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The Effect of Thermal History on the Maillard Reaction in a Glassy Matrix

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To test whether the extent of physical aging affected the reaction rate, Maillard reaction kinetics were studied in glassy model preservation systems subjected to two different thermal histories. The glass transition temperature and physical aging of the matrix were determined using differential scanning calorimetry, and the normalized heat capacities were modeled using the Tool−Narayanaswamy−Moynihan approach. Samples prepared using the different thermal histories initially had different degrees of aging, but these were practically indistinguishable after 10 h under the reaction conditions (65 °C); the samples underwent rapid structural relaxation at that temperature. The reaction of glucose and lysine in an amorphous trehalose/sucrose matrix was followed using spectrophotometric and chromatographic analysis. A difference in reaction rate could only be distinguished in the rate of consumption of glucose, which was approximately 20% faster in the minimally aged matrix; no significant differences were seen in any other indicator of reaction.

KEYWORDS: Glass transition; Maillard reaction; physical aging; kinetics; preservation

INTRODUCTION

Amorphous carbohydrates are widely used for the preservation of active ingredients, including proteins, in the glassy state against chemical and physical changes. It is proposed that the enormous viscosity of the glass ($>10^{12}$ Pa s) arrests diffusion on practical time scales, and as a consequence, physical and chemical stabilities are obtained. In practice, this view is an oversimplification. A glass in itself is not physically stable but will be subject to the process of physical aging through structural relaxation, which produces characteristic changes in mechanical behavior commonly referred to as embrittlement. In addition, in glassy carbohydrate mixtures, the diffusion of relatively small molecules can be more rapid than would be expected. Several studies have shown that chemical reactions may not be arrested in the glassy state and may still proceed to a measurable extent (*1*−*7*). The phenomenon is relevant to practical problems of food preservation, for example, the storage of skim milk and whey protein powders (*8*, *9*), and, more generally, in bioprocessing to the dry heat antiviral treatment of proteins for biomedical applications (*10*−*12*). In this article, we consider the relationship between the physical aging of a glassy matrix and its effect on chemical reaction in the glass. The aim of this research was to study the effect of physical aging on the rate of Maillard reaction, a reaction that is relevant to the stability of proteins in glassy carbohydrate matrices (*10*−*14*).

As a molten carbohydrate is slowly cooled, its viscosity and the associated structural relaxation time will progressively increase. The density of an undercooled carbohydrate liquid will increase with decreasing temperature. Vitrification at the glass transition temperature (T_g) effectively "locks in" a liquid structure, and the expected densification on cooling subsequently occurs at a rate related to the structural relaxation time. As a consequence of the slow densification, material properties, including compliance, stiffness, and brittleness (*15*), slowly evolve with time, with the whole process commonly known as physical aging.

The structural relaxation time is in part dependent on temperature and partly on liquid structure. Liquid structure is characterized on a temperature scale through the notion of a fictive temperature, T_f, the temperature at which a particular structure would be fully relaxed (*16*). Densification affects the energetics of interaction between molecules and the accessibility of liquid configurations, both of which can be probed in a calorimetric experiment. The calorimetric consequence of the slow structural relaxation, which occurs on aging in the glass, is observed as a peak in heat capacity preceding T_g or as an overshoot at T_g on subsequent heating.

There are various phenomenological approaches for describ-

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ing the observed time-dependent behavior. A widely applied model, which has a potentially useful predictive capability, is the Tool−Narayanaswamy−Moynihan (TNM) (*16*−*18*) model, which has been applied to polymeric systems. The dependence of structural relaxation on time, t, can be described by an empirical relaxation function, ϕ, of the stretched exponential form

$$\phi(t) = \exp[-(t/\tau_0)^{\beta}] \quad (1)$$

in which β ($0 < \beta \leq 1$) is a measure of its nonexponentiality. The parameter τ_0 is a characteristic time, which is dependent on both temperature, T, and, to an extent, liquid structure (characterized by T_f) and has been successfully obtained using the expression

$$\tau_0 = A \exp [x\Delta h^*/RT + (1 - x)\Delta h^*/RT_f] \quad (2)$$

where A, x ($0 < x \leq 1$), and Δh^* are constants (*16*, *19*, *20*). These relationships can be used to calculate the time dependence of T_f following a temperature step. For example, the steady cooling and heating of a sample can be considered as a succession of n such steps in which case

