Nonenzymatic Browning in Food Models in the Vicinity of the Glass Transition: Effects of Fructose, Glucose, and Xylose as Reducing Sugar

SATU M. LIEVONEN,*,† TOMMI J. LAAKSONEN,† AND YRJÖ H. ROOS‡

Department of Food Technology, P.O. Box 27, FIN-00014 University of Helsinki, Finland, and Department of Food Science, Food Technology and Nutrition, University College Cork, Cork, Ireland

Effects of a reducing sugar, fructose, glucose, or xylose, and glass transition on the nonenzymatic browning (NEB) rate in maltodextrin (MD), poly(vinylpyrrolidone) (PVP), and water systems were studied. Glass transition temperatures (T_g) were determined using DSC. Water contents were determined gravimetrically, and NEB rates were followed at several temperatures spectrophotometrically at 280 and 420 nm. Reducing sugar did not affect water contents, but xylose reduced the T_g of the solid models. Sugars showed decreasing NEB reactivity in the order xylose > fructose > glucose in every matrix material. The NEB reactivity and temperature dependence of the single sugars varied in different matrices. The NEB rates of the solid models increased at temperatures 10–20 °C above the T_g, and nonlinearity was observed in Arrhenius plots in the vicinity of T_g. The temperature dependence of nonenzymatic browning could also be modeled using the WLF equation.

INTRODUCTION

Nonenzymatic browning (Maillard reaction) is one of the most important chemical reactions in foods. It is a complex reaction series, which starts with a condensation of a free amino group and a reducing sugar and ends with the formation of brown melanoid pigments (*1*). Nonenzymatic browning has effects on the flavor, color, and texture of food materials. It also affects the nutritional quality and toxicological characteristics (*2*). Because of its wide consequences in foods and other biomaterials, nonenzymatic browning has been studied intensively. At present, nonenzymatic browning is known to depend on several factors such as time, temperature, moisture content, water activity, pH, concentration of reactants, and reactant type (*3*).

Increasing attention has recently been paid to the effect of the physical state of biomaterials on NEB kinetics. The reason for the augmented interest is an effort to optimize processing and storage conditions of dry and intermediate moisture foods (*4, 5*). These foods typically contain amorphous or semiamorphous regions, which change their physical state from brittle glass to elastic rubber at the glass transition temperature range. Below this range an amorphous material is in a highly viscous glassy state where molecular mobility and thereby chemical reactivity are suggested to be largely hindered. Keeping the amorphous materials at glassy state during processing and storage is therefore proposed to prevent deteriorative chemical

reactions, to maintain quality, and to extend the shelf life of products (*6*).

Studies relating nonenzymatic browning and physical state have concentrated on the examination of the effects of changes in matrix materials on reaction rates. The effect of the glass transition on NEB rates has mainly been studied in different model systems both at various temperature ranges (*7–15*) and at constant temperature (*16–19*). Special attention has been focused on the effects of different $T - T_g$ conditions (*7–10, 12*), different moisture contents or water activities (*7, 9, 16*), and concomitant physical changes such as collapse or crystallization (*7, 10, 13, 15, 18, 19*). Generally, the glass transition has been reported to possess an independent effect on nonenzymatic browning rates. The effect, however, has been relatively moderate. Increasing reaction rates have been observed, not at the glass transition temperature but 10–40 °C above the glass transition temperature (*7, 11*). Furthermore, nonenzymatic browning has been showed to proceed at the slow rate even well below the glass transition temperature (*7, 9, 11, 12*). Whereas the researchers have concentrated on studying the macroscopic changes in amorphous materials, the effects of other factors such as internal heterogeneities of materials and reactant size, concentration, and reactivity have been neglected so far.

The type and concentration of reactants are known to affect significantly nonenzymatic browning (*3, 20*). The studies relating physical state and nonenzymatic browning have attempted to use highly reactive model systems to facilitate the collection of browning data (*10*). Xylose, although commonly used because of its high reactivity, is rare in foods (*21*). It may be possible that NEB results obtained with the models using

* Corresponding author (telephone +358-9-191 58204; fax + 358-9-191 58651; e-mail satu.lievonen@helsinki.fi).
† University of Helsinki.
‡ University College Cork.

NEB Reaction: Effects of T_g and Sugars

J. Agric. Food Chem., Vol. 50, No. 24, 2002 **7035**

xylose as a reducing sugar are not generalized as such to real food systems in which less reactive sugars, glucose and fructose, are the most abundant monosaccharides. For instance, it could be possible that the model systems containing xylose as a reducing sugar might lead to overestimation of the NEB rates in glassy state foods. Furthermore, comparisons between reactivities of different sugars have usually been performed in aqueous model systems (*22−30*). Studies comparing the reactivities of sugars in different solid matrices are not as common. The purpose of the present study was to compare the effects of different reducing sugars and the glass transition on the NEB rates in maltodextrin-, poly(vinylpyrrolidone)-, and water-based food models.

