furthermore trehalose is shown to be incapable of taking part in the denaturation process of lysozyme under thermal stress.

Introduction

The extraordinary bioprotective properties of the glass-forming disaccharide trehalose have been recently the subject of attention not only under the aspect of the pure physical, biological and chemical research (Magazù *et al.*, 2004, 2005, 2006, 2007, 2008a, b; Cottone *et al.*, 2002; Cordone *et al.*, 2005; Lerbret *et al.*, 2005; Magazù & Migliardo, 2007; Varga *et al.*, 2008), but also because of the promising applicative implications (Guo *et al.*, 2000; Zhang *et al.*, 2003; Tanaka *et al.*, 2004; Hamaratoglu *et al.*, 2005; Nie *et al.*, 2005). Many organisms show remarkable surviving capabilities to environmental stress conditions by synthesizing trehalose, which allows them to enter into a state of 'suspended life' and to re-activate their vital functions when the external conditions again become favourable. Such organisms, called 'extremophiles', are useful model-systems because the adaptation strategies they have developed can suggest innovative methodologies to be used for stabilizing biological molecules (Crowe, 1975; Crowe *et al.*, 1984a, b, 1987; Van Dijck *et al.*, 1995; Robert *et al.*, 2000; Rothschild & Mancinelli, 2001; Schiraldi & De Rosa, 2002; van den Burg, 2003; Pakchung *et al.*, 2006; Unsworth *et al.*, 2007).

Trehalose has been demonstrated to possess much potential within the food industry (Pollock & Holmstrom, 1951; Roser, 1991a, b; Van Dijck *et al.*, 1995; Gleeson & Bishop, 2000a, b; Murray *et al.*, 2000; Oku & Nakamura, 2000; Ivy, 2001; Hiashiyama, 2002; Richards *et al.*, 2002; Salas-Mellado & Chang, 2003; Danielson & Rollin, 2004; Patist & Zoerb, 2005; Duong *et al.*, 2006; Zhou *et al.*, 2006). Being a non-reducing sugar, trehalose inhibits Maillard reactions, which can seriously affect the cereal production, and therefore it can be successfully used to preserve food products and it can maintain taste and flavour of cereals and freeze-dried ingredients added to cereals (Pollock & Holmstrom, 1951; Roser, 1991a, b; Van Dijck *et al.*, 1995; Hiashiyama, 2002). In commercial baker's yeast the trehalose content is widely believed to be a critical parameter that determines its stress resistance. Studies performed on the production of 'instant active dry yeast' have demonstrated that trehalose levels of

15–22% of the dry weight are common, even if a content of 4–5% (based on the dry weight of the yeast) protects the yeast cells during prefermentation, freezing, frozen storage and thawing, while for sweet dough and for savoury dough yeasts after 45 days frozen storage the addition of 10% trehalose in dough formulation produced higher survival yeast rates (Salas-Mellado & Chang, 2003).

Trehalose can be also used as a flavour enhancer in non-standardized, ready-to-eat meat and poultry products. Furthermore it effectively reduces or eliminates the metallic, bitter and astringent off-flavours associated with the use of such ingredients as sodium phosphate, salt, potassium lactate and sodium diacetate (Danielson & Rollin, 2004).

This review aims to present an overview of the molecular mechanisms responsible for the trehalose bioprotectant effectiveness in binary disaccharide/water and in ternary disaccharide/protein/water. More specifically attention is focused on the interaction between trehalose and water and between trehalose, water and lysozyme.

Experimental Procedure

Aqueous solutions of ultrapure α,α-trehalose, maltose and sucrose, purchased by Aldrich-Chemie (Aldrich-Chemie, St. Louis, MO, USA), were investigated at different concentrations ϕ, being ϕ the weight fraction $\phi = M_d n_d / (M_d n_d + M_w n_w)$; n_d, n_w, M_d and M_w are the mole numbers and the molecular weights of disaccharide and water, respectively. Care was taken in order to obtain stable, clear and dust-free samples; ample time of about 6 h was allowed for equilibration. In addition, the solutions were filtered with 0.45 μm Amicon filters (Amicon Filters, Millipore, Billerica, MA, USA) and stored in the dark to minimize biological and photochemical degradation.

The samples for Raman scattering measurements were sealed in optical quartz cells of inner diameter 5 mm and then mounted in an optical thermostat especially built to avoid any unwanted stray-light contribution. I_{VV} and I_{VH} Raman spectra were obtained by a high-resolution fully computerized Spex-Ramalog 5 triple monochromator (HORIBA Jobin Yvon Srl, Milano Italy) in a 90° scattering geometry. Vertically polarized radiation of an INNOVA 70 Series Ar–Kr gas-mixed laser operating in the range 4579–6764 Å was used as excitation source. To reduce fluorescence we chose the 6471 Å laser line. Each reported spectrum is the average of different scans. For each isotherm spectrum, the individual I_{VV} and I_{VH} scans were taken in an alternating sequence to ensure that a definite intensity relationship existed between the final I_{VV} and I_{VH} spectra of the some isotherm. Isotropic scattering intensities were calculated from the parallel and perpendicular, respect to the scattering plane, components of the scattered light by $I_{is} = I_{VV} - 4/3 I_{VH}$. All the spectral contributions were normalized with the C–H stretching band that does not suffer H-bond interactions. Finally, this contribution was removed from the OH-stretching contour.

Neutron diffraction (ND) measurements combined with H/D substitution have been performed on trehalose aqueous solutions (hydrated trehalose in H_2O, partially deuterated trehalose in 50% H_2O/50% D_2O and totally deuterated trehalose in D_2O), for temperature values of $T = 300$ and 340 K and for two concentration values corresponding to 40 and 20 water molecules for each of trehalose, by using the SANDALS diffractometer at ISIS facility (DRAL, UK). The technique of isotope substitution enables the water structure to be viewed directly by making use of the pronounced difference in neutron-scattering length between hydrogen and its isotope deuterium. The water protons can be labelled independently of the other atoms in the solution and so their relative correlation can be obtained independently. In this context the hydrogen-bonding interaction plays a key role as responsible of the characteristic form in the short and medium range of water. This technique allows observations as to whether this arrangement is altered significantly in the presence of a dissolve solute.