$$T_{f,n} = T_0 + \sum_{j=1}^{n} \Delta T_j \{1 - \exp [-(\sum_{k=j}^{n} \Delta T_k/Q\tau_{0,k})^{\beta}]\} \quad (3)$$

where Q is a heating or cooling rate and T_0 is a starting temperature where relaxation is sufficiently rapid that, in the initial stages of cooling, equilibrium is obtained. During annealing, the sample is held at a fixed temperature, T_a, for a certain length of time, and this is divided into 10 logarithmically spaced steps with eq 3 being modified as described

$$T_{f,n} = T_0 + \sum_{j=1}^{n_a} \Delta T_j \{1 - \exp [-(\sum_{k=n_a}^{n} \Delta t_{e,k}/\tau_{0,k})^{\beta}]\} \quad (4)$$

where $n_a + 10 \geq n > n_a$ and

$$\Delta t_{e,k} = t_e^{1/10} \quad k = n_a + 1$$

$$\Delta t_{e,k} = t_e^{(k-n_a)/10} - t_e^{(k-n_a-1)/10} \quad k > n_a + 1$$

For a calorimetric experiment, $T_{f,n}$ is then related to a normalized heat capacity, $C_{p,n}$, by

$$C_{p,n} = (T_{f,n} - T_{f,n-1})/(T_n - T_{n-1}) \quad (5)$$

These predicted values of $C_{p,n}$ can then be compared with those determined calorimetrically. This approach can then be extended in a straightforward way to predict the effects of more complex thermal histories.

By controlling the physical aging of glassy matrices, it is proposed that denser matrices with more restricted dynamics may result in enhanced preservation through the slower deterioration kinetics of encapsulated species. In this study, the aim is to test this proposal by following the rate of a model food relevant reaction in the glassy state, the Maillard reaction between glucose and lysine, after the glass has been aged to different extents using different thermal histories.

MATERIALS AND METHODS

Materials and Instrumentation. Trehalose dihydrate, sucrose, glucose, and lysine monohydrochloride were obtained from Sigma-Aldrich. High-performance liquid chromatography (HPLC) was performed on a Dionex gradient HPLC system (P580 pump, ASI-100 autosampler with PDA-100 photodiode array detector, and Shodex RI-71 refractive index detector). A Dionex CarboPac PA1 column was used with 50 mM NaOH in water (isocratic, 1 mL min^{-1}) as the eluant. Chromeleon chromatography software (version 6.40) running on a Windows PC was used for data recording and analysis. UV−visible spectroscopy was recorded on a Perkin-Elmer Lambda 25 spectrophotometer. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC 7 using PETA software running on a UNIX PC. Samples were freeze-dried using a Dura-Top/Dura-Dry refrigerated shelf freeze dryer combination (FTS Systems). A laboratory fan oven (Binder) was used for the storage of the samples at 50 and 65 °C, and a second laboratory oven (Memmert) was used for temporary storage at 50 °C.

Preparation of the Sample Mixture (*1*). Trehalose dihydrate (19.9 g), sucrose (7.5 g), and glucose (1.5 g) were dissolved in 250 mL of water. Lysine hydrochloride (3.0 g) was dissolved in a separate 50 mL of water, and the two solutions were stored on ice prior to mixing. Mixing these solutions gave a solution with a dry solids composition as follows: trehalose, 60% w/w; sucrose, 25% w/w; glucose, 5% w/w; and lysine, 10% w/w. The mixed solution (pH 7.3) was transferred to a stainless steel tray on the freeze dryer shelf (shelf temperature −29 °C) and allowed to freeze overnight before starting the condenser and pump. During primary drying (72 h), the product temperature (monitored by a PTFE-coated thermocouple) was typically 10 °C lower than the shelf temperature, due to evaporative cooling. After primary drying, the shelf temperature was increased in stages (−20 °C for 24 h, −5 °C for 24 h, followed by 25 °C for 72 h) over the remainder of the drying period. Drying was judged to be complete when the pressure in the drying chamber was the same as that in the condenser chamber (4.5 Pa). After drying, the mixture was removed from the freeze dryer and the brittle cake of material was crushed with the end of a spatula to a coarse powder, which was transferred to a vacuum desiccator over P_2O_5 for further drying.