MATERIALS AND METHODS

Materials. D-Fructose was purchased from Merck (Darmstadt, Germany). D-Glucose, D-xylose, l-lysine (free base), and poly(vinyl-pyrrolidone) (PVP-40, PVP) were from Sigma Chemical Co. (St. Louis, MO). Maltodextrin (Maltrin M-100, MD) was obtained from Grain Processing Corp., (Muscantine, IA). Distilled water was used in the preparation of models.

Preparation of Food Models. Both liquid and solid models having different reducing sugars were made. Water models were prepared by dissolving nonenzymatic browning reactants, lysine (5.0% w/w) and either fructose, glucose, or xylose (5.0% w/w), into water. Aliquots of 5 mL of the solutions were placed into glass ampules (5 mL), and the ampules were hermetically flame-sealed. Amorphous solid MD- or PVP-based food models were made by preparing solutions containing 20% total solids from the reactants, the matrix material, and distilled water. On the basis of pre-experiments, the exact amounts of the reactants, 1:1, were adjusted to be 10% w/w of the water that the models sorbed after freeze-drying when they were equilibrated into 0.33 water activity at 23−24 °C. Solutions of 50 g were frozen on Petri dishes (2−24 h at −20 °C and 24 h at −80 °C) and freeze-dried (48 h, *p* < 0.1 mbar) (Lyovac GT 2, Amsco Finn-Aqua GmbH, Hürth, Germany).

The freeze-dried MD- and PVP-based materials containing glucose were first stored in vacuum desiccators at 33% relative humidity (RH) for 24 h. After 24 h, the materials were ground, and aliquots of 1 g were transferred into glass ampules, which were stored in the vacuum desiccators at 33% RH for another 24 h. Then the ampules were flame-sealed. Grinding of the moist materials was difficult, and therefore the MD- and PVP-based materials containing fructose or xylose were ground immediately after freeze-drying. Ground powders on the Petri dishes were stored in vacuum desiccators at 33% RH for 24 h. Thereafter, aliquots of 1 g were transferred into glass ampules, and the ampules were stored in the vacuum desiccators at 33% RH for another 24 h. Then the ampules were flame-sealed.

Water Content Determination. Water contents of the solid food models were determined gravimetrically. Triplicate samples of 1 g of each freeze-dried food model, prepared in 20-mL glass vials, were stored in a vacuum desiccator over P_2O_5 for a week. After dehydration, the samples were stored in vacuum desiccators at 23−24 °C over a saturated salt solution of $MgCl_2$, which gave an RH of 33.0%. The samples were weighed at intervals until the weights leveled off. The water contents of the models leveled off within 24 h.

Differential Scanning Calorimetry (DSC). The glass transition temperatures for the MD- and PVP-based food models were determined using a DSC (Mettler TA4000 system with TC15 TA processor, DSC 30 measuring cell, and STAR^e Thermal Analysis System version 3.1 software; Mettler-Toledo AG, Schwerzenbach, Switzerland). The instrument was calibrated, and the T_g measurements were performed as described by Lievonen et al. (*11*). The glass transition temperature (T_g) was taken from the onset temperature of the glass transition temperature range. An average obtained for triplicate samples is reported as the glass transition temperature.

Nonenzymatic Browning. For NEB measurements, the flame-sealed ampules were stored at 10 °C intervals at eight temperatures from 30 to 100 °C. Triplicate samples were removed at intervals after sufficient color formation and stored at −80 °C before analysis. The extent of

Table 1. Water Contents and Glass Transition Temperatures of the MD- and PVP-Based Food Models Having either Fructose, Glucose, or Xylose as a Reducing Sugar

matrix	reducing sugar	water content^a ± SD at 24 °C (g/100 g of solids)	$T_g{}^a$ ± SD (°C)
MD	fructose	8.2 ± 0.02	58 ± 0.7
	glucose	8.2 ± 0.03	62 ± 1.0
	xylose	8.2 ± 0.02	58 ± 2.6
PVP	fructose	12.6 ± 0.02	55 ± 0.8
	glucose	12.9 ± 0.04	60 ± 0.1
	xylose	12.8 ± 0.01	51 ± 2.2

^{a} Each value is an average of triplicate samples.

browning was determined spectrophotometrically (Perkin-Elmer Lambda 2 UV−vis spectrometer; Norwalk, CT) from the optical density (OD) at 280 and 420 nm. Samples were dissolved with 20 mL of water/ethanol (3:1) solution and diluted when necessary to obtain absorbance signals on scale.

Data Analysis. Nonenzymatic browning of both the water models and the solid MD- or PVP-based models was modeled to be a pseudo-zero-order reaction as often reported in the literature (*3*). Rate constants, their 95% confidence limits (CL), and coefficients of determination (R^2) were calculated using a linear regression analysis. The statistical significance of the different browning rates of the different food models at each temperature was tested using a linear model. The statistical models were fitted using generalized least squares. The temperature dependence of the NEB reaction was modeled using the Arrhenius equation and the Williams−Landel−Ferry (WLF) equation.

