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Trehalose in hair care: Heat styling benefits at high humidity

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Synopsis

Human hair switches that have been treated with trehalose solution and straightened using hot irons show longevity of the straight style even in high-humidity conditions. This anti-humidity benefit is especially noticeable if the straight style has been created at low relative humidity. It is proposed that hot-iron straightening of trehalose-treated switches produces *in situ* glassy forms of the sugar that act as water sinks at high humidity to provide anti-humidity benefits. Adsorption isotherms and powder x-ray studies of different forms of trehalose and moisture uptake of hair treated with the sugar suggest that the ability of a glassy form of trehalose to regulate moisture in the fiber and consequently affect its viscoelastic properties is a major factor in providing long-lasting straight style in humid conditions.

INTRODUCTION

In the last few years with the advent of ever more sophisticated appliances and hair care products, it has become far easier to create desired hair styles. While in ambient conditions styles last for a few hours, at high humidity hair style loss is accelerated and consumer satisfaction recedes in only a matter of minutes. The retention of hair styles for a longer period, especially at high humidity, is therefore a big and hitherto unmet consumer need.

Trehalose, a naturally occurring osmolyte, is a non-reducing disaccharide formed by the 1→1 linkage of two D-glucose residues. Cells of many organisms, including bacteria, yeast, fungi, insects, invertebrates and plants, use trehalose to protect their proteins and biological membranes against extreme dehydration, desiccation, temperatures, and many unfavorable environmental conditions (1–8). For this reason trehalose is increasingly finding uses in medicine, pharmacy, food processing, and personal care products (9). Typical applications include trehalose as a stabilizer of proteins and as an excipient in pharmaceutical formulations.

In hair care, trehalose has found increasing use in the benefit areas of damage repair and style manageability (10). Here we look at the effect of trehalose in heat-styling benefits, particularly in providing style maintenance benefits in high-humidity conditions.

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MATERIALS AND METHODS

Trehalose was purchased from Sigma Aldrich. Dark brown European hair with a waviness number of 6 was used in the experiments (Figure 1).

In a typical experiment, hair was washed with base wash (12/1.6 of sodium laureth ether sulfate / cocoamidopropyl betaine) and when dry either soaked in or dosed with a solution of trehalose. Control switches were treated with an equal amount of water. Hair switches were straightened using GHD straighteners (Leeds, UK, BD20 0ES). Each switch was clamped to a stand, and with one hand a comb was slowly run through the switch while the iron held by the other hand followed the comb straightening the switch. After four passes of the iron, each switch was further combed five times.

In one set of experiments at high humidity, the treated switches were placed in a humidity chamber, the conditions were set to 30°C and 80% RH for three hours, and pictures were captured at regular intervals. From the pictures and in-house software the "volume" of the switches was measured from the projected area of the switch silhouette.

In another set of experiments switches were styled in a climatic room whose environmental conditions (temperature and humidity) could be altered and pictures obtained.

Adsorption isotherms were studied using a dynamic vapor sorption (DVS) kit by Surface Measurement Systems (SMS, UK, HA0 4PE). Powder x-ray studies were contracted out to Intertek, UK. Trehalose glass was made from a solution of trehalose dihydrate using a Buchi spray dryer (Buchi UK Ltd., Oldham, OL9 9QL).

RESULTS AND DISCUSSION

HUMIDITY CHAMBER EXPERIMENTS

Dark brown EU wavy #6 switches (as shown in Figure 1), treated with 2% trehalose solution and straightened using hot irons in the laboratory at conditions of 23°C and 40% RH, showed that straight style could be sustained at high humidity (80% RH and 30°C) even after three hours (Figure 2).

While control switches fluffed up to an area of ~14000 mm^2, from a starting point of ~5000 mm^2, trehalose- and heat-treated switches attained a final area of only ~7000 mm^2. This striking longevity benefit could only be obtained with trehalose and heat, i.e., switches straightened with hot irons without trehalose treatment did not show any anti-humidity benefit and those not straightened with hot irons but were trehalose-treated did show some but not the pronounced anti-humidity benefit seen here.

