Enhancement of the Foaming Properties of Protein Dried in the Presence of Trehalose

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Surface tension, foamability, and foam stability kinetics have been measured for the pure proteins bovine serum albumin (BSA) and β-lactoglobulin, before and after aqueous solutions of the proteins had been subjected to different drying conditions, and also for whey protein concentrate (WPC). Pure proteins were air-dried, at 78 or 88 °C, in the presence and absence of sucrose or trehalose, at a mass ratio of 5:1 sugar/protein. WPC was spray-dried in the presence of various sugars: trehalose, sucrose, lactose, and lactitol. Spray-drying WPC without sugars resulted in a dramatic decrease in the foam stability, whereas drying in the presence of sugars gave better retention of the original foaming properties. Trehalose in particular resulted in almost complete retention of the foam stability observed for the nondried WPC. Pure β-lactoglobulin showed similar behavior, but trehalose did not seem to afford the same protection to BSA.

Keywords: *Protein; drying; trehalose; foams*

INTRODUCTION

In recent years there has been a tremendous increase in interest in the biological effects of the sugar trehalose. This molecule is widely found in nature among species that are capable of withstanding conditions where they are exposed to conditions of lower water activity. Such species vary from cyanobacteria (Hill et al., 1997), hyperthermophilic (Ramos et al., 1997) and halophilic (Lippert and Galinski, 1992) bacteria, yeasts (Majara et al., 1996; Attfield et al., 1992), mushrooms (Yoshida and Fujimoto, 1994), tardigrades (Somme, 1996; Westh and Ramlov, 1991), nematodes (Womersley et al., 1998; Sastry and Agmon, 1997), desert "resurrection plants" (Muller et al., 1997), and cysts of shrimps (Clegg, 1997). These species frequently accumulate high concentrations of this sugar (through active metabolism) in response to low water activity or thermal stress prior to entering a state of almost perfect suspended animation. They may be able to remain in this state for many years, but with the ability to spring back into life once the environmental stress is removed. This ability to withstand harshly dehydrating conditions (anhydrobiosis) has been discussed (Potts, 1994) in relation to prokaryotic organisms in general and points to its possible importance to the development of life itself on this planet. Such is the diversity of anhydrobiotic organisms which accumulate trehalose that this suggests some fundamental property (or properties) of trehalose for which it has been selected to protect the biological infrastructure of such species. However, up to now, the exact mechanism(s) by which trehalose exerts these effects is (are) still the topic of healthy debate.

Indeed, trehalose is probably not the only molecule capable of exerting such strong protective effects: raffinose (Rossi et al., 1997; Suzuki et al., 1997; Muller et al., 1997), stachyose (Blackman et al., 1992), sucrose (Muller et al., 1997; Hill et al., 1997), and other sugar derivatives and polysaccharides (Hill et al., 1997) have also been shown to be more or equally important in some species under certain conditions. The problem of trying to unravel the effects in whole organisms is exhibited by various studies on yeast (Alexandre et al., 1998; Lewis et al., 1997), the viability of which is of course of great significance to the food and drink industry. It appears that trehalose may be important depending on the growth stage (Uritani et al., 1995) and thermal history (Piper, 1993) of the yeast cells, although recent work on mutant (Eleutherio et al., 1995) and nonmutant strains (Attfield et al., 1992) points more directly to trehalose as having a dominant effect. The story is more complicated, however, by the suggested interactions between heat shock proteins (Singer and Lindquist, 1998; Lee and Goldberg, 1998; Mager and Varela, 1993; Winkler et al., 1991) and trehalose in determining the lability of unfolded protein molecules. Thus, heat shock proteins may influence aggregation, gelation, etc., of food proteins, whereas the possibility of preserving whole fruits, vegetables, food ingredients, etc. (Roser, 1991a,b), in their fresh state by drying in the presence of trehalose has excited much interest. Some studies have indicated that such dried products retain practically all of their fresh flavor, aroma, texture, etc., on rehydration (Roser, 1991a). Disadvantages in the cost of trehalose and capital equipment outlay have to be offset by the added value of the resultant products. Various other studies have examined the effects of trehalose and other sugars on the stability of various biological molecules besides proteins and enzymes (Sampedro et al., 1998; Rossi et al., 1997a; Uritani et al., 1995), such as antibodies (Draber et al., 1995) and DNA (Anchordoquy et al., 1997; Colaco et al., 1992) in vitro. This has led to ways of maintaining the viability of various medical diagnostic kits in a dried state (without the need for refrigeration), but even in

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10.1021/jf990206n CCC: $18.00
Published on Web 11/12/1999

these relatively simple systems the mechanism of action of the sugar molecules is still not clear.