RESULTS AND DISCUSSION

Water Contents and Glass Transition Temperatures. The MD-based food models sorbed 8.2 g of water/100 g of solids on average when equilibrated into 0.33 water activity at 24 °C (**Table 1**). The PVP-based food models were more hygroscopic. Their average water sorption was 12.8 g of water/100 g of solids. The reducing sugar did not affect the water sorption behavior of the models. The glass transition temperatures of the models varied between 51 and 62 °C depending on the matrix material and the reducing sugar. As shown in **Table 1**, the glass transition temperatures of the MD-based food models were slightly higher than those of the PVP-based food models. The glass transition temperatures of both types of matrix materials were highest when glucose was used as a reducing sugar and decreased when either fructose or xylose was used. The effect of the reducing sugar on the T_g was more pronounced in the PVP-based food models than in the MD-based food models.

A survey of the literature showed that there were moderately large deviations between water content and glass transition temperature data obtained from different studies. The water contents of the present MD food models were higher and the glass transition temperatures were lower than previously reported for the plain maltodextrin (Maltrin M100) stored at 33% RH at 25 °C, which contained 5.4 g of water/100 g of solids and had a glass transition temperature of 66 °C (*31*). In agreement with the present study, Roos and Himberg (*9*) reported lower T_g values for the MD-based food models containing xylose and lysine (7% solids, 1:1) than for MD alone.

There are quite a number of recent publications reporting water contents and glass transition temperatures of different PVP-based model systems (*10*, *11*, *16*, *18*, *19*, *32*, *33*). The present results agreed well with the literature. The reported water contents of the most comparable PVP models with average molecular weights of 40000 and similar equilibrium conditions as in the present study varied between 9.5 and 12.5 g of water/
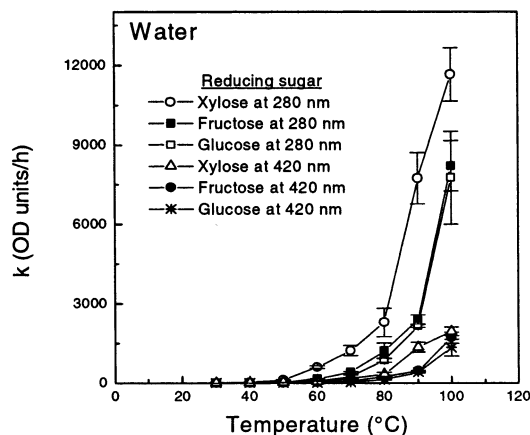
7036 *J. Agric. Food Chem.,* Vol. 50, No. 24, 2002

Lievonen et al.

Figure 1. Rate constants for the water models having different reducing sugars at 280 and 420 nm as a function of temperature. Error bars represent the 95% confidence limits.

100 g of solids, and the corresponding glass transition temperatures varied between 46 and 63 °C (*10, 16, 18, 33*). There are several reasons for the large variation between reported results. The method chosen for determining water content or glass transition temperature affects the results. Effective details are, for instance, heating rates during DSC scans, whether vacuum or nonevacuated desiccators are used, and whether the T_g value is taken as the onset or as the midpoint temperature of the glass transition temperature region (*11, 32*). In addition, every model system tends to have a unique composition of its own. Some authors (*33*) use dialyzed matrix materials, which raises the glass transition temperatures (*32*). On the other hand, even a small amount of NEB reactants may act as plasticizer by decreasing the T_g of the MD or PVP matrix (*31, 32*). Furthermore, the different compounds have different effects on the glass transition temperature. Buera et al. (*32*) reported that a 20% (w/w) addition of xylose into PVP decreased the T_g of the system 28 K, whereas the same addition of glucose changed the T_g only 12 K. This observation probably explains why the present food models having glucose as a reducing sugar had higher glass transition temperatures than those having xylose as a reducing sugar. Fructose has also a lower glass transition temperature than glucose, and therefore it works as a more effective plasticizer than glucose (*34*).

Nonenzymatic Browning. NEB rates, detected from optical density, were higher at 280 nm than at 420 nm and increased with increasing temperature (**Figure 1**). There was a lag period in optical densities of the water models, which was followed by a linear region (**Figure 2**). On the contrary, no lag phase was observed in the MD- and PVP-based models. Instead, optical densities first increased linearly and then leveled at a plateau as the reaction proceeded (**Figure 3**). The rate constants were determined from the linear region of the plots. The coefficients of determination of the reaction rate constant (R^2) varied from 0.803 to 0.995. The average 95% confidence limit of the rate constants was 16% of the actual rate constant value.

When effects of different sugars on browning rates were compared, the materials having xylose as a reducing sugar browned at the highest rate in every matrix material (**Figure 4**). The reaction rate difference between the models containing xylose and the models containing fructose or glucose was statistically significant ($p < 0.05$) at all temperatures and in every matrix at 280 nm. The same was true at 420 nm, with two exceptions (data not shown). The results agreed well with the literature, in which pentoses are generally considered to be more reactive than hexoses (*21*). It should also be noted that
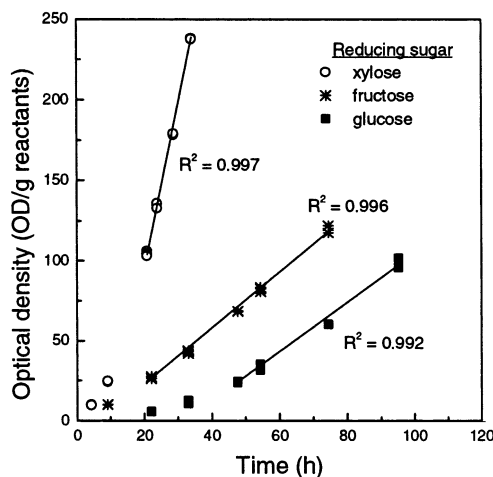