ADSORPTION ISOTHERMS AND POWDER X-RAY STUDIES

As increase in moisture content in hair fibers at high humidity and consequent plasticization of the hair is implicated in the loss of hair style, the first experimental check that was performed was to ascertain the amount of water taken up in the control and trehalose-treated (and ironed) switches. Two types of experiments were performed. In one a large batch of control and trehalose-heated switches was carefully weighed before and after the

Figure 1. A 2-gram, 25-cm European dark brown wavy #6 switch laid out horizontally. The root ends are on the left and the tip ends are on the right.

high-humidity experiment and the % increase in weight of moisture was calculated. In another experiment samples of hair from both sets of switches were studied using a dynamic vapor sorption kit and adsorption isotherms were obtained. Both sets of experiments showed that the amount of moisture uptake by the control ironed switches was almost identical (within experimental error) to trehalose-treated (and ironed) switches as shown in Figure 3.

This leads to the important finding that trehalose- and heat-treated switches take up nearly as much water as control heat-treated switches and yet display a distinct anti-humidity effect as seen in Figure 2. This further suggests that it is not the amount of water in the hair fiber but possibly how moisture affects the viscoelastic properties within the fiber or the surface effect at the array level that determines the anti-humidity effect.

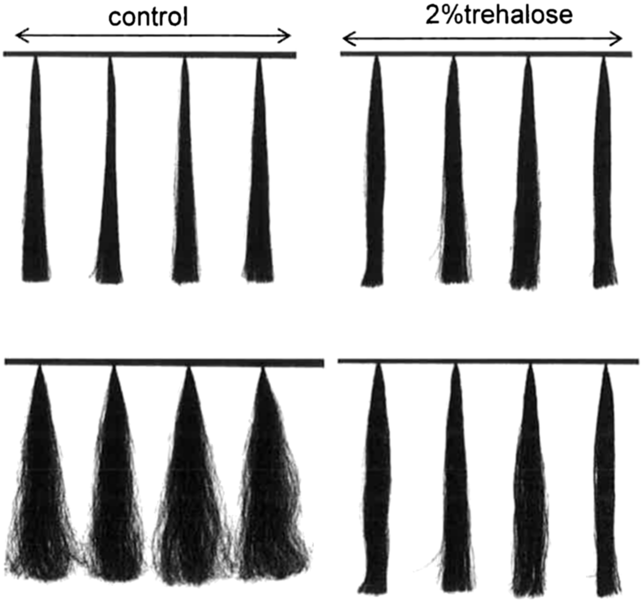

Figure 2. The two pictures at the top are immediately after straightening and combing, and the pictures below are after three hours at 30°C and 80% RH. Hair treated with trehalose (the two pictures on the right) show clear anti-humidity benefits. The two pictures on the left are control switches treated with water. These pictures were obtained in the humidity chamber kit. The switches were combed five times to remove any residue holding the switches together prior to the experiment.

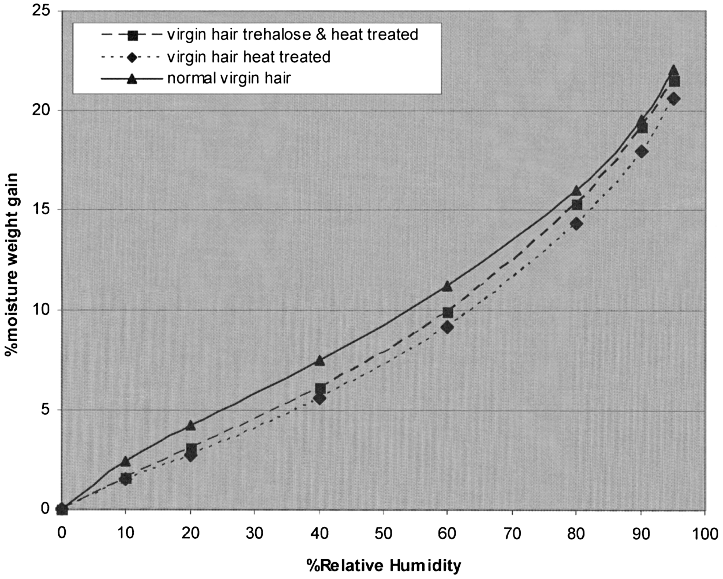

Figure 3. Adsorption isotherms at ~25°C of 2% trehalose-treated (and ironed) hair (dashed line), and control and ironed hair (dotted line) obtained using a DVS kit (within two days of the heat-styling treatment without any rinse or washing in between), showing very small differences in water uptake, with the trehalose-treated switches showing slightly higher moisture gain. Also given is the normal DVS curve (solid line) for untreated and un-ironed virgin hair. It is noticeable that the effect of heat treatment (200°C irons) has changed the shape of the DVS curves.

