

The rate of non-enzymatic browning reaction in model freeze–dried food system in the glassy state

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Abstract

The effect of glass transition on the rate of the non-enzymatic browning reaction (NBR) of glucose and lysine in different glassy matrices; trehalose, maltose, polyvinylpyrrolodone (PVP)-k90, -k30 and -k13~19, was investigated. The extent of NBR was determined by the absorbance at 280 nm/gram sample (ABS_{280}), and the rate of NBR was evaluated as a pseudo zero order reaction rate from the time course of ABS_{280} . In each glassy matrix, the NBR progressed at temperatures much lower than the glass transition temperature (T_g). Although the T_g of PVP matrix was much higher than that of trehalose or maltose, the NBR rate was much faster. These results suggest that the NBR rate in a glassy matrix cannot be understood by the T_g of matrix only. We should also consider the direct molecular interaction by hydrogen bond between NBR reactants and a glassy matrix.

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Keywords: Freeze dry; Glass transition; Non-enzymatic browning reaction; Molecular mobility below the T_g ; Hydrogen bond

Industrial relevance: Interestingly the authors show that the concept of glass transition cannot be applied to the non-enzymatic browning reactions in freeze-dried materials. They suggest that a matrix preventing non-enzymatic browning has to consist of a material exhibiting a high T_g and superior hydrogen bond forming ability (e.g. disaccharides).

1. Introduction

In the context of food preservation, glass transition phenomenon is one of high interest to many food researchers (Le Meste, Champion, Roudaut, Blond, & Simatos, 2002; Levine & Slade, 1988). Since a degrading chemical reaction or physical change in a food system during storage requires at least small molecular rearrangements, it is postulated that the limited molecular mobility in the glassy state results in long-term stability of foods. When a glassy material is heated to temperature above the glass transition temperature (T_g), a sharp decline in viscosity and structural collapses such as caking happen suddenly (Aguilera, del Valle, & Karel, 1995; Le Meste et al., 2002). This phenomenon, the glass transition, also takes

place at constant temperature, when the T_g of glassy material is reduced below the storage temperature by moisture uptake. So, as a criterion for storage stability of glassy foods, the relationship between the T_g value and the moisture content has been investigated for food related ingredients (Kawai, Suzuki, & Takai, 2002; Levine et al., 1988; Wang, 2000) and multi component real food systems (Khalloufi, El-Maslouhi, & Ratti, 2000).

On the other hand, non-enzymatic browning reactions (NBR), which is an amino–carbonyl reaction that occurs between amino acids and reduced sugars, have been studied. The progress of NBR during storage causes various quality changes, e.g. color, flavor, nutritional and functional properties (Burin, Buera, Hough, & Chirife, 2002; Labuza & Massaro, 1990; Maltini, Torreggiani, Venir, & Bertolo, 2003). Therefore, the prediction and control of the rate of NBR have to be considered in the context of storage stability of low moisture foods. For such purpose, the relation between NBR and the T_g of foods has been studied

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(Bell, Touma, White, & Chen, 1998; Buera & Karel, 1995; Karmas, Buera, & Karel, 1992; Lievonen, Laaksonen, & Roos, 1998; Lievonen, Laaksonen, & Roos, 2002; O'Brien, 1996; Roos & Himberg, 1994). From their studies, it is evident that reaction rates were much lower at temperature below T_g as compared with temperature above T_g . Therefore, the importance of T_g for the control of NBR has been emphasized. However, it should be noted that NBR has been observed even at temperatures below T_g (Schebor, Buera, Karel, & Chirife, 1999). That is, there is the observation that NBR is not always prevented in the glassy state.

To understand this problem in this study, the NBR in different glassy matrices during isothermal storage was investigated at temperature below the T_g . As a model freeze-dried food, a system composed of glucose, lysine and a glass matrix former was used. The glass matrix formers were trehalose, maltose, polyvinylpyrrolidone (PVP)-k90, -k30 and -k13~19.