Figure 2. Optical density development of the water solutions having different reducing sugars at 280 nm versus storage time at 50 °C. Regression lines with coefficients of determination (R^2) are also shown.
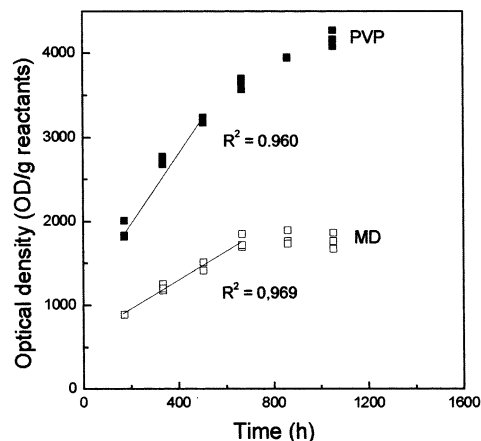

Figure 3. Optical density development of the MD- and PVP-based food models having xylose as a reducing sugar at 280 nm versus storage time at 50 °C. Regression lines with coefficients of determination (R^2) are also shown.

the molar concentration of xylose in the models was ~20% higher than that of fructose or glucose, which may have affected the results. The fructose-containing materials browned more quickly than the glucose-containing materials. The reaction rate difference between the fructose- or glucose-containing materials was not as straightforward as the difference between the materials containing xylose and the other sugars. The reaction rate difference between fructose and glucose was statistically significant ($p < 0.05$) at most temperature−matrix combinations. However, there were several situations (6 cases of 24 at 280 nm) in which statistical significance was not found. In addition, at the lowest temperature the MD- and PVP-based models containing glucose browned more quickly than those containing fructose did. In general, aldohexoses are considered to be more reactive than ketohexoses, because of their more electrophilic carbonyl groups (*22*). There are, however, large deviations between reactivities of single monosaccharides. For instance, fructose is considered to be a reactive sugar because it has a high concentration of acyclic form in aqueous solutions (*22, 27*). Despite these basic rules, there is no general agreement about the absolute reactivity order of fructose or glucose as NEB reactants in the literature. Contradictorily, both fructose (*27*) and glucose (*35*) have been reported to be the more reactive sugar. Differences in reaction conditions and methods used to

NEB Reaction: Effects of T_g and Sugars

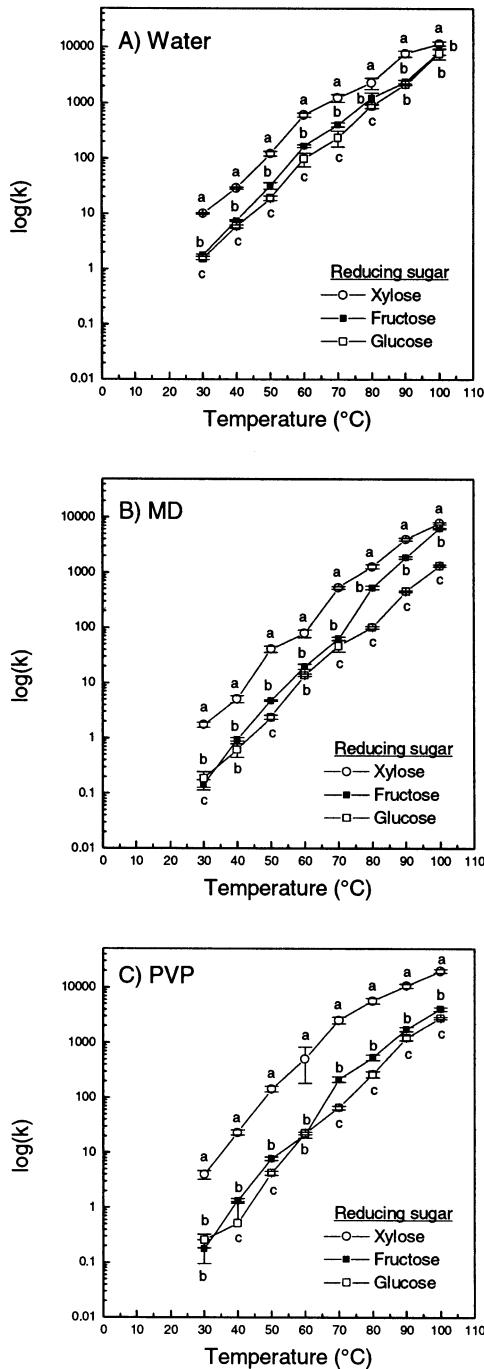*J. Agric. Food Chem.,* Vol. 50, No. 24, 2002 **7037**