Sample Preparation. The dry mixture was transferred to a glovebox (drybox) in a dry nitrogen atmosphere, and 20 portions of approximately 100−150 mg were placed into headspace GC vials (22 mL capacity, Chromacol). The headspace vials were sealed while under nitrogen, and each vial was randomly labeled with a sample number. Similarly, 20 preweighed 50 μL DSC sample pans were filled with approx 5 mg of the sample mixture and sealed under nitrogen. Each sample pan was placed in a labeled screw-cap glass vial, and the filled pans were reweighed to determine the mass of their contents.

Experimental Design. In an earlier paper, it was shown that the Maillard reaction (nonenzymatic browning) can occur in a glassy model system at detectable rates (*1*). In this study, the aim is to test whether the rate of Maillard reaction in the same freeze-dried glassy trehalose/sucrose/glucose/lysine mixture is affected by the state of physical aging. The aim of the experiment was therefore to impose two different thermal histories on sets of otherwise identical samples. These we term the minimally aged (MIN) set and the maximally aged (MAX) set. These would then be heated to a temperature where reaction had previously been observed in the glassy state (65 °C for samples with a water content of 1.1%). Analysis of samples taken at different times after the temperature was raised would allow any difference in the reaction rates to be determined.

To identify appropriate thermal histories for the MIN and MAX samples, their effect on the extent of physical aging was modeled using the TNM model. Relationships 2−5 were used to predict the result of imposing a series of temperature steps on the material. Because the sample matrix consisted of a mixture of disaccharides, parameters previously obtained for maltose were initially used ($\Delta h^* = 479$ kJ mol^{-1}; $x = 0.475$; $\beta = 0.4$) (*21*), with the value of $-\ln A$ ($= 158$) adjusted to take account of the difference in T_g between the maltose and the trehalose/sucrose-based mixture. The model predicted that 21 days (3 weeks) at 50 °C would impose a fictive temperature of ∼65 °C on the samples, the same temperature at which Maillard reaction was carried out, and so this would be a suitable aging history for maximal aging. In order that the MIN and MAX sets differed only in their degree of physical aging, both were subjected to the same 50 °C treatment, but the minimally aged (MIN) samples subsequently had

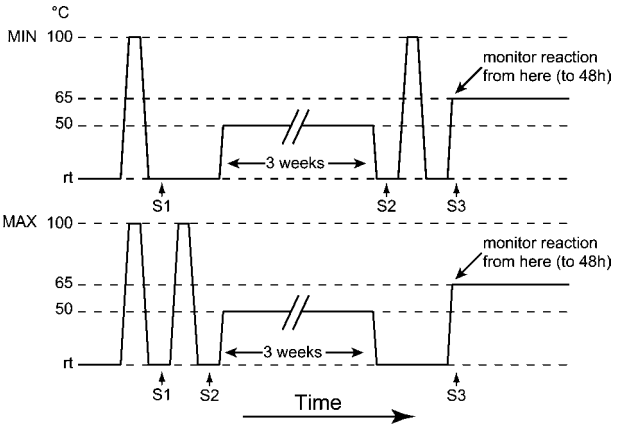

Figure 1. Schematic representation of the temperature steps imposed on the minimally aged (MIN, upper line) and maximally aged (MAX, lower line) sample set, including erasure steps (held for 5 min above T_g at 100 °C), the aging step (3 weeks at 50 °C), and the start of the reaction period (48 h at 65 °C). The points at which the first three samples were taken from each set are indicated by S1, S2, and S3; the last point representing the $t = 0$ point for the reaction in both sets.

their thermal history erased by heating briefly above T_g before both sets were heated to 65 °C for the reaction phase. Both sets of samples were also heated above T_g in an identical erasure step prior to the 50 °C aging step in order to remove any history from the vitrification and drying processes ("pre-erasure"). A second erasure step was also imposed on the maximally aged (MAX) set before the aging to balance the second erasure of the minimally aged set. In this way, both samples experienced the same steps in the heating cycles but in a different order, as summarized schematically in **Figure 1**.

