Maillard Reaction Rate in Various Glassy Matrices

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The Maillard Reaction (MR) rate below the glass transition temperature (T_g) for various model glassy food systems was studied at temperatures between 40 °C and 70 °C. As a sample, freeze-dried glucose and lysine systems embedded in various glassy matrices (e.g., polyvinylpyrrolidone and trehalose) were used, and the MR rate below the T_g was compared among the various glassy matrices. The extent of MR was estimated spectrophotometrically from the optical density at 280 nm (OD_{280}), and the MR rate (k_{280}) was determined as a pseudo zero order reaction rate from the time course of OD_{280}. Although k_{280} was described by the Arrhenius plot, the temperature dependence of k_{280} was almost the same and the intercept was different among the matrices. From the comparison of k_{280}, it was suggested that the MR rate in glassy matrix was affected not only by the T_g, but also by the hydrogen bonding between MR reactants and glassy matrix.

Key words: Maillard Reaction; freeze dry; glass transition; glassy matrix; hydrogen bond

Maillard Reaction (MR), known as an amino-carbonyl chemical reaction, has been widely studied. In the field of food science, it is well-known that MR causes not only a browning of color and flavor change, but also loss of nutritional or functional value. Since this is a serious problem for the shelf life of low-moisture dry food, glassy food is in a non-equilibrium state in which macroscopic molecular mobility is frozen. Once glassy food changes to the rubber state at the glass transition temperature (T_g), molecular mobility rapidly increases. Since molecular mobility usually affects the rate of physical change or chemical reaction, it is believed that the T_g value of glassy food is a critical factor for the consideration of storage stability. Actually, it has been reported that the rate of some undesirable physical changes (e.g., crystallization, and structural collapse) or chemical reactions (e.g., degradation, oxidation, and Maillard Reaction) have a close relation with T_g. That is, the rates below T_g are much slower than that above T_g. This observation itself is worthwhile, but it should be noted that MR progresses even below T_g, and in fact, the prediction and control of MR in glassy food remain difficult. As of today, there have been few reports focusing on differences in MR rate among various glassy matrices below T_g.

The purpose of this study was to clarify the effects of the glassy matrix on the MR rate by investigating the MR rate in various glassy matrices. The model freeze-dried food system composed of glucose, lysine, and glass matrix former was used. As glass matrix formers, raffinose, trehalose, dextran, and polyvinylpyrrolidone (PVP) -k90, -k30, and -k13–19 were used. The MR rates below T_g of various glass matrices were studied across a wide temperature range, and the relationship between the difference in the MR rate in the glassy matrix and the T_g was discussed.

Materials and Methods

Materials and sample preparation. Analytical grade d-glucose, dextran (MW = 10,000), and PVPk13–19 (MW = 10,000) were purchased from Sigma Co., Ltd. Lysine, PVPk90 (MW = 360,000), PVPk30 (MW = 40,000), and raffinose pentahydrate were purchased from Wako Pure Chem. Ind., Ltd., Japan. Reagent grade d-trehalose dihydrate was provided by Hayashibara Co., Ltd.

The reagent adjusted to the ratio of glucose:lysine:glass matrix former = 1:1:98% (w/w) was dissolved in 10% (w/w) aqueous solution, and the 2 ml solution was pipetted into a 10 ml glass vial. The solution was frozen in a freezer at approximately −50 °C for 12 h, and transferred to a pre-cooled freeze-drier. Freeze-drying was carried out from −40 °C to 20 °C at 3.0 × 10^{−2} Torr over a 2 d period. After freeze-drying, the residual moisture content was removed over diphosphorus pentaoxide in a vacuumed desiccator for one week at...
25 °C. Moreover, only for the PVPk30 system, a sample containing some residual moisture was also prepared over saturated potassium acetate (RH22%) in a desiccator for one week at 25 °C. The final moisture content was checked by the Karl–Fisher method.