Figure 4. Logarithms of rate constants with 95% confidence limits of nonenzymatic browning in (A) water, (B) maltodextrin, and (C) poly-(vinylpyrrolidone) models having different reducing sugars at 280 nm as a function of temperature. Statistically significant ($p < 0.05$) differences between NEB rates of the models at each temperature are indicated using different letters.
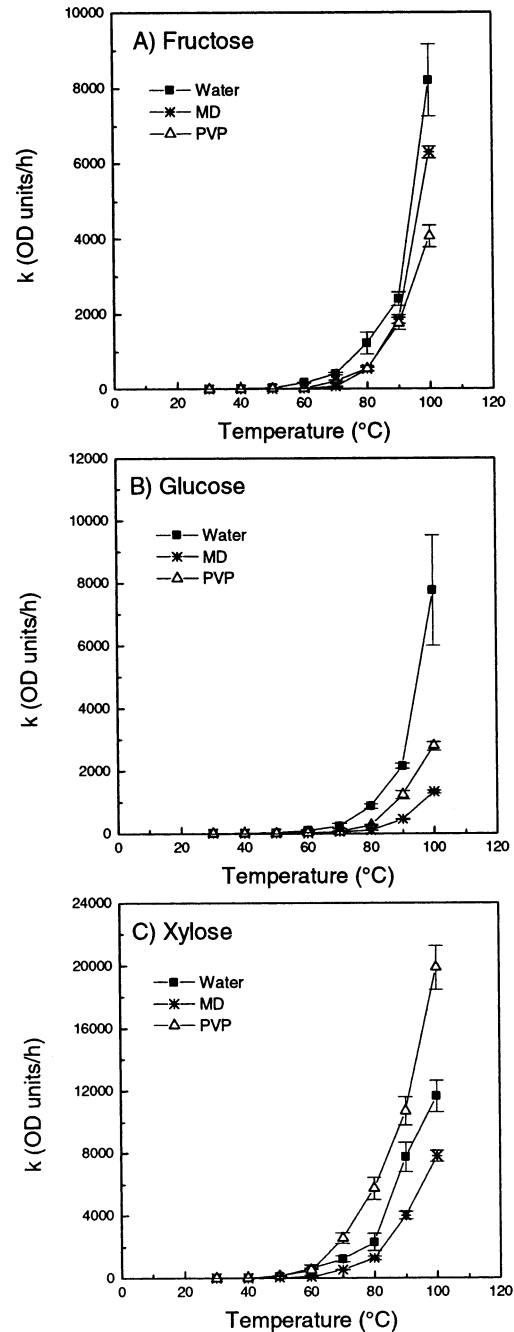

Figure 5. Comparison of rate constants with 95% confidence limits of different food models having (A) fructose, (B) glucose, and (C) xylose as a reducing sugar at 280 nm as a function of temperature.

follow the reaction are assumed to explain different results (*24, 29, 36, 37*). In the present study, xylose was found to be the most reactive sugar. The fructose-containing materials browned at the second fastest rate, but the rates were closer to the rates of the glucose-containing materials than the rates of xylose-containing materials.

When the browning behaviors of fructose, glucose, and xylose in the different matrices were compared, deviations in reaction rates were noticed (**Figure 5**). The NEB rate of both fructose and glucose was the highest in water. In solid food models, browning in fructose−PVP systems occurred more rapidly

below 80 °C, but then nonenzymatic browning was faster in the MD matrix. The influence of temperature on the NEB reaction in the fructose-containing MD-based models was distinctly greater than in the corresponding PVP-based models or in the water. The same kind of deep temperature dependence of nonenzymatic browning of fructose-containing materials was reported by Gögüs et al. (*30*) for grape juice and by Naranjo et al. (*36*), who studied the effects of reducing sugars on lysine loss in solid casein models. The PVP-based materials containing glucose and xylose browned more quickly than the corresponding MD matrices at every temperature, and no crossing over was seen. Xylose-containing materials browned in order PVP > water > MD. The higher browning rate in PVP than in water was unexpected, because diffusion of the reactants was planned to be freer in water than in PVP (*38*). The reaction rate

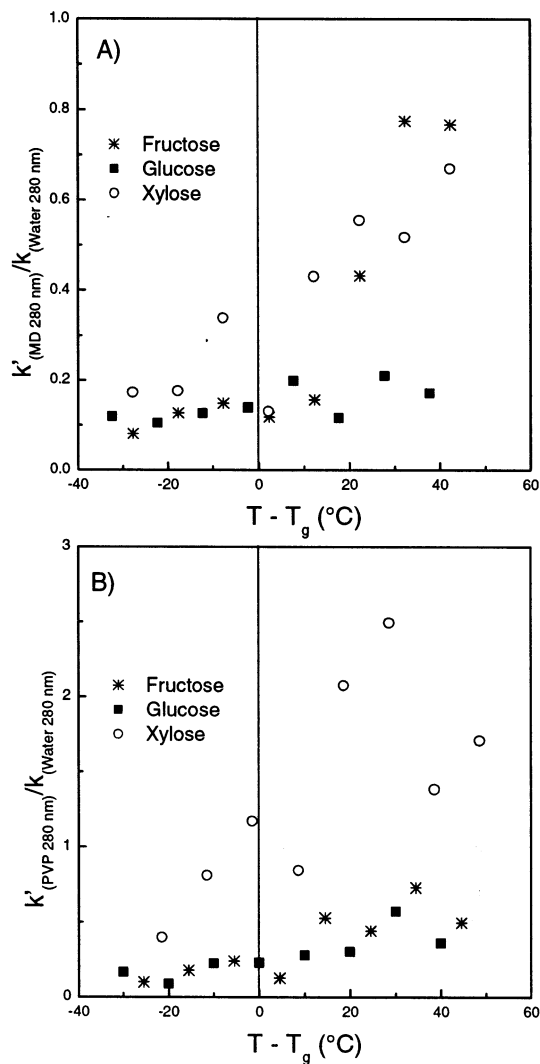**7038** *J. Agric. Food Chem.,* Vol. 50, No. 24, 2002