2. Materials and methods

2.1. Preparations

Lysine, PVPk30, and maltose monohydrate from WAKO Pure Chem. Ind. Ltd., and D-Glucose and PVPk13-19 from SIGMA Co. Ltd., were used. Reagent grade D-trehalose dihydrate was provided from Hayashibara Co. Ltd.

A 10% w/w aqueous solution of glucose:lysine:glass matrix former=1:1:98 (% w/w, dry basis) was prepared, and 2 ml fraction of each solution was put into 10 ml-glass vial. The solution was frozen at $-50\text{ }^\circ\text{C}$ for 12 h and transferred to a pre-cooled freeze-drier. The frozen formulation was freeze dried increasing temperature from $-40\text{ }^\circ\text{C}$ to $20\text{ }^\circ\text{C}$ at 3.0×10^{-2} Torr over a 2 day period. The residual moisture of the freeze-dried sample was removed over diphosphorus pentoxide in vacuum desiccator during 1 week at room temperature. The residual water content of the sample was below 0.5% w/w.

2.2. Non-enzymatic browning reaction

The freeze-dried sample was stored in an incubator at a controlled temperature from $40\text{--}70\text{ }^\circ\text{C}$ over a 2 month period. After storage, the sample was rehydrated using distilled water, and the extent of NBR was detected by measuring the absorbance at 280 nm/gram sample (SHIMADZU UV-160). The absorbance at 280 nm has been often used to detect the early stage of NBR (Flink, 1983; Karmas et al., 1992; Lievonen et al., 1998, 2002; O'Brien, 1996). The measurement of absorbance was performed about three samples obtained from the same sample preparation. The average value of three samples was the extent of NBR (ABS_{280}). Furthermore, the increase of ABS_{280} after storage, ΔABS_{280} , was calculated as the progress of NBR during storage.

2.3. Glass transition temperature

The T_g of the sample was examined by Differential Scanning Calorimetry (DSC) (SHIMADZU DSC-50). DSC was calibrated by pure water and indium. The sample (5–10 mg) was weighted and sealed into an aluminum DSC pan. The DSC heat-scan was performed from $0\text{ }^\circ\text{C}$ to maximally $200\text{ }^\circ\text{C}$ at a scan rate $5\text{ }^\circ\text{C}/\text{min}$. The T_g was determined from the onset temperature of the glass to rubber transition.

3. Results

The result of the DSC measurement is shown in Fig. 1. Each sample showed one endothermic shift from base line, the T_g value was determined from the onset point of the shift. The T_g values are summarized in Table 1. The obtained T_g values were slightly lower than those of the pure glassy matrices as reported in literature (Wang, 2000). This difference is caused by the plasticizing effect of NBR reactants and little residual water ($<0.5\%$ w/w). On the other hand, as known for amorphous polymers, the T_g s of PVPs increased with increasing molecular weight (effect of sub-entanglement molecular weight).

The time course of ΔABS_{280} for trehalose systems stored at $10\text{ }^\circ\text{C}$ intervals at the temperatures between 40 and $70\text{ }^\circ\text{C}$ is shown in Fig. 2 as a typical result. The ΔABS_{280} increased linearly with storage time, and the rate increased with increasing storage temperature. The results of other samples also showed the same trend though not shown here. The increase of ΔABS_{280} as a function of time could be represented by a pseudo zero order reaction, which is in line with available literature data (Karmas et al., 1992; Lievonen et al., 1998, 2002; O'Brien, 1996). The correlation coefficient (R^2) was always higher than 0.95. Since NBR is a complex process which generates different type of intermediates, the NBR rate cannot be defined intrinsically as a single reaction