Headspace GC vials were used for bulk samples of approximately 100−150 mg and sealed under nitrogen in order to maintain a dry environment and to prevent further uptake of water. These provided material for HPLC and UV spectroscopic analysis. In addition to the bulk samples, an equal number of 50 μL DSC pans was prepared for thermal analysis. The sampling schedule included the initial points marked S1 and S2 for each set, taken between each of the major temperature steps (**Figure 1**) and at S3 ($t = 0$ h). Subsequent samples were removed from the oven at 2 h intervals during the first 10 h at 65 °C and at 24 and 48 h. The labeled samples were placed in a freezer after collection for later analysis.

Pre-erasure and Thermal Aging. The headspace vials and DSC samples were arranged in two tube racks of 10 of each type of sample. The oven for the 50 °C aging was preheated while the sample pre-erasure was carried out. The two sets of samples were transferred in turn to a GC oven (Perkin-Elmer Autosystem XL GC), and the temperature of the oven was raised from 50 to 100 °C at 25 °C min^{-1} and held at this temperature for 5 min before cooling to 50 °C at 25 °C min^{-1}. The minimally aged (MIN) set was subjected to this heating cycle once and then transferred to the 50 °C oven. The maximally aged (MAX) set was cycled twice in succession before transfer to the oven. Both sets of samples were held at 50 °C for 3 weeks. One pair of samples (vial and DSC pan) was taken from the MIN set after the erasure cycle and placed in a labeled polythene bag in a −20 °C freezer. Similarly, two pairs of samples were collected from the MAX set, one after each of the two erasure cycles, and also stored in the freezer.

Second Erasure and Reaction Sampling. A second oven was set to 50 °C, and the temperature was allowed to stabilize overnight from the 20th day of the aging period. At the end of the 3 week aging period, the two sets of samples were transferred to this oven while the first oven was reequilibrated to 65 °C (the reaction temperature). The MIN set was transferred to the GC oven and subjected to a second erasure cycle, as described above. A second pair of samples (vial and DSC pan) was collected from the MIN set before the erasure cycle. Both MAX and MIN sets were then transferred to the 65 °C oven, and a pair of samples was taken from each ($t = 0$ samples). Pairs of samples were then taken from each set at 2 h intervals up to 10 h, with the remaining two pairs from each set being collected at 24 and 48 h. All samples were stored in labeled polythene bags in a −20 °C freezer until required for analysis.

UV−Vis Spectroscopy. Samples were taken from the freezer and allowed to warm to room temperature before transfer to the drybox. A four-figure balance (Sartorius) was used within the drybox to accurately weigh out samples (approximately 10−15 mg) into a series of 5 mL volumetric flasks. Aluminum foil was used on the base of the drybox, and a spatula connected to an earthed wire was used for each weighing in order to help dissipate static electricity; however, it was still necessary to wait several minutes before recording the weights for the balance reading to stabilize. Triplicate samples were taken for each sample point. The headspace vials were then recapped before removal from the drybox to preserve the samples for further measurement. Samples were dissolved in a 1:3 v/v mixture of ethanol:water and accurately diluted to 5 mL immediately before measurement. The absorbance was determined at 280 and 420 nm, according to Lievonen et al. (*4*, *22*). Absorbances were normalized to a concentration of 2 mg mL^{-1} for comparison of the data.

HPLC Calibration. Calibration solutions appropriate to the concentration of the four initial components of the mixtures were prepared by mixing aliquots of two solutions (for trehalose, sucrose, and lysine) or dilution with water (glucose), such that the initial concentration of each analyte in the sample was approximately in the middle of the range of concentrations prepared, assuming a concentration of 20 mg mL^{-1} of the mixture. In this way, solutions of trehalose dihydrate of 10 and 20 mg mL^{-1}, sucrose of 2 and 6 mg mL^{-1}, and lysine monohydrochloride of 1 and 3 mg mL^{-1} were mixed in proportions of 0:1.0, 0.2:0.8, 0.4:0.6, 0.6:0.4, 0.8:0.2, and 1.0:0 mL to give a set of six calibrant solutions for each analyte. Similarly, the glucose solution was diluted with water to produce a set of five calibrants. On HPLC analysis, these solutions gave four calibration graphs (using data from the refractive index detector channel), fitted to a linear equation with $R^2 \geq 0.9997$ in all cases.