Lievonen et al.

Figure 6. Effect of temperature difference ($T - T_g$) on the ratio of the rate constants in (A) MD-based and (B) PVP-based models and the rates constant of the water model at 280 nm.

Table 2. Activation Energies (E_a) of Nonenzymatic Browning Obtained from Optical Density at 280 and 420 nm for the Water-, MD-, or PVP-Based Food Models with Different Reducing Sugars[a]

matrix	reducing sugar	OD at 280 nm		OD at 420 nm	
		$E_a \pm$ 95% CL (kJ/mol)	R^2	$E_a \pm$ 95% CL (kJ/mol)	R^2
water	fructose	112 ± 8	0.995	114 ± 8	0.995
	glucose	114 ± 5	0.998	119 ± 7	0.997
	xylose	98 ± 10	0.989	101 ± 10	0.990
MD	fructose	144 ± 8	0.997	154 ± 16	0.995
	glucose	121 ± 7	0.996	121 ± 13	0.989
	xylose	117 ± 11	0.992	123 ± 15	0.984
PVP	fructose	137 ± 12	0.993	139 ± 12	0.992
	glucose	132 ± 11	0.993	135 ± 16	0.986
	xylose	116 ± 16	0.981	117 ± 16	0.981

[a] The overall activation energies of the solid MD- and PVP-based models were calculated for each entire data set, ignoring apparent breaks in the Arrhenius plots. Coefficients of determination (R^2) for the Arrhenius plots are also shown.

difference between the MD- and PVP-based food models agreed with our previous result (*11*). Because both solid materials were equilibrated into the same initial water activity and they had the same reactant concentration in sorbed water, we have suggested that different reaction rates may result in a possible phase separation in PVP matrix. A separate water–reactant phase in PVP-based food models would drive reactants into closer proximity to each other than in plain water and thus explain the browning difference between water and PVP.

Effect of the Glass Transition on Nonenzymatic Browning. Effect of the glass transition on the NEB rate of the solid models was evaluated by comparing ratios of the rate constants for the reaction in the MD- and PVP-based models over the rate constant in the comparable water system as a function of $T - T_g$ (**Figure 6**). The solid systems were thought to be diffusion-limited, and the water systems were considered to be diffusionally as free as possible (*38*). The ratio was assumed to approach unity above the T_g, if decreasing viscosity of the solid model systems would increase the molecular mobility of the NEB reactants. As expected, the ratios approached zero below the T_g. In agreement with the previously reported results (*7, 9, 11, 12*), the glassy state did not completely prevent the nonenzymatic browning reactions, which occurred slowly even 30 °C below the T_g. The ratios generally stayed at low level below T_g

and increased significantly ~10–20 °C above the T_g. There was, however, an exception. The ratios of the xylose-containing PVP-based models started increasing immediately well below the T_g. The increasing ratio of the xylose-containing PVP-based model was exceptional, because the rate constant of the PVP-based model exceeded the rate constant of the xylose-containing water system even at temperatures in which the PVP polymers were in mobility-restricted glassy state. A possible separation of a water-reactant phase in the PVP-based model, in addition to the high reactivity of xylose as a reducing sugar, may be due to the observed overwhelming NEB rate.

Temperature Dependence. The temperature dependence of nonenzymatic browning in the present food models was analyzed using the Arrhenius equation, which is commonly used for modeling the temperature dependence of chemical reactions. As seen in **Table 2** all of the food models could be modeled using the Arrhenius equation. A visually observed break in the vicinity of the glass transition temperature was seen in five of six Arrhenius plots. The break was usually seen from 2 to 12 °C above the T_g. In the MD-based food model having glucose as a reducing sugar, the break was below the T_g of the model. In the corresponding PVP-based model, no break was seen in the vicinity of the glass transition. The results at 420 nm were similar (the data are not shown). Decreasing viscosity of an amorphous material at the glass transition temperature region affects the activation energy of diffusion-controlled chemical reactions, and breaks or step changes in the Arrhenius plots are expected (*6, 38, 39*). The shape of the present Arrhenius plots agreed well with the data reported by Karmas et al. (*7*) for comparable model systems. Water activities of the MD- and PVP-based models increased linearly with temperature (*40*) and were not likely to affect observed deviations in Arrhenius plots.