Quasi elastic (QENS) and elastic neutron scattering (ENS) experiments have been carried out on hydrogenated trehalose, maltose and sucrose ($C_{12}H_{22}O_{11}$) in H_2O and on partially deuterated trehalose, maltose and sucrose ($C_{12}H_{14}D_8O_{11}$) in D_2O. All the three disaccharides possess hydrogen atoms, belonging to the OH groups, which exchange easily with the deuterium atoms of heavy water. Measurements were performed in a temperature range of $273 \div 353$ K (QENS experiment) and of $20 \div 310$ K (ENS experiment) on hydrogenated trehalose, maltose and sucrose in H_2O and on partially deuterated trehalose, maltose and sucrose in D_2O at a weight fraction values corresponding to 19 and 6 water (H_2O and D_2O) molecules for each disaccharide molecule. We estimated that in the deuterated solutions (at the investigated concentration) the coherent contribution to the total scattering cross-section is ∼5%. In the case of protonated samples, we focused attention on the incoherent scattering arising from the self-correlation function, which involves the motions of protons, the ratio between the incoherent cross-section, σ_i, and the scattering cross-section, σ_s, being $\sigma_i/\sigma_s = 0.94$.

The QENS experiment was carried out using the IRIS backscattering spectrometer at ISIS facility (DRAL). We measured sets of QENS spectra covering a Q, ω-domain

extending from $\eta\omega = -0.3$ to 0.6 meV (energy transfer) and $Q = 0.3$–$1.8\ \text{Å}^{-1}$ (momentum transfer). The detectors used give a mean energy resolution of $\Gamma = 8\ \mu\text{eV}$ of half-width at half-maximum as determined by reference to a standard vanadium plate. The raw spectra were corrected and normalized using the standard GENIE procedures.

The ENS measurements were performed using the IN13 spectrometer at ILL (Grenoble, France). The incident wavelength was 2.23 Å; the Q-range was 0.28–4.27 Å^{-1}; the elastic energy resolution (full-width and half-maximum) was 8 μeV and the resolution time was 0.1 ns. Raw data were corrected for cell scattering and detector response and normalized to unity at $Q = 0\ \text{Å}^{-1}$.

Small angle neutron scattering (SANS) findings have been obtained by using the LOQ spectrometer at the ISIS Pulse Neutron Facility (RAL, UK) at the temperature values of $T = 310$ and 333 K on lysozyme/D_2O and lysozyme/D_2O/trehalose mixtures at a lysozyme concentration of 10 mg mL^{-1} and for a lysozyme:trehalose mass ratio of 1:1. The Q-range covered by the LOQ spectrometer is from 0.007 Å^{-1} to 0.287 nm^{-1} and the wavelength resolution (full-width and half-maximum) is $8\% < \Delta\lambda/\lambda < 18\%$.

Results and discussion

Figure 1 shows an example of the isotropic spectra of the O–H stretching band for α,α-trehalose, maltose and sucrose aqueous solutions at $T = 293$ K at three concentrations, ϕ being the molar fraction. A remarkable observation resulting from an inspection of these Raman spectra, is that no additional Gaussian components at higher frequencies were required to fit the OH-stretching contour, that is, no components, whatever, near 3500 and 3620 cm^{-1} were required at high concentration. The higher frequency components arise when hydrogen bonds are broken and their absence in spectra indicates that free O–H groups are not present. As an example, the decomposition of the isotropic spectrum of the three disaccharides at $\phi = 0.09$ into an 'open' and a 'closed' contribution, is reported in Figure 2. The result of the decomposition of each spectrum into an 'open' [attributed to the O–H vibration in tetrabonded H_2O molecules that originate low-density patches in the system (stretched water)] and a 'closed' [corresponds to the O–H vibration of H_2O molecules that have a not fully developed hydrogen bond (distorted bond)] contribution (D'Arrigo *et al.*, 1981) is reported. The fractional open band intensity is expressed by

$$B(T,\phi) = \frac{\int I_{is}^{open}(\omega, T, \phi)d\omega}{\int I_{is}^{tot}(\omega, T, \phi)d\omega} \quad (1)$$

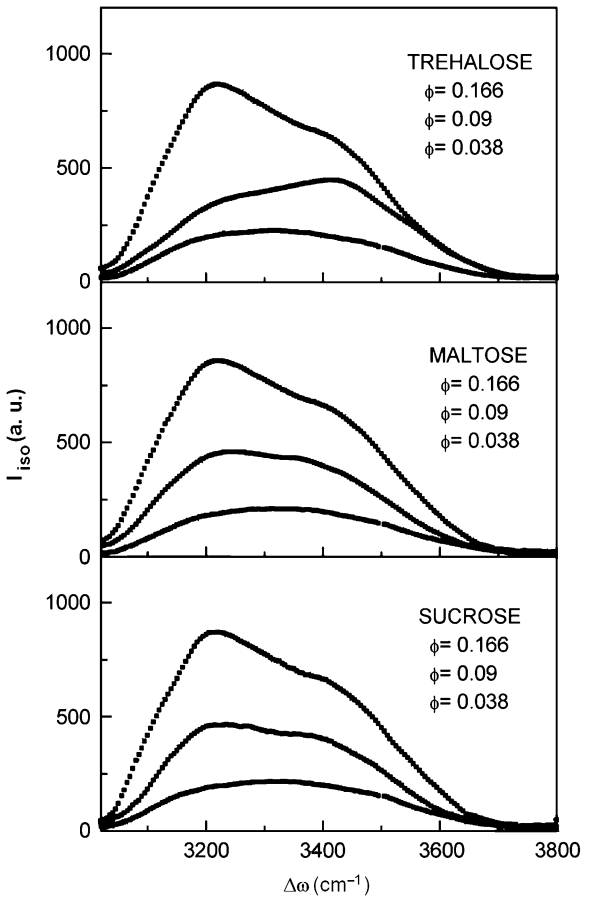

Figure 1 Comparison among the isotropic spectra, $I_{is}(\omega)$, of the O–H stretching band for trehalose, maltose and sucrose aqueous solutions at $T = 293$ K at three concentrations, being ϕ the molar fraction.

and it is shown in the insert as a function of the molar fraction for the three disaccharides in the concentration range $0 < \phi < 0.2$ at $T = 293$ K.