HPLC Determination of Reactants. Samples were again weighed by accurate balance in the drybox taking precautions against static electricity (see above). Approximately 20 mg samples were weighed accurately in triplicate into 1.5 mL capacity HPLC vials. Each sample was dissolved in 1 mL of water, capped with a septum, and stored in the HPLC autosampler carousel at 10 °C to await analysis. Analyses were performed on single injections of 50 μL. Data from the refractive index detector channel were used for all peak analyses and quantitation. The trehalose peak was used as an internal standard for calculation of the relative size of the glucose and lysine peak areas in each sample.

DSC Measurements. The T_g of samples was determined by calorimetry using a Perkin-Elmer DSC7 fitted with a robotic autosampler and an Intra-cooler II. The instrument was calibrated for temperature using the melting temperatures of indium (156.6 °C), dodecane (−9.65 °C), and octadecane (28.24 °C) and for heat flow using the heat of fusion of indium (28.45 J g^{-1}). The instrument was calibrated for heat capacity, C_p, using a sapphire standard. Panned samples were scanned in the DSC from 30 to 110 °C at 10 °C min^{-1} twice. The T_g for each sample was determined from the second heating run, as the midpoint of the sharp change in heat capacity, which occurs at T_g. Subtraction of the second run from the first and peak analysis allowed the enthalpy of the relaxation peak to be determined.

RESULTS AND DISCUSSION

DSC Studies. The midpoint T_g measurements (**Figure 2**) determined by DSC confirm that the glass transition is unchanged, within experimental error, at least for the first 24 h of reaction. The measured T_g of 82 °C indicates that this material is identical to one of the samples studied previously and so has a water content of around 1.1% w/w (*1*). The drop in T_g seen in the final (48 h) measurement may be due to plasticization by the reaction products, which may include water.

Figure 3a shows a plot of $C_{p,n}$ as a function of temperature for the trehalose mixture, which was aged for 21 days at 50 °C. The sample shows a marked overshoot in heat capacity

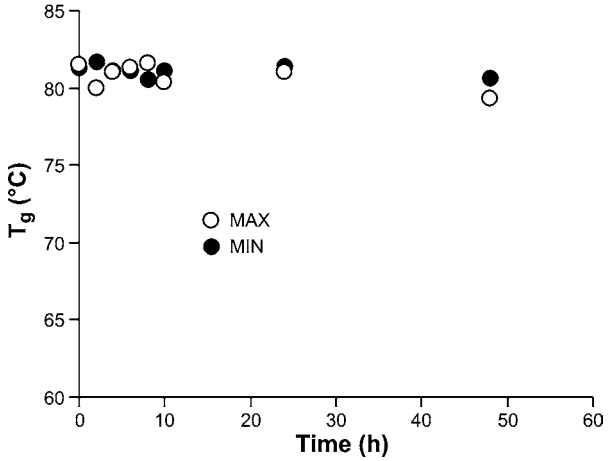

Figure 2. Glass transition temperatures (midpoint, determined by DSC) of samples plotted against reaction time, showing that T_g remained constant over at least the first 24 h of reaction.

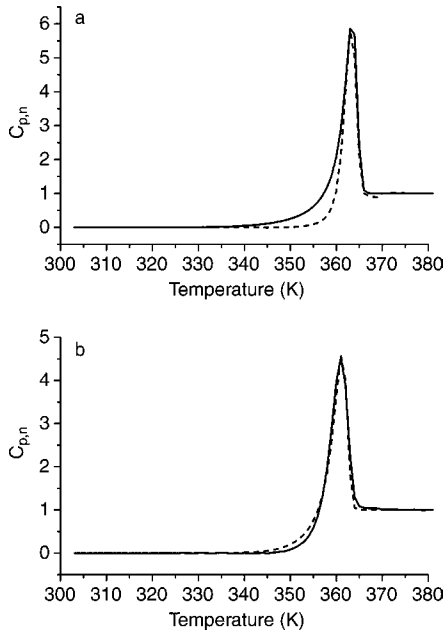

Figure 3. Experimental (solid lines) and predicted (dashed line) normalized heat capacities ($C_{p,n}$), plotted against absolute temperature, showing the relaxation peak, for (**a**) MAX 0 h sample (S3) (aged 21 days at 50 °C) and (**b**) MAX 24 h sample (aged 21 days at 50 °C, followed by 24 h at 65 °C).