What seems to have received relatively little attention to date is the possibility of using trehalose to add value to various proteinaceous ingredients, such as milk proteins, which are added as dried powders to various food products as emulsifying, foaming, gelling, dispersing, etc., agents. This paper is part of an ongoing study to investigate and understand how sugars such as trehalose affect the functional properties of such proteins. The effects of sugars, particularly trehalose, on commercial whey protein and its major component, β-lactoglobulin, have been studied because these proteins possess good ability to stabilize foams, which is a key functional property required of proteins in many foodstuffs.

MATERIALS AND METHODS

Bovine β-lactoglobulin (crystallized and lyophilized; lot 114H7055), bovine serum albumin (BSA) (essentially globulin free, lyophilized; lot 16H9314), AnalR trehalose (from *Saccharomyces cerevisiae*; lot 96H3790), AnalR sucrose (lot 95H09441), and AnalR lactose were purchased from Sigma Chemicals (Poole, Dorset, U.K.). A sample of 98% lactitol was a gift from Cortec Ltd. (Chester, U.K.). AnalR potassium dihydrogen orthophosphate, AnalR disodium hydrogen orthophosphate, and AnalR concentrated nitric acid were purchased from BDH Chemicals (Poole, Dorset, U.K.) and used for preparing buffer solutions. All water used was double-distilled, with a surface tension of 71.2 ± 0.3 mN m^{-1} at 30 °C. For drying and foaming experiments on the pure proteins the sample was dissolved in 5 mM phosphate buffer at pH 7.0 to give 0.1 wt % protein. Sugars were dissolved in the protein solution to give 0.5 wt % sugar. Fresh whey protein concentrate (WPC) was manufactured and supplied by a local dairy, using a commercial ultrafiltration unit. Two batches of WPC were supplied, each with a solids content of 13.2 wt %, 35% of which was protein. The same company also supplied a spray-dried whey protein product, dried on a commercial scale spray-dryer. Sugar was added to the WPC to give 10 wt % sugar (i.e., 23.2% solids in total) for spray-drying. One liter samples were spray-dried on a Kestner pilot scale spray-dryer with an inlet air temperature of 220 °C and the outlet air temperature controlled to between 80 and 90 °C.

All pieces of apparatus coming into contact with the pure protein solutions were cleaned by soaking in concentrated nitric acid and rinsing with copious amounts of double-distilled water and buffer.

Measurement of Foam Stability. Two methods of measuring foam stability were used. A "crude" shake test was used for the WPC samples, consisting of mechanical shaking of 30 mL of sample solution, thermostated at 30 °C, in a sealed glass bottle at 5 Hz for 30 s. The foam lifetime was taken as the time when the foam had collapsed to the point where a hole appeared in the bottom of the foam, so that a clear region of the macroscopic air−water interface was visible. WPC−sugar mixtures were diluted with buffer to give 5 wt % WPC solids before this crude shake test was performed. Measurements were repeated at least twice and were reproducible to ±3 min.

For the pure protein systems, to assess foam stability more exactly and reproducibly, the pressure decay method, originally proposed by Nishioka and Ross (1981), was used. The principle is based on the release of the slight excess Laplace pressure inside a bubble when it collapses. Thus, in a sealed container containing foam, the pressure inside the container rises as the foam collapses due to bursting, coalescence, or disproportionation of the bubbles, and the pressure rise is directly related to the decrease in the foam surface area (Nishioka and Ross, 1981). The apparatus that was used in these studies has been described in detail previously (Murray and Liang, 1999). Basically, it consists of a sample chamber in which the foam is produced connected to an identical reference chamber, containing no foam, via a very sensitive differential pressure transducer; the whole unit is immersed in a water bath thermostated at 30 ± 0.2 °C. For each experiment 20 mL of protein solution was injected into the sample chamber and the apparatus left for 1 h for thermal equilibration before the commencement of foaming. Nitrogen gas saturated with water was used to produce the foam, using a constant flow rate of 0.6 ± 0.05 dm^{-3} min^{-1} directed to 12 individual stainless jets of 0.1 mm internal diameter, via a flow equalization chamber. Nitrogen was bubbled through the sample solution, via the jets, for 20 ± 1 s before valves were closed and the pressure difference between the sample and reference chambers monitored. This system gave a more uniform and reproducible initial bubble size distribution than the earlier version of this apparatus used by Dickinson et al. (1993), which aids interpretation of the foam collapse kinetics (Monsalve and Schechter, 1983; Nishioka et al., 1996).