A likely hypothesis for the above striking anti-humidity observation is that we think the water uptake ability of trehalose is in its different solid-state forms. While trehalose is normally found in its dihydrate crystalline form, it can under certain conditions exist in an amorphous or glassy form, and these forms can interconvert depending on temperature and humidity (11–13).

Here we have investigated the sorption isotherms of two forms of trehalose, one of which is the crystalline dihydrate and the other a "part-crystalline/part-glassy" trehalose form (Figure 4). Concurrently, the dihydrate and the part glassy samples were studied using powder x-ray methods (Figure 5). From the powder x-ray graphs in Figure 5, it is clear that the one sample is far more crystalline than the other.

In the literature (14), the water uptake profile of trehalose glass shows that the glassy form picks up water up to ~12% of its weight until ~50% RH (this is approximately 60% more than the water uptake shown here in Figure 4 for the part-crystalline/part-glassy material, suggesting that this sample may contain 40% crystalline material). Further increase in %RH results in a slight drop in the water uptake, and then a steady state is reached corresponding to a stable form. The final figure suggests that the part-crystalline/part-glassy material has fully converted to the dihydrate crystal, which as Figure 4 (dashed line) shows, does not pick up any water. Irrespective of the actual amount picked up by the part-glassy material, the important finding here is that water is taken up in this material until about 50% RH and a further increase in %RH results in a slight drop in moisture uptake initially, with no further moisture gain.

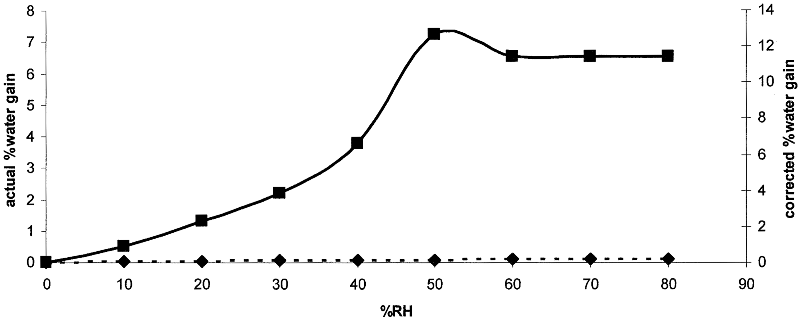

Figure 4. Water uptake DVS curves of trehalose dihydrate (dashed line) and trehalose part-crystalline/part-glassy material (solid line). The dihydrate form shows no water uptake until 80% RH. However, the part-glassy form shows moisture uptake until 50% RH and no further uptake after 60% RH. The "corrected" values on the right hand y-axis assume approximately 40% crystalline material in the part-crystalline/part-glassy sample.

Here it is hypothesized that the straightening process of a hair switch soaked in trehalose solution produces *in situ* trehalose glasses on or in the hair fiber during the heat drying process. These glasses then scavenge water at high humidity—water that may have otherwise disturbed the "water wave" (15,16) leading to loss of straight hair style. That trehalose glasses act as water sinks and convert to dihydrate crystals is well documented; here the trehalose glasses would pick up about 11–12% of their own weight in water and "lock" them in their di-hydrate crystal form (14). This could explain the finding that both sets of switches take up the same amount of water—it is just that in one case some of the water (albeit a very small proportion) is locked in trehalose di-hydrate crystal and not available to disturb the styling water wave. Recent evidence (17,18) suggests that trehalose glasses, depending on their starting point, might pick up nearly four waters per molecule just prior to crystallization.

Thus if trehalose is in a glassy state below 50% RH, it can take up water, and as the humidity goes above 50% any glass present converts to a stable dihydrate crystal after which there would be no further pickup of water. What this implies is that the relative humidity conditions during the straightening style creation process may be important for the anti-humidity effect observed.