The most noteworthy feature is that, by decreasing the water content, one observes a marked decrease of the 'open' area contribution which tends to a plateau value (Branca *et al.*, 1999). These findings are consistent with the picture of a disaccharides destructuring effect on the H_2O-tetrabonded network of molecules that originates low-density conformations similar to that of supercooled water (Lerbret *et al.*, 2005; Magazù *et al.*, 2005, 2007, 2008b). In addition, as it is shown in the figure, for the same concentration the integrated area values decrease. This allows us to state that a more marked destructuring effect occurs in the presence of trehalose rather than in the presence of sucrose or maltose.

The Raman scattering findings have been confirmed by the ND results. It is known that the peaks at 2.3 and 3.8 Å in the partial distribution function g_{HH} are indicative of intermolecular orientational correlations and are associated

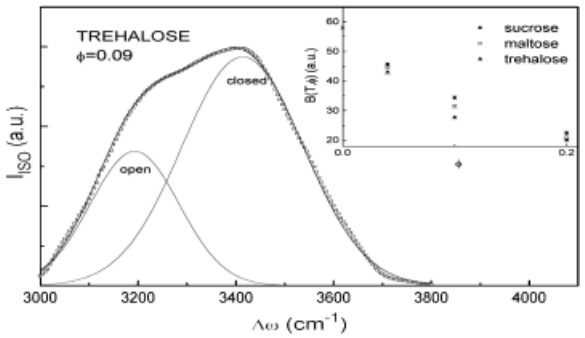

Figure 2 Decomposition of the isotropic spectra of the three disaccharides at $\phi = 0.09$ into an 'open' and a 'closed' contribution. In the insert the fractional open band intensity as a function of the molar fraction for the three disaccharides in the concentration range $0 < \phi < 0.2$ at $T = 293$ K is shown.

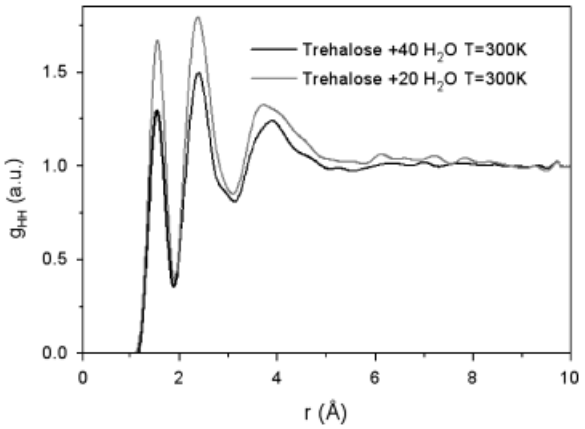

Figure 3 Partial distribution function g_{HH} for trehalose aqueous solutions at the concentration values corresponding to 40 and 20 H_2O molecules for each one of trehalose at $T = 300$ K.

with the tetrahedral coordination of water molecules (Soper & Luzar, 1992; Soper, 2000). As it is evident in Figure 3, for trehalose aqueous solutions at a concentration value corresponding to 40 H_2O molecules for each one of trehalose at $T = 300$ K, these peaks are shifted to slightly larger r values, as in the case of pure water at elevated temperatures. Furthermore the peak at 4.5 Å in g_{OO} of pure water is associated with the 'degree of tetrahedrality' (Soper & Luzar, 1992; Soper, 2000). In trehalose+40 H_2O at $T = 300$ K, shown in Figure 4, this peak is absent and the general trend is significantly distorted (Pagnotta *et al.*, 2008).

The described Raman and ND results point out the trehalose capability for distorting the hydrogen-bonded network of water. In the food industry this function of trehalose has important consequences. Because trehalose is a hydrogen bond donor, its destructuring effect on the tetra-

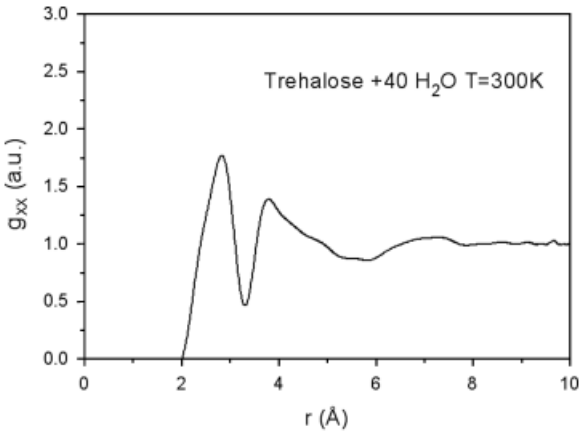

Figure 4 Partial distribution function g_{OO} for trehalose aqueous solutions at a concentration value corresponding to 40 H_2O molecules for each one of trehalose at $T = 300$ K.

hedral hydrogen bond network of water has a significant effect in reducing the probability of ice formation and in stabilizing biomolecules in food products during freeze- and air-drying processes (Pollock & Holmstrom, 1951; Roser, 1991a, b; Van Dijck *et al.*, 1995; Hiashiyama, 2002; Richards *et al.*, 2002; Zhou *et al.*, 2006). As an example, trehalose has been shown to effectively prevent the protein denaturation of tilapia surimi during frozen storage (Zhou *et al.*, 2006). Trehalose (8% w/w) appeared to deliver better cryoprotection than the commercial blend (sucrose/sorbitol, 1:1), therefore, trehalose can be used as alternative cryoprotectants in surimi due to its low sweetness and caloric value.

Let us analyse the dynamical properties of disaccharide/water solutions. It is important to notice that in the investigated mixtures no protons from bulk water are present only protons of the disaccharide molecule and of its hydration shells (Magazù *et al.*, 2006, 2008b; Magazù & Migliardo, 2007).