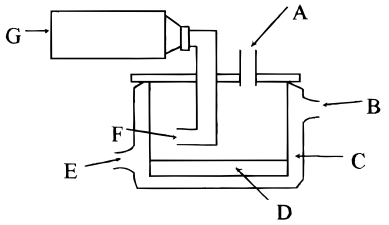

Figure 1. Schematic diagram of the dehydration device: outlet of hot air (A); outlet of water bath (B); water bath (C); sample solution (D); inlet of water bath (E); outlet of hot air side the glass tank (F); inlet of hot air (G).

Controlled Dehydration Device. Because it was too expensive to dry the same quantities of the pure proteins on the spray-dryer and also because the time−temperature conditions in the spray-dryer were not so well-defined, a simple device was constructed to dry the pure samples under more precisely controlled conditions. This is illustrated in Figure 1.

Basically, the dehydration device consisted of a jacketed glass dish (internal diameter = 10 cm), thermostated by circulating either water or oil through the jacket. For each drying run a small volume (e.g., 10 mL) of protein solution was placed in the dish and hot air from a domestic hair dryer was circulated over the solution to carry moisture away. The temperature of the hot air from the dryer was constant at 40 ± 1 °C. For drying "at 78 °C", the jacket temperature was 80 ± 0.2 °C, the resulting air temperature in the dish was 66 ± 2 °C, and the sample solution temperature stabilized at 78 ± 1 °C after 17 ± 2 min when drying was conducted for a further 50 ± 2 min. For drying "at 88 °C", the jacket temperature was 108 ± 0.2 °C, the resulting air temperature in the dish was 80 ± 2 °C, and the sample solution temperature stabilized at 88 ± 1 °C after 25 ± 2 min when drying was conducted for a further 70 ± 2 min. After these drying times, the samples were glassy or crystalline solids of constant mass.

Surface Tension Measurements. The surface tensions of protein solutions were measured using the Wilhelmy plate method and a Krüss K10ST digital tensiometer, thermostated at 30.0 ± 0.1 °C. A roughened platinum plate was used, cleaned with nitric acid and allowed to air-dry before flaming in a Bunsen burner. After pouring the protein solution into the glass sample dish, the solution was left to come to thermal equilibrium for 5 min and then the surface aspirated to create a fresh interface and then surface tension monitored. Measurements were repeated at least twice and were reproducible to ±0.4 mN m^{-1}.

RESULTS

Nishioka et al. (1981, 1996) and Yu and Damodaran (1991) have discussed in detail the pressure decay kinetics in relation to the mechanisms of foam collapse. The difference in pressure, ΔP, between the foam at zero time (before collapse) and the pressure at subsequent times rises until it reaches a maximum, ΔP_{max}, when

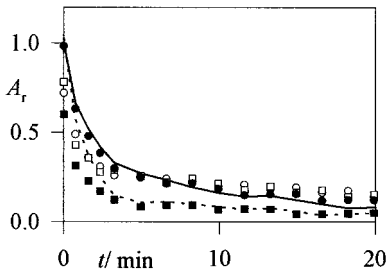

Figure 2. Relative foam area, A_r, versus time, t, for 0.1 wt % β-lactoglobulin: (—) native (undried) protein; (- - -) dried at 78 °C with no sugar; (○) native + 0.5 wt % trehalose; (□) native + 0.5 wt % sucrose; (●) dried at 78 °C with 0.5 wt % trehalose; (■) dried at 78 °C with 0.5 wt % sucrose.

the foam has completely collapsed (i.e., all of the bubbles have disappeared). It can be shown that the surface area, A, of the foam at any given time, t, during the collapse is given by

$$A(t) = 3V(\Delta P_{max} - \Delta P)/(2\gamma) \quad (1)$$

where γ is the interfacial tension and V is the total volume of the cell, which here was the same for each experiment.

Because the principal aim of this study was to compare the effects of various drying treatments with the foaming behavior of the undried, "native", pure protein, we have chosen to represent the data as relative to the behavior of the pure, undried system. Thus, in Figures 2−4 the relative foam area, A_r, has been calculated from

$$A_r(t) = (\Delta P'_{max} - \Delta P')/\Delta P_{max} \quad (2)$$

where ΔP_{max} refers to the decay curve for the undried pure protein in the absence of any sugars and ($\Delta P'_{max} - \Delta P'$) refers to the decay curve for the system being compared with the undried pure protein. All measurements were repeated at least twice and A_r was reproducible to ±0.06. Comparison of systems using A_r calculated in this way also assumes that γ is the same for the systems being compared. At a bulk concentration 0.1 wt % this was the case for an individual protein, irrespective of protein treatment (see surface pressure results below). The advantage of this representation is that both foamability (the maximum amount of foam produced) and foam stability relative to the native sample can be easily compared on a single graph, such as Figure 2. Higher values of A_r are interpreted as "more foam surface present", either due to more bubbles and/or smaller bubbles—and this may be taken as a measure of the foamability of the solution. Visual observations suggested that all of the systems studied here using the multiple jet apparatus at least started with very similar bubble sizes, except where the foams were very unstable. A more rapid fall in A_r with time indicates a more rapid loss of foam surface area—and so the rate of change of A_r may be taken as a measure of foam stability.