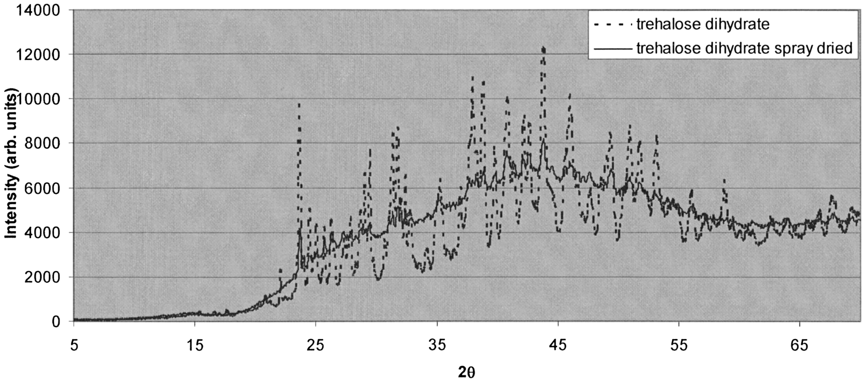

Figure 5. Powder x-ray patterns of trehalose dehydrate crystal (dashed line) and part-crystalline/part-glassy material (solid line).

CLIMATIC ROOM EXPERIMENTS

The finding above suggests an important testable concept, i.e., for anti-humidity action to take effect trehalose must be in an anhydrous or glassy form below 50% RH. To test this, experiments were performed under controlled conditions of 30% RH and ~20°C and 60% RH and ~20°C. Control hair switches (those "treated" only with water) and hair switches treated with trehalose were styled using straightening irons at the two conditions given above in a climatic room. Subsequently the humidity and temperature in the testing area were increased to a final state of 80% RH and ~30°C. The switches continued to remain in the climatic room while the conditions were being changed. The results are given in Figures 6 and 7. In both experiments, control (i.e., water-treated only) switches are the first and the last switches and flank the six trehalose-treated switches in between. It can be seen that trehalose-treated switches styled at lower %RH show anti-humidity benefits (Figure 6, right) while those styled at higher %RH do not (Figure 7, right). It is proposed that in the latter case, as the conditions were being changed to high-humidity conditions, any glassy material formed during the styling phase would have converted to the crystal form even before the high-humidity conditions of 80% set in and thus would not have shown anti-humidity benefits.

Clearly Figures 6 and 7 offer compelling evidence that the anti-humidity benefit obtained from trehalose and heat is intimately related to the starting styling conditions. Trehalose-treated and hot-iron straight-styled switches at lower relative humidity conditions show noticeable anti-humidity benefits. Thus if one assumes that the use of hot irons (~200°C) in the straightening process converts the trehalose to a glassy form *in situ*, this suggests that the ability of the trehalose glassy form to pick up water below 50% RH and convert to a stable dihydrate crystal above 50% RH is linked to the anti-humidity activity observed.

DISCUSSION ON MECHANISM OF ACTION OF TREHALOSE

Here we have presented evidence for trehalose- and heat-treated switches showing anti-humidity benefits compared to the control. Evidence has also been presented that glassy forms of trehalose can pick up water compared to the crystal form and that hair treated with trehalose and heat has similar overall water-uptake properties compared to the control. Finally, it has been shown that if the starting condition during style creation is at low relative humidity, only then is the anti-humidity benefit seen. Here we summarize the mechanism of action of trehalose to give anti-humidity benefits.

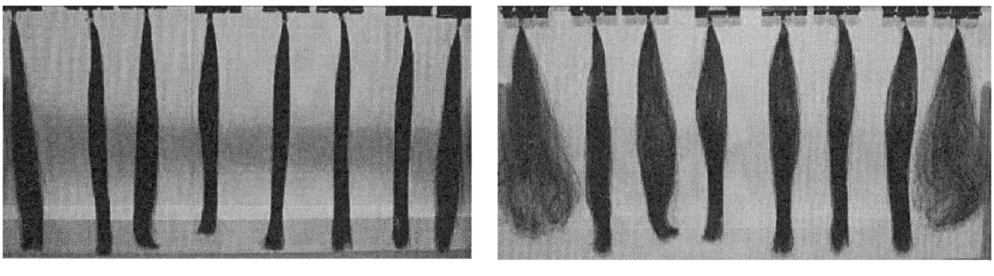

Figure 6. Left: after styling with straighteners at ~20°C and 30% RH. Right: After changing the conditions in the room to high humidity at ~30°C and 80% RH. In both pictures the first and the last switches are control (treated with water) and fluff up at high humidity, i.e., do not show the anti-humidity benefit. All switches in the middle are treated with 2% trehalose solution and show the anti-humidity benefit.