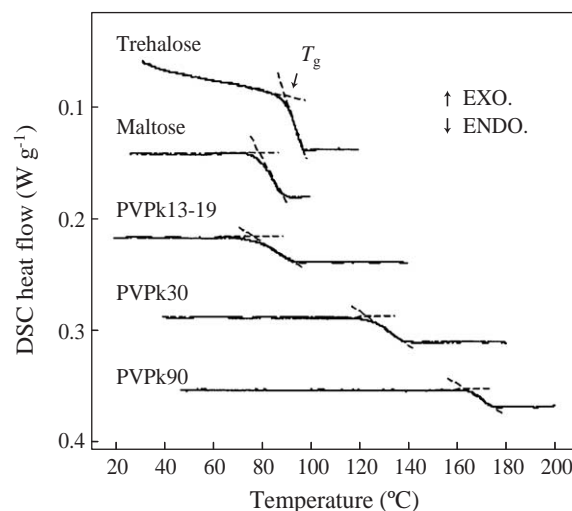


Fig. 1. DSC curves of the NBR samples.

Table 1
The evaluated T_g , k_0 and ΔE_{280}

Matrix	T_g °C	k_0 g ⁻¹ s ⁻¹	ΔE_{280} kJ mol ⁻¹
Trehalos	90.3	2.0×10^{28}	145.0
Maltos	79.6	1.4×10^{27}	140.2
PVPk13-19	81.2	3.2×10^{28}	143.3
PVPk30	131.7	2.9×10^{27}	138.4
PVPk90	164.9	2.0×10^{27}	138.3

process. However, it has been confirmed experimentally that the pseudo zero order reaction is the most successful description for the NBR (O'Brien, 1996). In this study, the reaction rate constant (k_{280}) was calculated from the change of ΔABS_{280} .

Moreover, the temperature dependency of rate constant k_{280} was analyzed using the Arrhenius equation as shown in Fig. 3. In all cases, the k_{280} could be well described by the Arrhenius equations. It was found that the temperature dependency of the k_{280} value for each sample showed no significant difference, while their intercepts shifted. Thus, the activation energy (ΔE_{280}) for NBR in each glassy system was similar and thus independent of the type of matrix used, of which values were in the range from 138.3 to 145.0 kJ mol⁻¹. This value is in agreement with literature values reported for other system (Lievonon et al., 1998). On the other hand, frequency factors, (k_0 s) were between 1.4×10^{27} and 3.2×10^{28} g⁻¹ s⁻¹ which were apparently different among different samples indicating that at a given temperature the reaction rate is dependent on the system. These values are also summarized in Table 1.

4. Discussion

4.1. The relationship between T_g and rate of NBR

Since the molecular mobility at temperature below T_g is very low, it is thought that the NBR rate also becomes low at

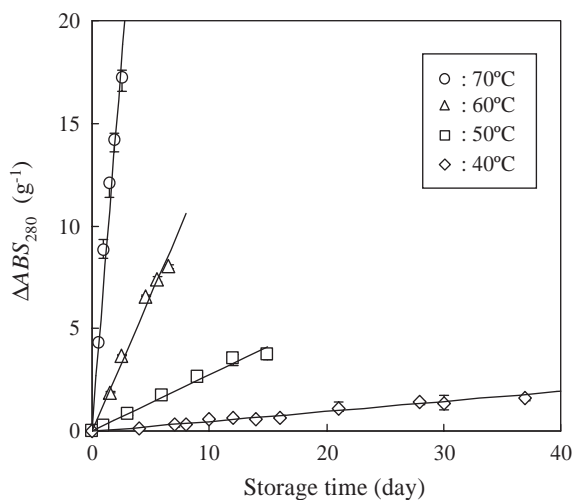


Fig. 2. Time course of ΔABS_{280} for trehalose sample stored at various temperatures.