Figure 2 shows the A_r versus time results obtained for native (undried) β-lactoglobulin, β-lactoglobulin with added trehalose or sucrose, and β-lactoglobulin dried at 78 °C, with and without trehalose or sucrose. All protein concentrations were 0.1 wt % and sugar concentrations 0.5 wt %. It is seen that adding trehalose or sucrose at this level reduced the foamability of the native protein.

Table 1. Summary of the Maximum Foam Surface Areas, A_{max}, and the Time, $t_{1/2}$, for the Area To Decay to Half A_{max} for the Various Systems Dried or Undried with Trehalose (+ T) or Sucrose (+ S)

	β-lactoglobulin		BSA	
system	$t_{1/2}$/min	$A_{max} \times 10^{-3}$/cm^2	$t_{1/2}$/min	$A_{max} \times 10^{-3}$/cm^2
native	1.7	3.4	4.1	4.7
native + T	1.6	2.4	2.4	4.3
native + S	1.2	2.6	5.4	3.8
78 °C drying	1.0	3.4	7.0	3.1
78 °C drying + T	1.5	3.3	2.8	3.8
78 °C drying + S	0.9	2.0	2.4	3.3
88 °C drying	1.3	1.9	<0.3	
88 °C drying + T	1.4	3.1	<0.3	
88 °C drying + S	1.3	2.2	<0.3	

Drying β-lactoglobulin at 78 °C without sugar did not appear to affect the foamability greatly, but there was a marked decrease in foam stability, A_r falling much more rapidly compared to the native protein. When the protein was dried in the presence of sugar, the most striking feature was the similarity in the behavior of the system dried with trehalose to the native protein, whereas drying with sucrose appeared to give no particular enhancement of the foamability or foam stability compared to the protein dried without sugar. In the presence of both sugars, the β-lactoglobulin plus sugar formed a glassy material on drying, which was easy to redissolve.

An alternative way of representing the foam decay results is to plot $\Delta P/\Delta P_{max}$ versus time as a measure of the relative change in foam area with time, independent of the initial amount of foam. The principal mechanisms by which foam area is lost are coalescence and gas diffusion (disproportionation) between bubbles. A number of workers [e.g., Yu and Damodaran (1991) and Nishioka et al. (1996)] have tried to interpret the decay kinetics of $\Delta P/\Delta P_{max}$ plots in terms of these different mechanisms. In practice, however, it is difficult to unequivocally attribute the parameters describing the decay kinetics to specific contributions from these two mechanisms, because both occur simultaneously and may be affected by other factors such as thin film draining, vibrations, dust, etc. Here the decay kinetics have been summarized by tabulating the time, $t_{1/2}$, for the foam area to decay to half its maximum value. The $t_{1/2}$ values for the different systems are shown in Table 1, along with the corresponding maximum values of A and A_{max}, calculated from eq 1 at $t = 0$. The results in Table 1 reinforce the picture that drying β-lactoglobulin with trehalose appears to give a protein which closely resembles the native protein in terms of its foaming behavior.

Figure 3 shows the corresponding results obtained for BSA. It is seen that adding trehalose to BSA resulted in a slight decrease in foamability, whereas adding sucrose resulted in a further slight decrease in foamability. Drying the BSA on its own resulted in a decrease in foamability similar to that seen with the addition of sugars, to give the lowest foam volume of the BSA systems studied, but the $t_{1/2}$ values in Table 1 show that there was some increase in foam stability, in contrast to the behavior with β-lactoglobulin. The foamability of BSA dried with trehalose was somewhat higher, although the foamability was slightly less than that of BSA plus trehalose which had not been dried, and the foam stability was similar to that of BSA dried without

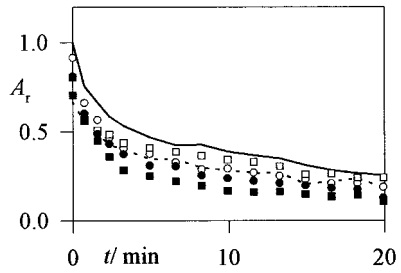

Figure 3. Relative foam area, A_r, versus time, t, for 0.1 wt % BSA: (—) native (undried) protein; (- - -) dried at 78 °C with no sugar; (○) native + 0.5 wt % trehalose; (□) native + 0.5 wt % sucrose; (■) dried at 78 °C with 0.5 wt % trehalose; (■) dried at 78 °C with 0.5 wt % sucrose.