indicative of the process of physical aging. Included for comparison is the predicted heat capacity response using the parameters $\Delta h^* = 479$ kJ mol^{-1}, $-\ln A = 159.7$, $x = 0.47$, $\beta = 0.464$, and an estimated T_f of ~61 °C. These parameters, similar to those describing amorphous maltose (*21*), give a reasonable description of the observed behavior. On heating the mixture with a T_f of ~ 61 °C to the reaction temperature (65 °C), it is expected that the liquid structure will further evolve with time. **Figure 3b** shows a corresponding plot for a trehalose mixture, which had been aged for 21 days at 50 °C followed by 1 day at 65 °C. The overshoot in heat capacity is substantially reduced, indicative of a limited erasure of physical aging on heating to a temperature higher (65 °C) than the estimated T_f (61 °C) of the stored sample. The parameters x and β required to describe the data are 0.59 and 0.635 and are indicative of a narrowing in spread of relaxation on subsequent aging at the higher temperature. The graph of observed relaxation enthalpy as a function of time (**Figure 4**) shows the general features of

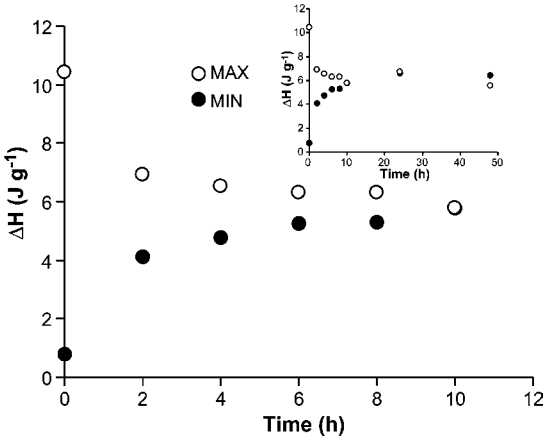

Figure 4. Relaxation enthalpies determined for the panned samples from the Maillard reaction sequence, as determined by DSC over the initial 10 h reaction period, showing further changes during the reaction period. The inset shows the same data including the 24 and 48 h points.

the observed aging response. The MAX aging history has resulted in a much higher relaxation enthalpy peak in the initial member of this set (lower fictive temperature), which decreases on aging at 65 °C. The MIN samples show an initially small relaxation enthalpy, which increases with time. During the course of the experiment, the relaxation enthalpy of the two sets of sample converged, so while the initial states were clearly different, the two sets of samples appeared more or less indistinguishable after 10 or 12 h at 65 °C. This means that a different thermal state is only maintained for a few hours and the samples are very different for only the first few data points.

Extent of Reaction. The progress of reaction was monitored by both HPLC for the disappearance of the starting materials and spectroscopic observation at 280 and 420 nm for the appearance of colored products (*4*, *22*). The development of an absorbance at 280 nm is said to be indicative of the early stages of Maillard reaction and may be due to the presence of furfural type compounds, while absorbance at 420 nm should be associated with visible coloration or browning of the material (*23*, *24*). It can be seen from **Figure 5** that despite some visible coloration of the samples to the naked eye, only a very slight increase in absorbance at 420 nm occurred, while a consistent and significant increase in absorbance at 280 nm was seen. Unfortunately, the data are not good enough at the beginning of the run for the critical initial rate of color development to be compared for the two sets. The curves for both sets of samples however appear to follow the same trend for both wavelengths, with a tendency for the rate of color development to slow as the absorbance reaches a plateau, possibly indicating a limiting extent of reaction due to restricted diffusion or other cause. It could be argued that the absorbance in the MAX set is always greater than that of the MIN samples at 280 nm; however, because the absorbance values are not identical at the start of the run, this is likely to be from another cause of systematic error; shifting all of the MAX data by the difference between the $t = 0$ h samples would result in all of the error bars of both sets of data to overlap. While there may be no significant differences between the data sets, they do provide further spectroscopic data on the Maillard reaction proceeding in the glassy state, which confirms previous reports (*1*, *3*−*5*).