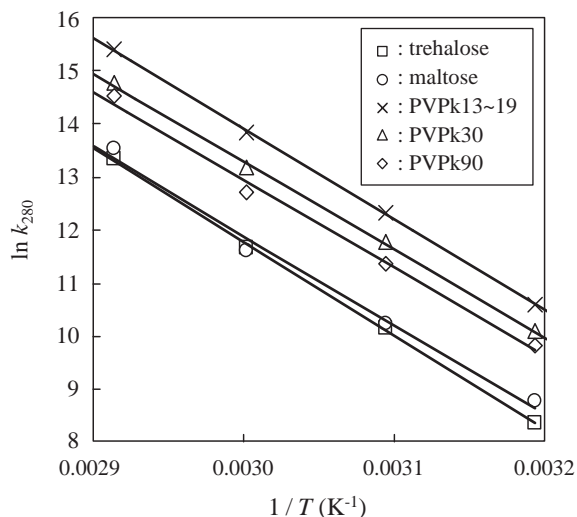


Fig. 3. Arrhenius plot of k_{280} at temperature below T_g .

temperature below T_g . Moreover, as T_g of the glassy matrix is high, the NBR rate will become lower at a given storage temperature. In polymeric matrices (PVP-k90, -k30 and -k13~19), as the T_g of glassy matrix was high, the NBR rate was lower. On the other hand, since disaccharides (i.e. trehalose and maltose) showed an almost equal T_g , no apparent difference in k_{280} could be found. However, when the k_{280} was compared to systems of polymers and disaccharides, the k_{280} s in polymeric matrices were higher than those in disaccharide systems, despite the fact that T_g s of the polymeric matrices were higher than those for disaccharides. This result suggests that the NBR rate in a glassy matrix cannot be understood by the T_g of matrix only. Other properties of the glassy material have to be considered.

4.2. Molecular mobility at temperature below T_g

Many studies on the enthalpy relaxation time of glassy materials have been conducted (Duddu, Zhang, & Dal Monte, 1997; Hancock, Shamblin, & Zografis, 1995; Kawai, Hagiwara, Takai, & Suzuki, 2004; Liu, Rigsbee, Stotz, & Pikal, 2002). The enthalpy relaxation time relates to the macroscopic molecular mobility below T_g , therefore, it has been considered that the understanding of enthalpy relaxation processes would help the prediction of decreased reaction rate at temperature below T_g (Duddu et al., 1997). However, in our previous study, it was found that the enthalpy relaxation of pure glassy trehalose or maltose could not be detected within a few days storage at the $(T_g - 40)$ °C (Kawai et al., 2004), while the NBR in a glassy trehalose or maltose matrix could fairly progress under the same storage condition as shown in this study. That is, the time scale of the enthalpy relaxation phenomena and the NBR reaction are quite different. The same trends could be confirmed from analyzing the NBR rate in the glassy PVPk90 matrix and the enthalpy

relaxation for this system as shown by Hancock et al., 1995. Therefore, it could be stated that the NBR rate in the glassy matrices is not directly related to its enthalpy relaxation time.

On the other hand, the β -relaxation, which is detected from dielectric relaxation measurements, has been suggested to reflect the local mobility such as the rotational or vibrational movement of the side chain or group (Noel, Parker, & Ring, 2000). This β -relaxation is known to progress fast even at temperature below the T_g , so that the correlation between the NBR rate below the T_g and the β -relaxation has been suggested (Lievonon & Roos, 2003). However, it seems to be doubtful that such local mobility in the glassy matrix reflects the NBR rate. Actually, Lievonon, and Roos (2003) investigated the dielectric properties of model samples for NBR. They concluded that the β -relaxation did not explain the difference in NBR rates as observed at temperature below the T_g . From these discussions, it is concluded that the molecular mobility of a glassy matrix is not a critical factor for preventing NBR.