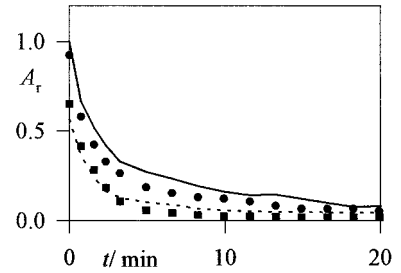

Figure 4. Relative foam area, A_r, versus time, t, for 0.1 wt % β-lactoglobulin: (—) native (undried) protein; (- - -) dried at 88 °C with no sugar; (●) dried at 88 °C with 0.5 wt % trehalose; (■) dried at 88 °C with 0.5 wt % sucrose.

trehalose. Drying BSA with sucrose gave a low foamability, similar to the BSA dried on its own, and an even lower foam stability than BSA dried on its own. The data in Table 1 emphasize that the foam stability ($t_{1/2}$) and foamability (A_{max}) of all the undried BSA systems were higher than those of the undried β-lactoglobulin systems. During dehydration of BSA in the absence of sugars some protein precipitation (coagulation) occurred, which made the dehydrated BSA more difficult to redissolve completely. In the presence of both sugars, BSA plus sugar formed a glassy material, which was easier to redissolve, and no insoluble protein was visible.

Figure 4 shows the behavior of β-lactoglobulin after drying at the higher temperature of 88 °C. Compared with the behavior after drying at 78 °C (Figure 2), drying the protein the absence of sugars caused an even greater loss of foamability and foam stability. Drying at 88 °C with sucrose gave no significant improvement in foamability or foam stability, in contrast to drying with trehalose, which gave a product with almost the same foamability as the native protein and only slightly lower foam stability. This is quite striking, given that the protein was subjected to this temperature for at least 45 min. When BSA was dried under the same conditions, the BSA dried either with or without sugars exhibited very poor foamability and the foam collapsed too rapidly (i.e., completely in <30 s) for any meaningful measurements to be made. Also, the dried material did not appear to be very soluble.

In summary, the foaming results obtained showed that adding trehalose to native protein gave no dramatic improvement in foam stability or foamability but that it could successfully prevent the significant reduction of these functional properties on dehydration at high temperatures.

As noted earlier, the surface pressures of all the systems shown in Figures 2−4 were not significantly

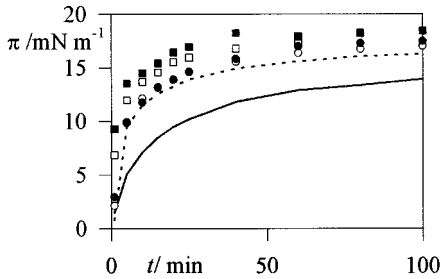

Figure 5. Surface pressure, π, versus time, t, for 10^{-3} wt % β-lactoglobulin: (—) native (undried) protein; (- - -) dried at 78 °C with no sugar; (○) native + 5×10^{-3} wt % trehalose; (□) native + 5×10^{-3} wt % sucrose; (●) dried at 78 °C with trehalose; (■) dried at 78 °C with sucrose.

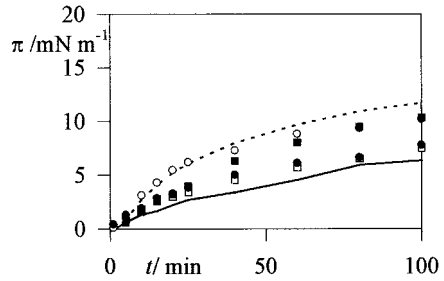

Figure 6. Surface pressure, π, versus time, t, for 10^{-3} wt % BSA: (—) native (undried) protein; (- - -) dried at 78 °C with no sugar; (○) native + 5×10^{-3} wt % trehalose; (□) native + 5×10^{-3} wt % sucrose; (●) dried at 78 °C with trehalose; (■) dried at 78 °C with sucrose.