While the development of colored products is more commonly associated with Maillard reaction (nonenzymatic browning), the early stages of reaction may not be associated with color formation but can be monitored by the loss of starting materials by HPLC measurement of the concentration of each

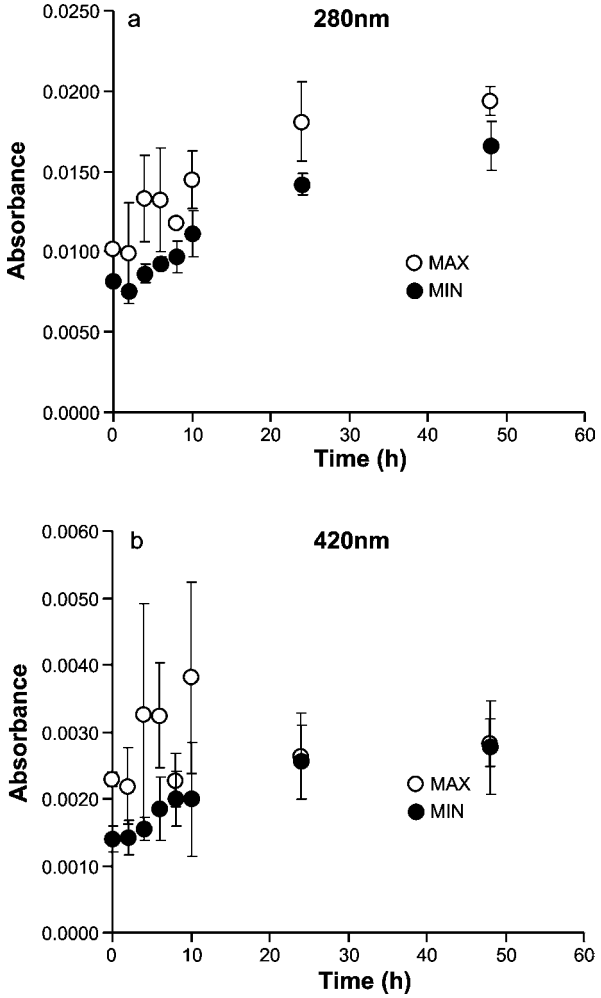

Figure 5. Absorbance of the samples in 1:3 ethanol:water solution as a function of reaction time at 65 °C, normalized to 2 mg mL^{-1} total concentration of mixture. Data are the means of three samplings per time point, and error bars represent one standard deviation from the mean.

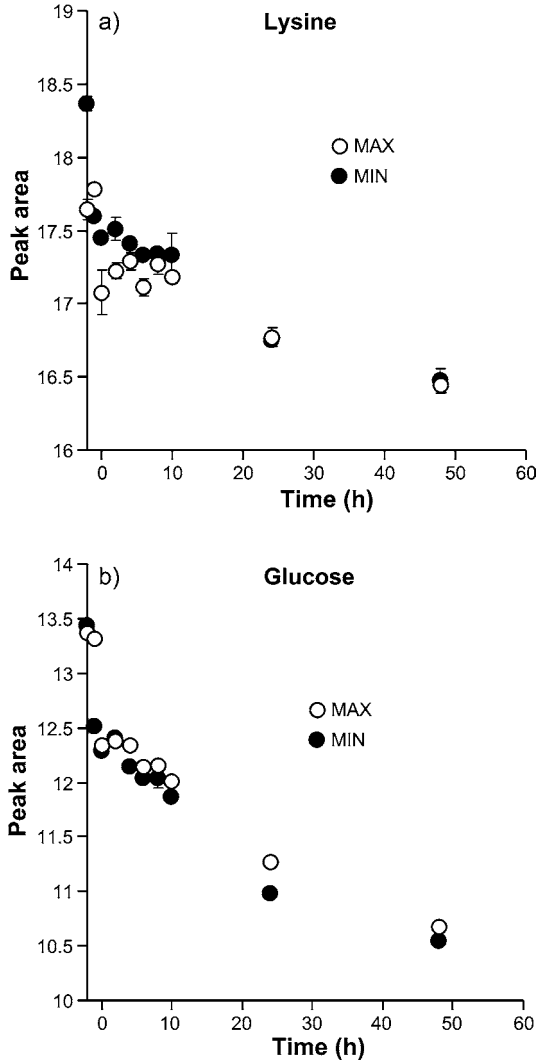

Figure 6. Relative consumption of lysine and glucose plotted as a function of reaction time at 65 °C. Peak areas were normalized according to the trehalose peak area and are the mean of three determinations per time point; error bars represent one standard deviation from the mean. The −2 and −1 h points represent the sampling points (S1) and (S2) in each set, as shown in Figure 1, respectively.