4.3. Molecular interactions in the glassy matrix

It has been known that hydrogen bond between sugar (hydroxyl group) and protein (carbonyl and primary NH group) is induced by freeze-drying a solution containing both components (Carpenter & Crowe, 1989; Crowe, Leslie, & Crowe, 1994). This phenomenon has been often understood as a water substitution effect of sugar to protein. We did not confirm the evidence of hydrogen bond in this study, however, it is reasonable to consider that sugars and amino acid formed hydrogen bond during sample preparation through freeze-dry. Based on the hydrogen interaction between sugar and amino acid, we propose a mechanism for NBR in a glassy freeze-dried system as shown in Fig. 4. Glucose and lysine, which are NBR reactants, form the intermolecular hydrogen bond by freeze-drying (Fig. 4-a). Then, if the NBR reactants are embedded in a glassy disaccharide (e.g. trehalose and maltose), the direct hydrogen bond between NBR reactants become hindered by the involvement of disaccharide as glassy materials. Moreover, each intermolecular hydrogen bond would form the hydrogen network which will introduce a cooperativity of molecular mobility (Fig. 4-b). On the other hand, in the glassy polymer matrix, the molecular steric hindrance due to large molecules can not form enough hydrogen bonds with NBR reactants (Crowe et al., 1994). Consequently, the polymer matrix hinders the hydrogen bonding network so that the reactants would be relatively free even in the glassy matrix (Fig. 4-c). Therefore, the average mean NBR rate in polymer system is higher than those in glassy disaccharide matrices.

It seems that this interpretation can explain successfully why the NBR can progress in the glassy matrix, and why the

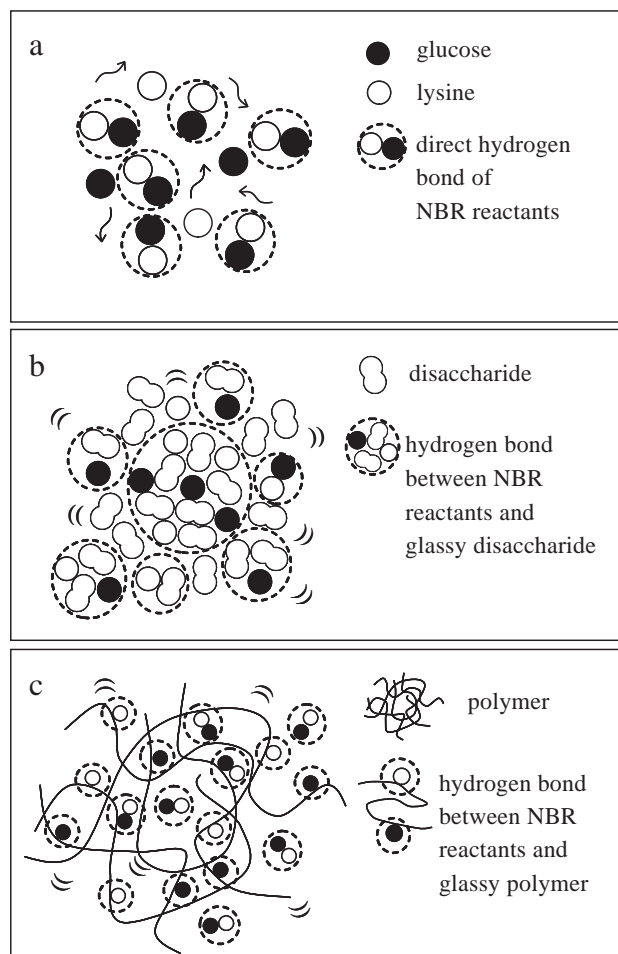


Fig. 4. Model of NBR in different glassy matrices. (a: no matrix, b: disaccharide matrix, c: polymeric matrix) In the disaccharide matrix, the diffusion and the direct hydrogen bond of NBR reactants are prevented, however, the direct hydrogen bond of NBR reactants is caused partially in the polymeric matrix.

NBR rate in the glassy polymeric matrix was faster than those in disaccharide systems. Nevertheless, the T_g is still an influencing factor for the NBR rate as concluded from a comparison of k_{280} among PVP matrices. Therefore, it is concluded that a glass matrix preventing NBR has to consist of a material which shows both characteristics, i.e. a high T_g and superior hydrogen bond forming ability such as disaccharides.

5. Conclusion

The fundamental concept of glass transition has been widely accepted. However, the glass transition concept cannot be applied to the NBR in freeze-dried materials. We propose an improved mechanism for preventing NBR in glassy materials. The direct molecular interaction by hydrogen bond and the slow molecular mobility introduced by the glass transition governs the NBR rate in glassy materials at temperature below T_g .

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