different. Surface pressure, π, was defined in the usual way as

$$\pi = \gamma_0 - \gamma \quad (3)$$

where γ_0 was the surface tension of the pure water and γ was the surface tension in the presence of the protein and/or sugar. For these systems, at 0.1 wt % protein, the equilibrium π values were all 20.5 ± 1 mN m^{-1} and were reached within 5−10 min. The π of 0.5 wt % trehalose or sucrose was <2 mN m^{-1} after 60 min, so any contribution from surface active impurities in the sugars was negligible. However, during the production of foam there is rapidly produced a considerably greater surface area than in the surface tension apparatus, in equilibrium with a thin film of aqueous solution, so that adsorption from solution may be more limited by diffusion and the local supply of protein. Surface pressure measurements were therefore also made at a lower protein concentration, diluting them to 10^{-3} wt % protein, $\pm 5 \times 10^{-3}$ wt % sugar. Figures 5 and 6 show the behavior of the surface pressure versus time for the β-lactoglobulin and BSA systems, respectively, including the systems dried at 78 °C.

Comparison of Figures 5 and 6 shows that β-lactoglobulin was more surface active than BSA in almost all cases, measured at this low protein concentration. Figure 7 shows the surface pressure results for β-lactoglobulin dried at the higher temperature of 88 °C. Comparison with Figure 5 (protein dried at 78 °C) shows the same pattern of behavior: the protein dried on its own and the protein dried with sucrose were more surface active than the native protein, whereas the protein dried with trehalose exhibited surface active behavior closer to that of the native protein.

Because the effects of sugars on the pure proteins were significant, some preliminary measurements were

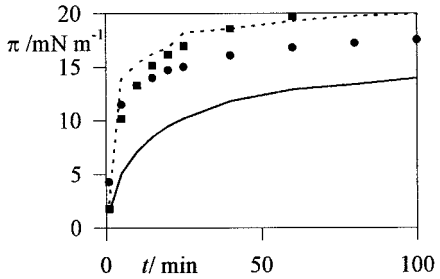

Figure 7. Surface pressure, π, versus time, t, for 10^{-3} wt % β-lactoglobulin: (—) native (undried) protein; (- - -) dried at 88 °C with no sugar; (●) dried at 88 °C with trehalose; (■) dried at 88 °C with sucrose.

Table 2. Foam Lifetimes (via Shake Test) on WPC: Batch 1, Sugars Added Then Foam Lifetime Measured; Batch 2, Including after Spray-Drying in the Presence of the Sugar (As Described in the Text), Then Rehydrated (*)

	foam lifetime/min	
WPC system	batch 1	batch 2
commercially spray-dried	0.5	0.5
fresh	19.5	14.3
fresh + no sugar		1.2*
fresh + sucrose	13.0	6.5*
fresh + trehalose	10.2	13.8*
fresh + lactose	12.1	1.8*
fresh + lactitol	11.2	7.5*

performed on the commercial whey protein subjected to spray-drying. Table 2 shows the foam lifetimes (measured via the shake test) of 5 wt % WPC (batch 1) ± 10 wt % of various sugar compounds. Also shown is the foam lifetime for the same batch of WPC supplied as a spray-dried powder. It is seen that the addition of all sugars gave rise to a decrease in the foam stability of the fresh WPC. Most striking, however, was the very poor foam stability of the commercial spray-dried powder compared to the other samples. Table 2 also shows the effects of adding the same sugars to the second batch of WPC and then spray-drying these mixtures. Spray-drying WPC (batch 2) under these conditions gave a product that exhibited slightly better foam stability than the commercial spray-dried WPC (batch 2), but still considerably lower than the fresh WPC (batch 2). On the other hand, spray-drying with the sugars gave much greater retention of the foam stabilizing properties of the fresh WPC (batch 2). This was most noticeable for trehalose, which gave approximately twice the foam stability of systems dried with sucrose and almost the same foam stability as the fresh (undried) WPC (batch 2). Thus, similar protective effects of trehalose were observed as with the pure β-lactoglobulin. The effects of sugars in protecting the foaming of spray-dried protein were much more striking than with the proteins dried in the dehydration device. This may reflect the more "destructive" conditions during spray-drying, e.g., exposure of the protein to higher temperatures.

DISCUSSION

Several studies have observed that sucrose enhances the foam stability of proteins at concentrations >15 w/v % (Halling, 1981; Waniska and Kinsella, 1979). This was explained as being due to the increased viscosity of the bulk phase, retarding thinning of lamellae around the bubbles. However, other studies (Arakawa et al., 1982; Poole et al., 1984; Howell and Taylor, 1995) reported no improvement (or a decrease) in foam stability in the presence of sucrose. The above results also suggest that sucrose, unlike trehalose, has no dramatic effect on foam stability with both native proteins, and foamability may be slightly curtailed. In fact, at the concentrations of sugar used here (0.5 wt %) there is no significant increase the viscosity of bulk phase and, furthermore, there is no significant difference in the viscosities of sucrose and trehalose solutions up to 30 wt %, so that bulk viscosity cannot be used to explain the differences in behavior observed between sucrose and trehalose.