**DSC analysis.** The $T_g$ of the sample was examined by DSC (Shimadzu DSC-50). For calibration of temperature and heat capacity in DSC measurement, indium (Wako) and distilled water were used. As a reference material, α-alumina powder for standard material (Shimadzu) was used. 5–10 mg of the sample was weighted and sealed in an aluminum DSC pan. Each sample was heat-scanned from 0 °C to a maximum of 200 °C at 5 °C/min.

**Estimation of extent of MR.** The freeze-dried sample was stored at 10 °C intervals at temperatures between 40 °C and 70 °C. After storage, the sample was rehydrated to 0.04 sample-g/ml with distilled water, and the extent of MR was estimated spectrophotometrically (Shimadzu UV-160) by optical density at 280 nm ($OD_{280}$). $OD_{280}$, which is considered to be due to the formulation of furfural compounds, is often used to detect the early stage of MR.16–18,21,22) Similarly, optical density at 420 nm, which quantifies the yellow and browning pigments formed at the final stage of MR, is also used, but only $OD_{280}$, which can detect even slight progress in MR, was used in this study. For the average of the three measurements, the difference in $OD_{280}$ before and after storage ($\Delta OD_{280}$) was estimated as the extent of MR during storage.

**Results**

**Glass transition temperature**

The DSC thermogram of each sample, as shown in Fig. 1, indicated one endothermic shift of the base line by glass transition, and each $T_g$ was determined from the onset point of the shift. The $T_g$s obtained are listed with the $T_g$s of the each glassy matrix reported in several papers23–25) in Table 1. Although the $T_g$s in the literature showed slightly different values, it was found that the $T_g$s obtained were approximately 25 °C lower than the $T_g$s of each glassy matrix. This result is due to the plasticizing effect by MR reactants. Furthermore, it is well-known that residual moisture plays the role of a superior plasticizer for the glassy matrix. The moisture content of each sample prepared over diphosphorus pentaoxide in the vacuumed desiccator was confirmed to be below 0.5% (w/w), and the PVPk30 sample prepared over RH22% contained 9.3% (w/w) residual moisture. Therefore, it is reasonable that the $T_g$ of the PVPk30 sample prepared over RH22% was much lower (approximately 59 °C) than that prepared over diphosphorus pentaoxide. On the other hand, the same as a general amorphous polymer, the $T_g$s of the PVP group became higher with increases in the molecular weight by the effects of sub-entanglement molecular weight.

**Estimation of MR rate**

As a typical result, the time courses of $\Delta OD_{280}$ for raffinose samples stored at 40 °C–70 °C are shown in Fig. 2. $\Delta OD_{280}$ increased linearly with the storage time, and the gradient (i.e., the increasing rate of $\Delta OD_{280}$) became larger with the storage temperature. The time and temperature dependence of $\Delta OD_{280}$ for other samples also showed the same trend as that of the raffinose sample, though this is not shown here.

For every sample, the MR rate ($k_{280}$) was estimated successfully as a pseudo zero order reaction rate ($R^2 > 0.94$). Since MR is a complex reaction process which generates many kinds of intermediate, the MR rate cannot be defined intrinsically as a single process. However, it has been confirmed experimentally that the pseudo zero order reaction formula is the most successful description for the MR rate, when the extent of MR was detected by $OD_{280}$.21) Thus, in this study, $k_{280}$ obtained by the pseudo zero order reaction formula of $\Delta OD_{280}$ was treated experimentally as a MR rate in accordance with the previous studies.16–18,21)

Moreover, the temperature dependence of $k_{280}$ was described well by the Arrhenius formula in the temper-

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (sample) (°C)</th>
<th>$T_g$ (matrix) (°C)</th>
<th>$\ln A$</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trehalose</td>
<td>90.3</td>
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<td>65.0</td>
<td>145.0</td>
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<td>115.4</td>
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<tr>
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<td>131.7</td>
<td>170.5</td>
<td>63.2</td>
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<tr>
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<td>70.7</td>
<td>156.0</td>
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<tr>
<td>PVPk90</td>
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<td>62.8</td>
<td>138.3</td>
</tr>
<tr>
<td>Dextran</td>
<td>157.7</td>
<td>unknown</td>
<td>58.8</td>
<td>130.9</td>
</tr>
</tbody>
</table>

List of $T_g$ values estimated by DSC measurement, $T_g$ values of each glassy matrix reported by reference, $\ln A$, and $\Delta E$ obtained by Arrhenius plot of $k_{280}$.