The spectra have been analysed using the fitting function:

$$\begin{aligned} S_{\mathrm{inc}}(Q,\omega) = & A(Q)\left\{ f_{\mathrm{disaccharide}}\left[F(Q)\frac{1}{\pi}\frac{\Gamma_1(Q)}{\Gamma_1^2(Q)+\omega^2} \right.\right. \\ & \left.\left. +(1-F(Q))\frac{1}{\pi}\frac{\Gamma_2(Q)}{\Gamma_2^2(Q)+\omega^2}\right] \right. \\ & \left. + f_{\mathrm{hydr}}\frac{1}{\pi}\frac{\Gamma_3(Q)}{\Gamma_3^2(Q)+\omega^2} \right. \end{aligned} \qquad (2)$$

where the first two terms refer to the translational and rotational contribution of hydrated disaccharide ($f_{\mathrm{disaccharide}}$ and f_{hydr} represent fraction factors of the total scattering from disaccharide and its strongly bonded water molecules), and the third one refers to hydration water ($f_{\mathrm{disaccharide}} + f_{\mathrm{hydr}} = 1$). Therefore information on the

diffusive dynamics of disaccharide can be obtained by analysing the disaccharide+D_2O spectra analysis for which the f_{hydr} results negligible.

The line width of the translational contribution as a function of Q^2 follows a typical random jump diffusion (RJD) model, as shown in Figure 5 (Magazù *et al.*, 2006, 2008b; Magazù & Migliardo, 2007):

$$\Gamma_1(Q) = D_s Q^2/(1 + D_s Q^2 \tau) \quad (3)$$

where D_s is the self-diffusion coefficient of the molecule and τ is the residence time.

The jump diffusion model assumes that the diffusive particle remains in a given site for a time τ, where it vibrates around a centre of equilibrium. After τ, it moves rapidly to a new position separated by the vector $\boldsymbol{l}$ from its original site.

The RJD model furnishes for the diffusion coefficient and the residence time values of $D_s = 1.58 \times 10^{-6}\,cm^2\,s^{-1}$ and $\tau = 12.9$ ps for trehalose and $D_s = 2.19 \times 10^{-6}\,cm^2\,s^{-1}$ and $\tau = 11.8$ ps for sucrose at $T = 320$ K. Furthermore from the relation $\langle l^2 \rangle = 6D\tau$, we can estimate the mean jump length $\langle l \rangle$ values, obtaining the value $\langle l^2 \rangle^{1/2} = 1.2$ Å for the two disaccharides. As far as the rotational contribution is concerned, the fitting procedure furnishes Γ_2 values nearly constant with Q ($\Gamma_2 \sim 59$ μeV for trehalose and $\Gamma_2 \sim 89$ μeV for sucrose), in agreement with the rotational model requirement. As far as water dynamics are concerned, the $\Gamma_3(Q)$ component dominates changes in broadening for all Q. In Figure 6 the $\Gamma_3(Q)$ behaviour, that in this model is referred to as hydration water, is reported together with the best fit according to the RJD model. For the diffusion coefficient of water in the investigated solutions we obtained at $T = 320$ K the value of $D_w = 1.49 \times 10^{-5}\,cm^2\,s^{-1}$ for trehalose solution and $D_w = 1.68 \times 10^{-5}\,cm^2\,s^{-1}$ for sucrose solution, with the values of residence times of $\tau = 3.4$ and 2.8 ps for trehalose and sucrose solutions, respectively, obtaining for the mean jump length $\langle l \rangle$ the value $\langle l^2 \rangle^{1/2} = 1.7$ Å. The whole water dynamics resembles that of water at ~267 K in the case of trehalose solution and at ~277 K in the case of sucrose solution, indicating that the water has a diffusive behaviour strongly triggered by the disaccharide molecules and suffers of a noticeable freezing effect (Magazù *et al.*, 2006, 2008b; Magazù & Migliardo, 2007). In Figure 7 the diffusion coefficient and residence time as a function of temperature for trehalose in comparison with water is shown.

These QENS findings confirm the strong influence of trehalose on water, so highlighting also the alterations of

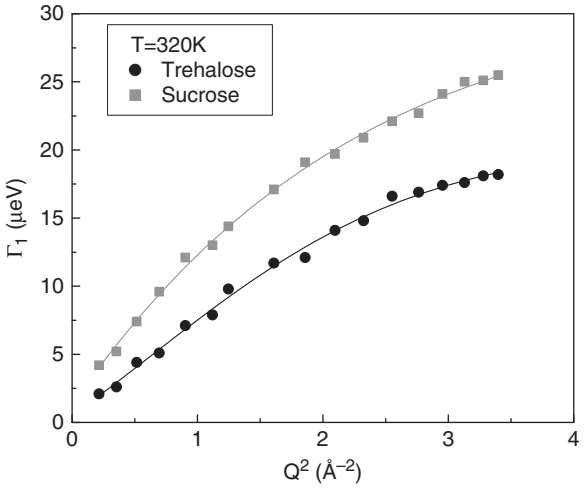

Figure 5 Line width of the translational contribution as a function of Q^2 for trehalose and sucrose at $T = 320$ K. The fitting lines are obtained according to the random jump diffusion model [equation (3)].

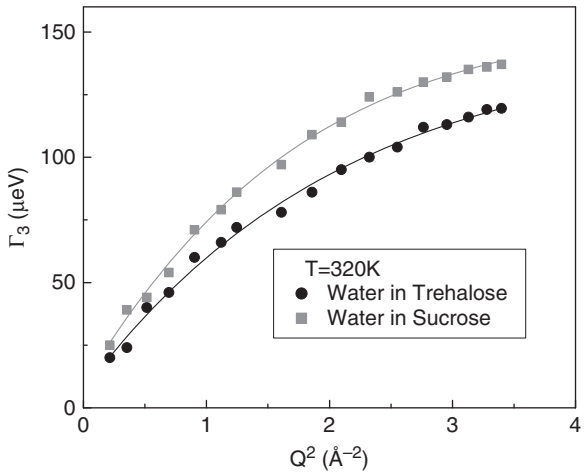

Figure 6 Line width of the translational contribution as a function of Q^2 for water at $T = 320$ K. The fitting lines are obtained according to the random jump diffusion model [equation (3)].