There do not appear to be any simple relationships between the rate of rise in π or the "final" value of π (after 100 min) and the foamability or foam stability results described above. Figures 5 and 6 show that β-lactoglobulin was more surface active than BSA in almost all cases. This is probably related to the fact that BSA is more highly cross-linked, with 17 disulfide bonds, whereas β-lactoglobulin has only two disulfide bonds and more hydrophobic side chains (Kinsella and Whitehead, 1989). Thus, β-lactoglobulin can more readily unfold and adsorb at the interface. However, undried BSA gave higher foamability at the higher concentration of 0.1 wt % (see Table 1), so that rate of transport to the interface and/or other factors, such as interfacial rheology, may be more important in determining the foamability of undried BSA and β-lactoglobulin. It is seen that the proteins dried either in the presence or in the absence of sugars at 78 °C were more surface active than the native protein, in terms of both the rate of rise in π and the value of π after 100 min. An increase in surface activity on heating can be explained by increased unfolding of the protein and exposure of the hydrophobic residues (Kitabatake, 1982; Graham and Phillips, 1979), provided the heating is not excessive and causes a high degree of cross-linking and consequently insoluble, high molecular weight polymers. Carrett et al. (1988) observed that sucrose may promote the denaturation of BSA and β-lactoglobulin but that it inhibits their subsequent coagulation.

An increase in the surface activity of native proteins due to the presence of such low bulk concentrations of sugar is more difficult to explain, but this effect has also been noted by Antipova and Semenova (1997) and Patino and Niño (1995) for sucrose. One explanation that has been suggested by Antipova and Semenova (1997), Patino and Niño (1995), Xie and Timasheff (1997), Lin and Timasheff (1996), and Kita et al. (1994) for any observed increase in surface activity of protein in the presence of polyhydroxy cosolvents is based on the observed exclusion of such compounds from the surface of proteins. There is therefore an excess of water at the protein surface compared to its concentration in the bulk, due to preferential hydration of the protein. Thus, the existence of sugar in aqueous solution limits protein unfolding in the bulk and therefore protein−protein interactions leading to aggregation in the bulk and loss of surface activity. For a protein with more hydrophobic side chains, such as β-lactoglobulin, the effect of sugars is more pronounced because the free energy required to unfold the protein is low compared to BSA. On the other hand, a higher surface activity implies an increased tendency to unfold, although it is perhaps difficult to compare the solvent conditions at the interface with those in the bulk solution. It is interesting to note that both proteins when dried with

trehalose generally exhibited surface activity which was closer to that of the native (undried) protein than the protein dried under any other conditions, consistent with idea that trehalose preserves the native protein structure on drying. Adding sugars generally gave a decreased foam stability compared to adding sugars and then drying with them. This points to a different type of interaction between the sugars and the proteins in solution at room temperature and the interactions that occur during or after the drying process.

The effects of drying β-lactoglobulin at the higher temperature (Figure 4) and the spray-drying of the WPC (Table 2) highlight the ability of trehalose in particular to protect protein foam functionality. Drying pure β-lactoglobulin with sucrose gave no significant improvement in foamability or foam stability, whereas drying with trehalose gave a product with almost the same foamability as the native protein and only slightly lower foam stability. When BSA was dried at this temperature, however, with or without either sugar, there was no measurable foam formation at all. This may be due to the larger number of disulfide bonds in BSA than β-lactoglobulin, as pointed out above, which makes BSA more susceptible to irreversible disulfide cross-linking during such treatment, with loss of protein solubility and functionality. This may also explain why the effects of trehalose were not so marked for BSA dried at the lower temperature (Figure 3), compared to the more marked protective effects of trehalose on the foaming of β-lactoglobulin. Several explanations have been put forward for how sugars such as trehalose may specifically protect delicate biological molecules from destruction by heating and drying, discussed as follows.