Since trehalose is a sugar, it is tempting to assume that the non-fluffing of the hair array could be due to the stickiness of the sugar, increasing tack and adhesiveness on the surface and holding the array in place even at high humidity. Here this is partly prevented by ensuring that the switches are combed at least five times after the styling process to remove any surface "bonds" or "welds" forming and holding the array together. Moreover, after high-humidity, tactile tests show that there is a very small crisp/dry coating on the surface, suggesting possibly the presence of the trehalose dihydrate crystal.

Apart from sugar stickiness, another possibility could be the difference in diffusion of water in fibers treated with trehalose and heat. The adsorption isotherms shown by the DVS curves in Figure 3 enable calculation of diffusion coefficients using simplified methods (19). It is found that there is no difference in the diffusion coefficients between control heat-treated and trehalose heat-treated hair, though both are significantly smaller than with normal (non-heat-treated) control hair.

In the literature the remarkable properties of trehalose in protecting living cells against extreme desiccation, etc., is thought to occur because trehalose works as a water replacement molecule or is a vitrification agent in the dry state (20–28). Here a simple moisture uptake of the glassy form of trehalose seems to correlate well with the effect seen in hair.

At normal room temperature the glass transition relative humidity of hair (RH_g) is around 65–70% RH. Above this RH_g, water uptake in hair increases rapidly, hair is plasticized, and style loss is accelerated. The effect of trehalose glass taking up water, however small, seems to increase the aging of the hair polymer, giving rise to preservation of straight style and consequently the anti-humidity benefit.

Further evidence of the effect of trehalose was seen on switches that were repeatedly subjected to heat straightening and high-humidity exposure without any wash in between, but with a mild rinse by spritzing with water from a wash bottle. Switches originally treated with trehalose continued to show low fluffing at high humidity compared to the control for about three cycles. Here we think that in the trehalose-treated switches during the styling and high-humidity cycle, trehalose changes forms from glass to crystal to glass again to give rise to continued straight style longevity. A complete rinse or a full washing process results in the loss of much of the continued straight style benefits.

A likely scenario for the mechanism of action for trehalose- and heat-treated hair could be the following. Trehalose is distributed at different parts of the hair composite from solution, and when subjected to the straightening procedure with high-temperature irons, it

Figure 7. Left: After styling with straighteners at ~20°C and 60% RH. Right: After changing the conditions in the room to high humidity at ~30°C and 80% RH. In both pictures the first and the last switches are treated with water. All switches in the middle are treated with 2% trehalose solution. None of the switches shows the anti-humidity benefit.

produces trehalose glasses *in situ* at their current locations. The trehalose glasses formed pick up water as the humidity rises and thus reduce the amount of moisture available to disturb the style. Furthermore, the trehalose glasses may act as pore blockers and sterically hinder water from reaching certain parts of the hair. Finally, molecular trehalose may itself stabilize the styled configuration through effects related to hair protein binding (28) and/or structuring water (29). The striking anti-humidity benefits as demonstrated in this paper may be a result of one or more mechanisms described above. More work needs to be done to completely resolve the mechanism of action.

In summary, here we see a macroscopic anti-humidity benefit at the hair array level that seems intimately related to the water uptake and solid state polymorphism of trehalose. Clearly, glassy trehalose regulates moisture in the fiber and affects the viscoelastic behavior and subsequent aging characteristics of the hair polymer in some way that, though not fully understood, seems to be related to the water uptake properties of this form of trehalose. We think that the presence of the trehalose glass and its ability to regulate moisture in the hair is the major contributing factor for the anti-humidity effect.

CONCLUSION

Hair treated with trehalose and heat-straightened shows long-lasting hair style retention even at high humidity compared to hair treated with water, provided that the conditions at the style creation stage are suitable, i.e., low-humidity ($<50\%$) conditions. The formation of trehalose glasses *in situ* during the heat straightening process on hair treated with trehalose may be responsible for the anti-humidity effect seen. Trehalose glasses regulate moisture in the fiber at high humidity, giving longer-lasting style benefits.

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