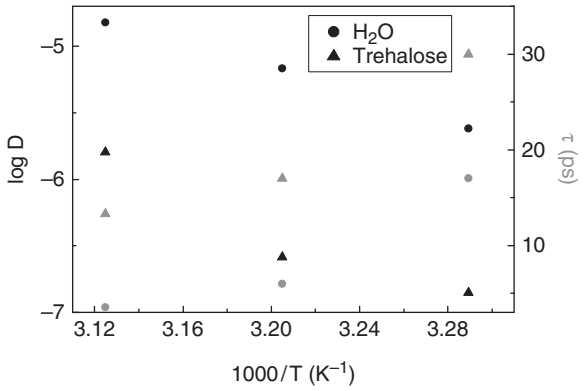

Figure 7 Diffusion coefficient and residence time as a function of temperature for trehalose in comparison with water.

water dynamics in presence of trehalose. This property, together with the capability to affect the structural arrangement of water, can be summarized by the kosmotrope nature of trehalose solute, which imposes a different order to water molecules. This peculiarity is related to the cryoprotective effect of trehalose in cryopreservation. It is known (Roser, 1991a; Hiashiyama, 2002; Richards *et al.*, 2002; Patist & Zoerb, 2005; Duong *et al.*, 2006; Zhou *et al.*, 2006) that in the food industry some microorganism cultures, before being used for industrial fermentations, are stored under particular conditions which increase the sensitivity of microorganisms to cryogenic stress resulting in significant cell injury and death and ultimately reducing productivity or activity. For example, it has been shown that the survival of *Lactobacillus acidophilus* increased concomitantly with increasing concentration of trehalose in a dose-dependent manner with survival appearing to be optimal in 20% trehalose (Duong *et al.*, 2006).

Let us analyse another effect of the presence of trehalose on the water dynamics. It is known that the ENS intensity is a combination of several Gaussian functions:

$$I(Q,t) \cong \sum_{\alpha=1}^{N} x_\alpha \exp\left[-\frac{Q^2}{3}\langle r_\alpha^2\rangle[1 - C_\alpha(t)]\right] \tag{4}$$

where $\langle r_\alpha^2\rangle$ is the equilibrium mean-square displacement, $C_\alpha(t)$ stationary position relaxation function. The incoherent dynamic structure factor $S_{\mathrm{inc}}(Q,\omega)$ is composed by two contributions: an elastic contribution $S_{\mathrm{inc}}^{\mathrm{el}}(Q) = S_{\mathrm{inc}}(Q, \omega = 0) = I(Q,\infty)\delta(\omega) \cong I(Q,\tau)$ (τ being the experimental resolution time), and a quasielastic contribution that involves energies $\eta\omega > 0$ (Magazù *et al.*, 2004, 2008a, b; Magazù & Migliardo, 2007; Varga *et al.*, 2008).

The mean-square displacement, $\langle u^2\rangle$, which takes into account fluctuations of all particles in the investigated system, is given by

$$\langle u^2\rangle = -3\frac{d\{\ln[S_{\mathrm{inc}}^{\mathrm{el}}(Q)]\}}{dQ^2}\Big|_{Q=0} = \sum_{\alpha=1}^{N} x_\alpha\langle r_\alpha^2\rangle[1 - C_\alpha(\tau)] \tag{5}$$

Nonetheless, for a given experiment $C_\alpha(\tau)$ is a constant that rescales the observed mean-square displacement, and hence $C_\alpha(\tau) = 0$ can be assumed. For simplicity in the present analysis the assumption that all particles are dynamically equivalent will be made, therefore in the previous equations x_α has been assumed to be equal to 1.

Figure 8 shows the mean-square displacements for trehalose and sucrose/H_2O mixtures for two concentration values as a function of temperature. If we define the mean-square amplitude $\langle u^2\rangle_{\mathrm{loc}}$ is defined as the difference between the mean-square displacement of the ordered and disordered phase:

$$\langle u^2\rangle_{\mathrm{loc}} = \langle u^2\rangle_{\mathrm{anharm}} - \langle u^2\rangle_{\mathrm{harm}} \tag{6}$$

A new operative definition to characterize the 'fragility' degree by ENS can be introduced:

$$M = \frac{d(u_0^2/\langle u^2\rangle_{\mathrm{loc}})}{d(T_g/T)}\Big|_{T=T_g^+} \tag{7}$$

From equation (7) using the parameter fit $\langle u^2\rangle_{\mathrm{loc}}$, we evaluate a fragility parameter M for all the investigated systems, obtaining the result shown in Figure 9. It is significant to observe the reported values indicate that the present operative definition for fragility furnishes an excellent direct proportionality between M and m. Although in

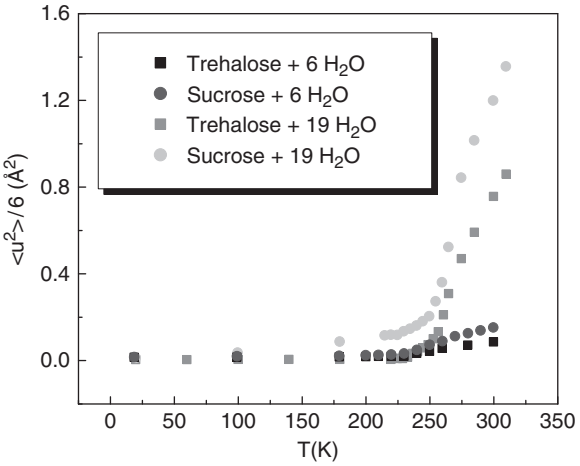

Figure 8 Mean square displacements for disaccharides/H_2O mixtures.