component in the mixture. The trehalose and sucrose components are nonreducing and therefore not capable of participating in the reaction with lysine, so the peak of either one can be used as an internal standard in the quantification of the glucose and lysine peaks. Trehalose was chosen as the internal standard peak as it showed the better peak shape; however, a plot of the trehalose peak areas against those of sucrose confirmed that a constant ratio was maintained for all samples; therefore, either disaccharide peak could have been used as standard. From the HPLC data (RI detector), one sample was chosen as the arbitrary standard for the size of the trehalose peak and all other samples were normalized to this standard. While it is possible to convert these data to concentrations using calibration curves, it is simpler to compare concentrations by relative peak area, as shown in **Figure 6**. A direct comparison of the consumption of the reagents is complicated by the stoichiometry of the reaction, while the lysine graph shows definite curvature; the consumption of glucose appears to be approximately linear for the first 24 h of reaction. While Bell et al. (*25*) observed second-order kinetics for the consumption of glucose in the reaction with glycine in poly(vinylpyrrolidone) matrices, comparison with our data is complicated by the different stoichiometries of the reacting entities (lysine is a diamine while glycine is a monoamine) with our system showing distinct deviation from second-order kinetics as a consequence. Furthermore, the conditions used here also differ in using an excess of amino acid over glucose, following the approach of Roos and co-workers (*3*, *4*) while the study of Bell et al. used 1:1 stoichiometry. The inclusion of data taken from the initial two points of each data set shows that some material was consumed during the 21 days at 50 °C, as might be expected. A linear fit of the glucose data over the 0−24 h interval suggests that the rate of glucose consumption is around 20% faster in the MIN set than the MAX (a similar difference in rate is also observed even if the 24 h point is disregarded), but this result is not supported by any of the other measures of reaction. The rates of disappearance of glucose calculated from these linear fits are 0.018 mol dm^{-3} day^{-1} for the MIN data set and 0.015 mol dm^{-3} day^{-1} for the MAX set, both of which compare well with the rate of glucose loss reported by Craig et al. (*1*) for the equivalent material, which is 0.022 mol dm^{-3} day^{-1}.

The initial condensation step of the reaction produces a stoichiometric amount of water and so the absolute reaction rates indicate that an increase in the water concentration of the sample of about 0.030−0.036 M occurs during the first 48 h of reaction. Assuming a density of 1400 kg m^{-3} means that this corresponds to a mass concentration of 0.04−0.05% w/w, a relatively small amount as compared to the water content of the sample. The

diffusive mobility of water in carbohydrate glasses (*26*) means that any heterogeneity occurring due to local production of water is likely to be short-lived at molecular length scales. Diffusion coefficients of 10^{-14}−10^{-16} m^2 s^{-1} mean that on the 1 s time scale the mean squared displacement of water molecules will be in the range of 24−240 nm.

In conclusion, it was shown that the rate of Maillard reaction could be followed in a relatively low water system in the glassy region having imposed two different thermal histories on the material. The reaction was monitored by consumption of glucose and lysine and the development of colored products. Rapid structural relaxation at the reaction temperature meant that the differences in aging history were not maintained for more than the initial monitoring period. The effect of aging on reaction rate is small, no greater than 20% in the current study, with the minimally aged material exhibiting the faster rate of glucose consumption; no significant differences in rate could be found for any other measures of reaction. The results suggest that densification of the matrix upon physical aging slows the rate of reaction, possibly through lowering the rate of diffusion of the reactants, but the effect is not very large.

While this study employed an elevated temperature to allow changes due to reaction over a relatively short time period, a longer term study would be necessary, using a similar experimental design, to probe the effect of the densification of the matrix on the storage stability of encapsulated ingredients and pharmaceuticals under more practical conditions.

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