The activation energy of the NEB reaction was calculated for each model system studied. The calculated activation energies of the different models varied from 98 to 154 kJ/mol and were typical of NEB reactions (*3, 41*). As seen in **Table 2**, the activation energies of the water models with xylose were lower than those with fructose or glucose. Fructose in the water matrix did not show such a steep temperature dependence as in the maltodextrin matrix. The activation energies of the solid food models were calculated both for each entire data set (**Table 2**) and separately for regions above and below the visually observed break in Arrhenius plots. The calculated activation energies above and below the break, their 95% confidence limits,
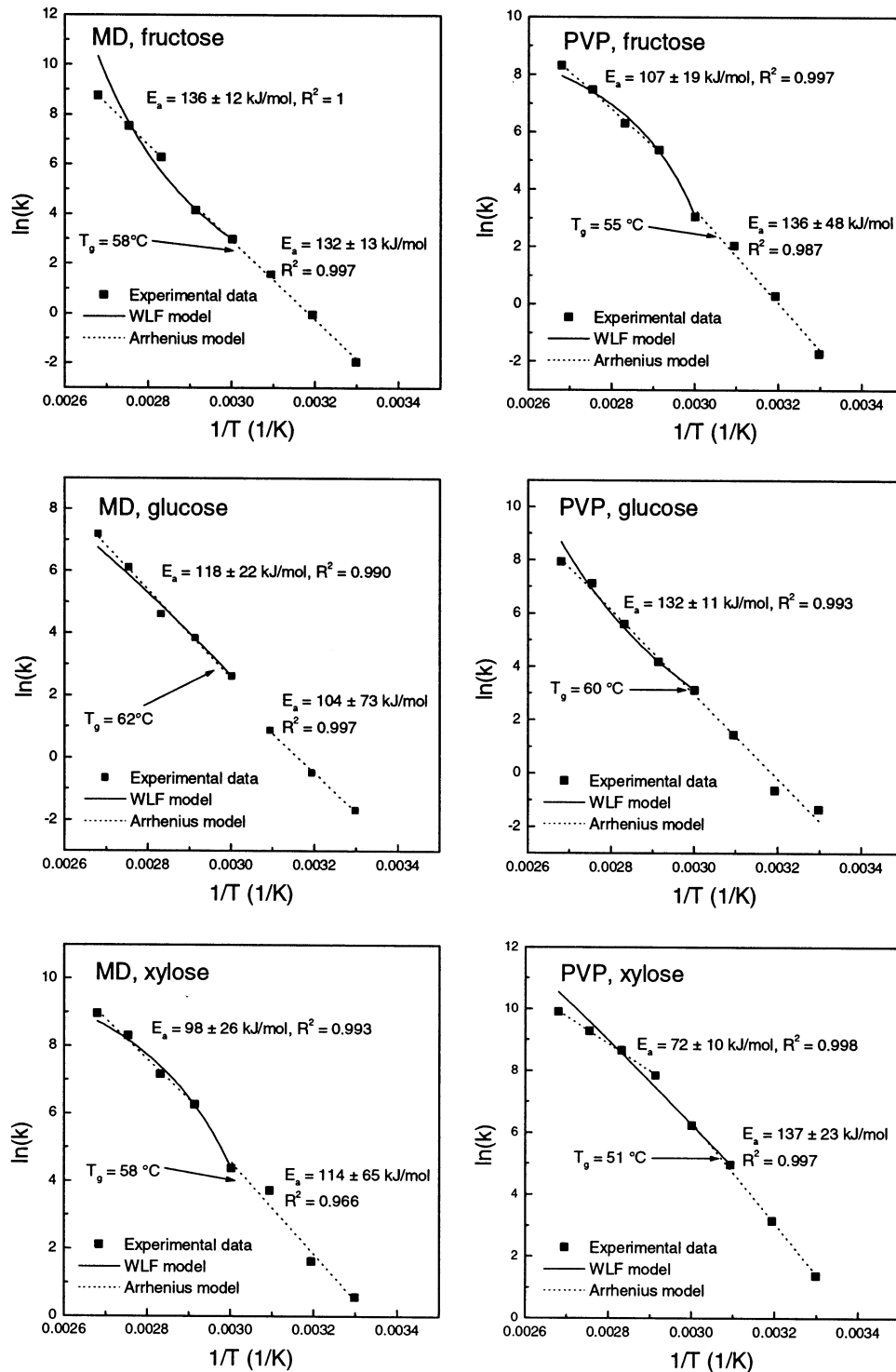
NEB Reaction: Effects of T_g and Sugars

J. Agric. Food Chem., Vol. 50, No. 24, 2002 **7039**

Figure 7. Arrhenius plots and WLF plots of the MD-based and PVP-based food models having different reducing sugars as determined from optical density at 280 nm. The breaks in Arrhenius plots, glass transition temperatures, activation energies (E_a) with their 95% confidence limits below and above the T_g, and coefficients of determination (R^2) are also shown.

and coefficients of determination for the browning reaction at 280 nm are shown in **Figure 7**. The results calculated at 420 nm agreed with the results at 280 nm (data not shown). The present results seemed to coincide with the results of Karmas et al. (*7*), but no definite conclusions on the difference between the activation energies below and above the break in Arrhenius plots were drawn because of fairly large 95% confidence limits. The small differences between the activation energies for the entire data sets and the activation energies below and above the glass transition indicated that changes in the physical state

of the matrix materials had only a moderate effect on nonenzymatic browning kinetics.