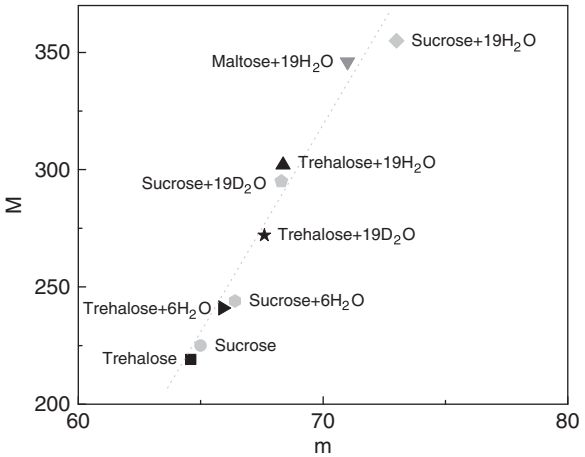

Figure 9 Fragility parameter M as a function of m.

principle it is not necessary, it can be useful to characterize a wide temperature range both for a good numeric evaluation of fragility, through m and M, and to highlight possible anomalies. The new procedure allows to compare M values for different compounds but also establishes a connection between a macroscopic parameter (viscosity) and a nanoscopic one (mean-square displacement).

Although all the three disaccharides/H_2O mixtures are considered as 'intermediate' systems in the Angell strong-fragile classification scheme (Angell *et al.*, 1994; Angell, 1997), the trehalose/H_2O mixture is stronger than the other two investigated systems and shows a higher glass transition temperature T_g in comparison with its homologous. This means that that the trehalose/H_2O mixture is characterized with respect to the maltose/H_2O and sucrose/H_2O mixtures by a higher resistance to local structural changes when temperature decreases towards the glass transition value. In this context the 'strongest' character of trehalose/H_2O mixture can justify its better effect with respect to maltose and sucrose/H_2O mixtures for encapsulating biostructures with protective glassy shell (Magazù *et al.*, 2004, 2008a, b; Magazù & Migliardo, 2007; Varga *et al.*, 2008).

The results described above which describe the glass-forming properties of trehalose are strictly linked to the action of the disaccharide as a shelf life extending compound (Hiashiyama, 2002; Richards *et al.*, 2002; Patist & Zoerb, 2005). One of the properties of food products, which determine their stability, is T_g. With increasing temperature, a food system passes from a stable state (below T_g) to a state where the system is more and more unstable following the increase of the temperature gap between the glass transition and the storage temperature. By overcoming T_g, in the case of dried proteins embedded in a glass, for example, molecular motions are physically constrained. Because water can act as a strong plasticizer, as highlighted by the ENS results, the T_g decreases with increasing water and therefore one can conclude that the high T_g value of trehalose increases the bioprotective effectiveness of the disaccharide in the anhydrous state. These results point to a cryptobiotic action for trehalose, which is capable of protecting food products in a more rigid glassy shell, so preserving also their safety.

Let us investigate the effect of the presence of trehalose on the conformational properties of a well-known protein, lysozyme, under thermal stress.

The SANS coherent scattering cross-section can be written as (Lindner, 1991; King, 1999)

$$\left(\frac{d\sigma}{d\Omega}\right)_{coh} = N\Delta(\underline{Q}) + N\langle|P_i(\underline{Q})|\rangle^2 S(\underline{Q}) \qquad (8)$$

where the term $\langle[P(\underline{Q})]^2\rangle$ is the form factor and contains information about the size, the shape and the internal structure of the molecule.

The Guinier relation, $\Phi(Q) = \Delta\rho^2(V')^2 \exp\left(-\frac{Q^2R_g^2}{3}\right)$, where V' is the volume of the particle and R_g is the gyration radius, can be used to get only information about the size, for Q values smaller than the inverse of the characteristic dimensions of the particle. Figure 10 shows the Guinier plot for lysozyme/D_2O/trehalose solutions for $T = 310$ and 333 K. The values obtained for the gyration radius are: $R_g = 11.8$ Å for lysozyme/D_2O solutions at $T = 310$ K and $R_g = 12.6$ Å at $T = 333$ K, and $R_g = 16.2$ Å for lysozyme/D_2O/trehalose solutions at $T = 310$ K and $R_g = 16.4$ Å at $T = 333$ K.

Because an increase of the dimensions of the protein in presence of trehalose is evident, we can conclude that trehalose creates a continuous network among the units of lysozyme. In addition, by increasing the temperature, the value of the gyration radius is not changed, this

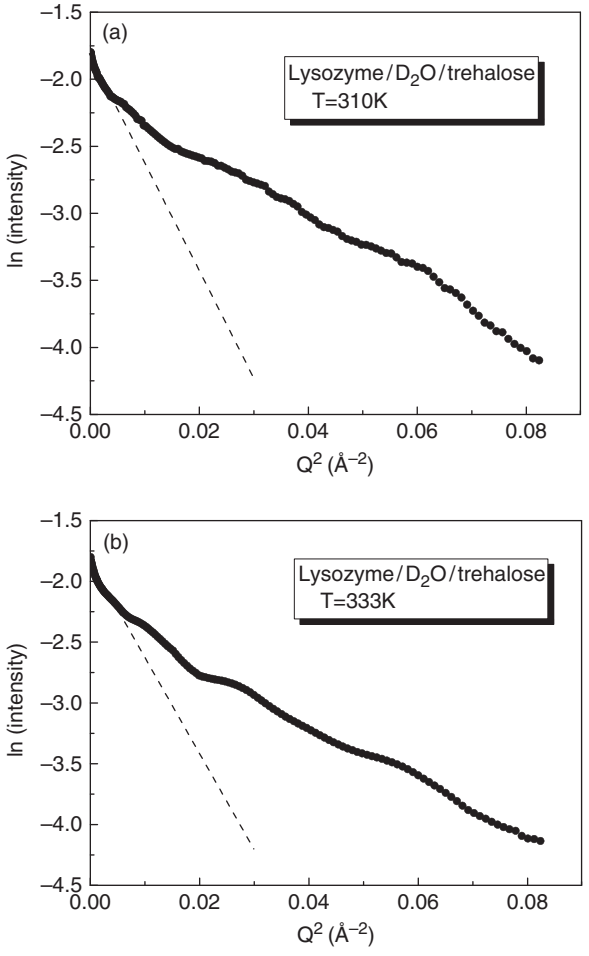

Figure 10 Guinier plots for lysozyme/D_2O/trehalose solutions for (a) $T = 310$ K and (b) $T = 333$ K.

circumstance emphasizing the stabilizing effect of trehalose on lysozyme.