**Table 1.** $T_g$ and Arrhenius Parameters

![DSC Thermogram of Each Sample.](image-url)

**Fig. 1.** DSC Thermogram of Each Sample.

The $T_g$ of sample was investigated by DSC, and the heating curve for each sample is shown. The arrows show the $T_g$.
ature ranges measured, as shown in Fig. 3. From the Arrhenius plot, it was found that the declines were almost parallel, and only their intercepts shifted. Thus, the activation energy ($E_a$) for MR in each glassy matrix system was almost independent of the kind of matrix, and the values were in the range of 130 to 156 kJ/mol. Since these values were in agreement with literature values reported for other MR systems,\textsuperscript{17} it is thought that the $E_a$s obtained in this work were in the reasonable range. In contrast, the pre-exponential parameters ($A$) were different depending on the material. These values are also summarized in Table 1.

**Discussion**

*Comparison of MR rates in various glassy matrices*

A comparison of $k_{280}$ among the four kinds of PVP matrix showed that each $k_{280}$ was lower in the order PVPk90, PVPk30, PVPk13–19, PVPk30 including 9.3% (w/w) moisture, which corresponded to the order of $T_g$. These results suggest that the MR rate in the glassy matrix relates to the $T_g$ of the matrix, as expected. But when the $k_{280}$s were compared between PVPk90 and the other glassy matrices (viz., trehalose, raffinose, and dextran), though the $T_g$ of the PVPk90 matrix ($T_g = 164.9 \degree$C) was the highest among the glassy matrices, the $k_{280}$ of the PVPk90 matrix was higher than that of the trehalose, raffinose, or dextran matrix. Similar trends were also found from the comparison of $k_{280}$s between dextran ($T_g = 157.7 \degree$C) and low molecular sugars such as trehalose ($T_g = 90.3 \degree$C) and raffinose ($T_g = 93.0 \degree$C). These results suggest that the MR rate in glassy matrix cannot be predicted universally by only the $T_g$ of matrix. The other factor for MR rate in glassy matrix is discussed in the following section.

*Difference of hydrogen bonding in glassy matrix*

The difference in glassy matrix between low molecular sugar and polymer has been discussed extensively for freeze-dried protein as follows.\textsuperscript{26–28} When protein aqueous solution is freeze-dried in the presence of low molecular sugar compounds, they can form intermolecular hydrogen bonds between the hydroxyl group in sugar and carbonyl and/or amino group in protein.\textsuperscript{26–28} Thus, the hydrogen bond formed between protein and sugar in the glassy matrix plays the role both of stabilizing the protein structure and of cooperative immobilizing.\textsuperscript{26–28} But the hydrogen bond between protein and polymer is restricted by molecular steric hindrance,\textsuperscript{26–28} so that glassy polymer cannot work as an effective protein stabilizer. A similar view may be applied to the result in this study. That is, the mobility of MR reactants (viz., lysine and glucose) are restrained by hydrogen bonding in the glassy low molecular sugar matrix, but hydrogen bonding is hindered in the polymer matrix.\textsuperscript{26–28} Furthermore, since dextran consists of glucose, which has some hydroxyl groups, the ability to form hydrogen bonding of dextran is superior to that of PVP, which has only one carbonyl group per monomer. From these observations, it is thought that the difference in the dynamic restraint of MR reactants by hydrogen bonding in the glassy matrix affect the MR rate in glassy matrix.

In conclusion, we suggest that the MR rate in glassy matrix is affected not only by the $T_g$ of the matrix, but also by the molecular interaction by hydrogen bonding between MR reactants and the glassy matrix. Although the relation between the MR rate and $T_g$ of glassy matrix has been investigated by many researches, only the importance of $T_g$ has been pointed out. Additionally, we propose the importance of knowledge of molecular interaction in the glassy matrix in the current study.
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References