(i) The Glassy State Explanation. With the formation of a glassy state around a protein, all physical and chemical transformations will be enormously slowed as result of the very high viscosity of the system (Gottfried et al., 1996). Work has therefore highlighted the special properties of trehalose in relation to its ability to form a glass (Sun and Davidson, 1998; Naini et al., 1998; Crowe et al., 1998; Ding et al., 1996), rather than a crystalline state (Sussich et al., 1998; Suzuki et al., 1997), on removal of water and the unique properties of the glass itself. For instance, trehalose has one of the highest glass transition temperatures of similar disaccharides, and the glass does not easily transform to the crystalline state even if moisture is present (Sussich et al., 1998). Some workers have demonstrated enhanced stabilizing effects of trehalose through manipulation of the glassy state by mixing trehalose with other species such as borate (Miller et al., 1998) and sucrose and polysaccharides (Hill et al., 1997). The main problem with the glassy state explanation is that many materials which form glasses equally easily, for example, dextrin, afford very little anhydrobiotic protection (Crowe et al., 1998) to proteins, etc., although perhaps not enough is known in detail about the glassy state in such mixed systems.

(ii) Water Replacement Explanation. The water content of some anhydrobiotic organisms in their dry state is so low that the water of hydration of proteins, etc., must be at least partially disrupted or removed. Another explanation of the protective properties of sugars and other polyhydroxy compounds is therefore that the sugars "bind" to the macromolecules and replace the water of hydration (Crowe et al., 1996). Although this is a neat explanation, unfortunately, as yet there is little direct evidence to support it (Cleland and Jones, 1996; Belton and Gil, 1994), and it is seen to be at odds with the water exclusion hypothesis already mentioned (see also below) to explain the effects on the surface pressure of protein solutions. The discrepancy with the water exclusion idea has been discussed by Crowe et al. (1997). In a slight modification of the idea, Sastry and Agmon (1997) recently reported that trehalose protects by preventing the escape of a vital *few* internal water molecules in myoglobin, whereas Butler and Falke (1996) reported that trehalose and various other sugars reduce local fluctuations in the polypeptide backbone of proteins. Rather than direct interactions between trehalose and proteins, etc., Crowe et al. (1994) have suggested that direct binding of trehalose to phospholipids can occur, which helps to maintain membrane fluidity (Oliver et al., 1998; Somme, 1996; Belton and Gil, 1994; Crowe and Crowe, 1991) and that this is just as important in maintaining the integrity of organisms during severe dehydration.

(iii) Water Exclusion Explanation. The water replacement explanation is also at odds with the thermodynamic description due to Timasheff and co-workers (Lee and Timasheff, 1981; Arakawa and Timasheff, 1982; Galinski, 1993; Kita et al., 1994; Lin and Timasheff, 1996; Xie and Timasheff, 1997). These workers explain the stabilizing effects of sugars as being due to a higher (positive) free energy difference between the native and unfolded state when sugars are present in aqueous solution, which is exhibited by the preferential exclusion of sugar molecules from the surface of proteins and the increase the surface tension of water when sugars are present.

(iv) Maillard Browning Explanation. Trehalose is a nonreducing sugar and so will not readily undergo nonenzymatic browning with amino-containing compounds, such as proteins. It is also relatively stable to heat and slow to caramelize. This has led some workers (Schebor et al., 1997; Colaco et al., 1996; O'Brien, 1996) to propose that this resistance to browning must be another aspect of the success of trehalose protecting macromolecules during drying (which also concentrates reactants) and heating. However, this idea per se cannot explain the marked effects of trehalose over other nonreducing, polyhydroxy compounds.

In summarizing the evidence to date, it seems likely that a combination of the above explanations will be required to explain all of the in vitro effects, such as those observed here on dried whey proteins, whereas in vivo effects may be mediated through other agents, such as heat shock proteins and other metabolites. With respect to the use of such technology to preserve protein ingredients for use in foodstuffs (Roser, 1991a,b), other aspects such as particle size and morphology on drying may need to be investigated, because the limited amount of work in this area (Broadhead et al., 1994) indicates that this may also be important.

CONCLUSIONS

The experiments on the pure and commercial whey proteins illustrate the ability of trehalose to protect the functionality of the protein during drying and high-temperature treatment, this being measured as the retention of foaming properties closer to the original, native protein. These results throw no further light on the mechanism by which trehalose exerts this protective action, but confirm that trehalose is much more effective

than other sugars of similar structure, such as sucrose. The protective effects are not the same for all proteins, however, and trehalose appeared to afford β-lactoglobulin much better protection than BSA. The systems studied here had a high ratio of sugar to protein. Apart from other considerations, use of such high sugar concentrations (of trehalose in particular) in this way would be prohibitively expensive on a commercial scale. Further work is required to investigate whether this ratio can be reduced and trehalose still give protective effects. This may be the case, because surface pressure measurements at low sugar and low protein concentrations suggest there are interactions between the two components which affect protein surface activity, although the reasons for this are not clear.

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Received for review March 3, 1999. Revised manuscript received July 27, 1999. Accepted October 5, 1999.

JF990206N