It is known that trehalose effectively stabilizes food products, so preventing protein denaturation, starch retrogradation and lipid degradation (Crowe *et al*., 1987; Van Dijck *et al*., 1995; Richards *et al*., 2002; Patist & Zoerb, 2005) and maintaining food safety and quality. In this context the SANS results point out to increased stability of lysozyme under thermal stress in the presence of trehalose, so justifying the key-role of this disaccharide in food industry.

Conclusions

In this review the bioprotective effectiveness of trehalose was investigated by moving from the analysis of relevant properties of trehalose to link the effect of its presence on the structural and dynamical properties of water and on protein denaturation to the usefulness of trehalose in food industry, which in turn, increases food safety and quality.

It has been emphasized by Raman and neuron scattering (NS) findings that trehalose is capable of imposing an order on the tetrahedral hydrogen bond network of water, so revealing a strong kosmotrope character. This aspect of trehalose action is confirmed by the QENS results, which reveal that the dynamics of disaccharides and water are strongly coupled, because the diffusion of water in trehalose solutions is strongly slowed down. These results clarify the mechanisms responsible for the cryobiotic effect of trehalose and explain its role in freezing, freeze-drying and lyophilization processes used in food production and storage.

The glass-forming properties of trehalose have been described by the ENS results, which show the cryptobiotic capability of trehalose in protecting biomolecules in rigid glassy shell. These properties are closely linked with the capability of trehalose for inhibiting the denaturation processes of biomolecules. The SANS results which were presented highlight the capability of trehalose for significantly inhibiting the swelling of lysozyme induced by thermal stress.

The experimental evidence has identifies the stabilizing action of trehalose in food products which justifies its use for maintaining the food quality and safety.

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References

Angell C.A. (1997) Glassforming liquids with microscopic to macroscopic two-state complexity. *Progress of Theoretical Physics*, **126**, 1–7.

Angell C.A., Poole P.H., Shao J. (1994) Glassforming liquids, anomalous liquids and polyamorphism in liquids and biopolymers. *II Nuovo Cimento D*, **16**, 993–1025.

Branca C., Magazù S., Maisano G., Migliardo P. (1999) Anomalous cryoprotective effectiveness of trehalose: Raman scattering evidences. *Journal of Chemical Physics*, **111**, 281–287.

Cordone L., Cottone G., Giuffrida S., Palazzo G., Venturoli G., Viappiani C. (2005) Internal dynamics and protein-matrix coupling in trehalose coated proteins. *Biochimica Biophysica Acta*, **1749**, 252–281.

Cottone G., Cicciotti G., Cordone L. (2002) Protein-trehalose-water structures in trehalose coated carboxy-myoglobin. *Journal of Chemical Physics*, **117**, 9862–9866.

Crowe J.H. (1975) The physiology of cryptobiosis in tardigrades. In: R. Higgins (ed.) International symposium on tardigrades, Italy, June 17–19, 1974. Mem. Ist. Ital. Idrobiol. 32 (Suppl.), 37–59.

Crowe J.H., Crowe L.M., Carpenter J.F., Wistrom C. (1987) Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochemical Journal*, **242**, 1–10.

Crowe J.H., Crowe L.M., Chapman D. (1984a) Infra-red spectroscopic studies on interactions of water and carbohydrates with a biological membrane. *Archives of Biochemical Biophysics*, **232**, 400–407.

Crowe J.H., Crowe L.M., Chapman D. (1984b) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science*, **223**, 701–703.

Danielson M., Rollin S.T. (2004) USDA determines Cargill's ascend trehalose suitable for meat products. Report to Cargill, Cargill Health and Food Technologies (H&FT).

D'Arrigo G., Maisano G., Mallamace F., Migliardo P., Wanderlingh F. (1981) Raman scattering and structure of normal and supercooled water. *Journal of Chemical Physics*, **75**, 4264–4271.

Duong T., Barrangou R., Michael Russell W., Klaenhammer T.R. (2006) Characterization of the *tre* locus and analysis of trehalose cryoprotection in *Lactobacillus acidophilus* NCFM. *Applied and Environmental Microbiology*, **72**, 1218–1225.

Gleeson M., Bishop N.C. (2000a) Studies on the potential use of trehalose as an ingredient of sports beverage; Study 1 the Glycaemic index of trehalose. Report to British Sugar, University of Birmingham, UK.

Gleeson M., Bishop N.C. (2000b) Studies on the potential use of trehalose as an ingredient of sports beverage; Study 2 effects of trehalose, glucose, or placebo ingestion on metabolic responses, perception of effort and endurance performance during constant load cycling to fatigue. Report to British Sugar, University of Birmingham, UK.

Guo N., Puhlev I., Brown D.R., Mansbridge J., Levine F. (2000) Trehalose production confers desiccation tolerance on human cells. *Nature Biotechnology*, **18**, 168–171.

Hamaratoglu F., Eroglu A., Toner M., Sadler K.C. (2005) Cryopreservation of starfish oocytes. *Cryobiology*, **50**, 38–47.

Hiashiyama T. (2002) Novel function and application of trehalose. *Pure Applied Chemistry*, **74**, 123–129.

Ivy J.L. (2001) The glycaemic index and performance enhancing capacity of trehalose. Report to Cargill, University of Texas, USA.

King S.M. (1999). Small angle neutron scattering. *In Modern Techniques for Polymer Characterisation* eds Pethrick R.A., Dawkins J.V. John Wiley and Sons, UK. pp. 171–232.

Lerbret A., Bordat P., Affouard F., Descamps M., Migliardo F. (2005) How homogeneous are the trehalose, maltose and sucrose water solutions? An insight from molecular dynamics simulations. *Journal of Physical Chemistry B*, **109**, 11046–11053.

Lindner P. (1991). *Neutron, X-Ray and Light Scattering*. Th. Zemb, The Netherlands.

Magazù S., Maisano G., Migliardo F., Benedetto A. (2008a) Elastic incoherent neutron scattering on systems of biophysical interest: mean square displacement evaluation from self-distribution function. *Journal of Physical Chemistry B*, **112**, 8936–8942.

Magazù S., Maisano G., Migliardo F., Mondelli C. (2004) Mean square displacement relationship in bioprotectant systems by elastic neutron scattering. *Biophysics Journal*, **86**, 3241–3249.

Magazù S., Migliardo F. (2007) Elastic, quasi elastic and inelastic neutron scattering studies on hydrogen-bonded systems of biophysical interest. *Chemical Physics Research Journal*, **1**, 37–43.

Magazù S., Migliardo F., Ramirez-Cuesta A.J. (2005) Inelastic neutron scattering study on bioprotectant systems. *Journal of Royal Society Interface*, **2**, 527–531.

Magazù S., Migliardo F., Ramirez-Cuesta A.J. (2007) Changes in water vibrational modes induced by bioprotectant systems. *Biophysical Chemistry*, **125**, 138–142.

Magazù S., Migliardo F., Telling M.T.F. (2006) α,α-trehalose/water solutions. VIII. Study of diffusive dynamics of water by high-resolution quasi elastic neutron scattering. *Journal of Physical Chemistry B*, **110**, 1020–1026.

Magazù S., Migliardo F., Telling M.T.F. (2008b) Structural and dynamical properties of water in sugar mixtures. *Food Chemistry*, **106**, 1460–1467.

Murray I.A., Coupland K., Smith J.A., Ansell D., Long R.A. (2000) Intestinal trehalase in a UK population; establishing a normal range and the effect of disease. *British Journal of Nutrition*, **83**, 241–245.

Nie Y., de Pablo J.J., Palecek S.P. (2005) Platelet cryopreservation using a trehalose and phosphate formulation. *Biotechnology Bioeng*, **92**, 79–90.

Oku T., Nakamura S. (2000) Estimation of intestinal trehalase activity from a laxative threshold of trehalose and lactulose on healthy female subjects. *European Journal of Clinical Nutrition*, **54**, 783–788.

Pagnotta S.E., Ricci M.A., Bruni F., McLain S., Magazù S. (2008) Water structure around trehalose. *Chemical Physics*, **345**, 159–163.

Pakchung A.A.H., Simpson P.J.L., Codd R. (2006) Life on earth. Extremophiles continue to move the goal posts. *Environmental Chemistry*, **3**, 77–93.

Patist A., Zoerb H. (2005) Preservation mechanisms of trehalose in food and biosystems. *Colloids and Surfaces B: Biointerfaces*, **40**, 107–113.

Pollock G.E., Holmstrom C.D. (1951) The trehalose content and the quality of active dry yeast. *Cereal Chemistry*, **28**, 498–505.

Richards A.B., Krakowka S., Dexter L.B., Schmid H., Wolterbeek A.P.M., Waalkens-Berendsen D.H., Shigoyuki A., Kurimoto M. (2002) Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. *Food and Chemical Toxicology*, **40**, 871–898.

Robert H., Le Marrec C., Blanco C., Jebbar M. (2000) Glycine betaine, carnitine, and choline enhance salinity tolerance and prevent the accumulation of sodium to a level inhibiting growth of *Tetragenococcus halophila*. *Applied Environmental Microbogy*, **66**, 509–517.

Roser B.J. (1991a) Trehalose drying: a novel replacement for freeze-drying. *Biopharmacy*, **5**, 44–53.

Roser B.J. (1991b) Trehalose, a new approach to premium dried foods. *Trends Food Science Technology*, **2**, 166–169.

Rothschild L.J., Mancinelli R.L. (2001) Life in extreme environments. *Nature*, **409**, 1092–1101.

Salas-Mellado M.M., Chang Y.K. (2003) Effect of formulation on the quality of frozen bread dough. *Brazilian Archives of Biology and Technology*, **46**, 461–468.

Schiraldi C., De Rosa M. (2002) The production of biocatalysts and biomolecules from extremophiles. *Trends in Biotechnology*, **20**, 515–521.

Soper A.K. (2000) Probing the structure of water around biological molecules: concepts, constructs and consequences. *Physical B*, **276–278**, 12–26.

Soper A.K., Luzar A. (1992) A neutron diffraction study of dimethyl sulphoxide–water mixtures. *Journal of Chemical Physics*, **97**, 1320–1326.

Tanaka M., Machida Y., Niu S., Ikeda T., Jana N.R., Doi H., Kurosawa M., Nekooki M., Nukina N. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nature Medicine*, **10**, 148–154.

Unsworth L.D., van der Oost J., Koutsopoulos S. (2007) Hyperthermophilic enzymes – stability, activity and implementation strategies for high temperature applications. *FEBS Journal*, **274**, 4044–4056.

van den Burg B. (2003) Extremophiles as a source for novel enzymes. *Current Opinion in Microbiology*, **6**, 213–218.

Van Dijck P., Colavizza D., Smet P., Thevelein J.M. (1995) Differential importance of trehalose in stress resistance in fermenting and nonfermenting *Saccharomyces cerevisiae* cells. *Applied Environmental Microbiology*, **61**, 109–115.

Varga B., Migliardo F., Takacs E., Vertessy B., Magazù S., Mondelli C. (2008) Neutron scattering studies on dUTPase complex in presence of bioprotectant systems. *Chemical Physics*, **345**, 250–256.

Zhang X.B., Li K., Yau K.H., Tsang K.S., Fok T.F., Li C.K., Lee S.M., Yuen P.M. (2003) Trehalose ameliorates the cryopreservation of cord blood in a preclinical system and increases the recovery of CFUs, long-term culture-initiating cells, and nonobese diabetic-SCID repopulating cells. *Transfusion*, **43**, 265–272.

Zhou A., Benjakul S., Pan K., Gong J., Liu X. (2006) Cryoprotective effects of trehalose and sodium lactate on tilapia (Sarotherodon nilotica) surimi during frozen storage. *Food Chemistry*, **96**, 96–103.