The Williams–Landel–Ferry equation (*42*) has been suggested as an alternative method for modeling the temperature dependence of viscosity-related chemical reactions above the glass transition. It has been assumed to be particularly suitable in temperatures immediately above the glass transition where deviations from Arrhenius-type temperature dependence are usually noticed (*6*, *39*). In fact, several authors have reported attempts to apply the WLF equation to the NEB data (*7*, *9*, *11*,

7040 *J. Agric. Food Chem.,* Vol. 50, No. 24, 2002

Lievonen et al.

Table 3. WLF Coefficients of the MD- and PVP-Based Food Models

matrix	reducing sugar	T_g	T_{ref}	280 nm			420 nm		
				C_1	C_2	R^2	C_1	C_2	R^2
MD	fructose	58	60	−4.48	−96.1	0.980	−5.40	−107.7	0.983
	glucose	62	60	9.58	172.9	0.976	7.62	120.4	0.978
	xylose	58	60	3.41	31.9	0.981	3.47	28.0	0.979
PVP	fructose	55	60	3.46	25.0	0.972	3.52	28.0	0.985
	glucose	60	60	−6.18	−142.4	0.995	−21.60	−410.3	0.994
	xylose	51	50	13.85	235.1	0.983	9.62	151.6	0.978

43−45). The best fits have been reached when system-dependent constants have been calculated instead of using the universal constants. In the present study, the WLF constants, C_1 and C_2 were calculated for each solid model using the linearized equation

$$\left[\log\frac{k_{ref}}{k}\right]^{-1} = \frac{-C_2}{C_1(T - T_{ref})} - \frac{1}{C_1} \tag{1}$$

where k_{ref} and k are reaction rate constants at reference temperature T_{ref} and temperature T, respectively, as suggested by Nelson and Labuza (39). The experimental temperature closest to the measured glass transition temperature was chosen as a reference temperature. Using the system-specific constants shown in **Table 3**, the temperature dependence of nonenzymatic browning of four of six solid food models could be modeled using the WLF equation. The negative WLF constant values for the MD−fructose and the PVP−glucose systems were not within the allowable range, and the WLF model did not fit the experimental data as shown in **Figure 7** (39, 46). Changing the reference temperature to 10 °C higher did not improve the general fit of the WLF equation. As shown in **Table 3** and **Figure 7**, the calculated constants and the suitability of the WLF model varied significantly even in systems with minor compositional differences. The result gave further evidence against the use of the universal WLF constants (39, 43). The choice of the reference temperature proved to be essential, because the best fit of the WLF equation was achieved with those two models in which the reference temperature happens to be the start point of the break in the Arrhenius plot. If the break were an indication of changing reaction mechanism, decreasing viscosity, and increasing molecular mobility, the WLF equation would be suitable for describing the changing temperature dependence of the NEB reaction.

As a summary, the results of the present study comparing fructose, glucose, and xylose as NEB reactants in three different matrices in the vicinity of the glass transition strongly suggest that there are variations in reactivity, not only between different sugars but also between the same sugar at different temperatures and in different matrices. As the results obtained with simplified food models are generalized into real foods, great care should be used.

LITERATURE CITED

(1) Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928−943.

(2) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631−653.

(3) Labuza, T. P.; Baisier, W. M. The kinetics of nonenzymatic browning. In *Physical Chemistry of Foods*; Schwartzberg, H. G., Hartel, R. W., Eds.; Dekker: New York, 1992; pp 595−649.

(4) Bhandari, B. R.; Howes, T. Implication of glass transition for the drying and stability of dried foods. *J. Food Eng.* **1999**, *40*, 71−79.

(5) Tolstoguzov, V. B. The importance of glassy biopolymer components in food. *Nahrung* **2000**, *44*, 76−84.

(6) Slade, L.; Levine, H. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 115−360.

(7) Karmas, R.; Buera, M. P.; Karel, M. Effect of glass transition on rates of nonenzymatic browning in food systems. *J. Agric. Food Chem.* **1992**, *40*, 873−879.

(8) Karmas, R.; Karel, M. The effect of glass transition on Maillard browning in food models. In *Maillard Reactions in Chemistry, Food, and Health*; Labuza, T. P., Reineccius, G. A., Monnier, V., O'Brien, J., Baynes, J., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1994; pp 182−187.

(9) Roos, Y. H.; Himberg, M.-J. Nonenzymatic browning behavior, as related to glass transition, of a food model at chilling temperatures. *J. Agric. Food Chem.* **1994**, *42*, 893−898.

(10) Buera, M. P.; Karel, M. Effect of physical changes on the rates of nonenzymic browning and related reactions. *Food Chem.* **1995**, *52*, 167−173.

(11) Lievonen, S. M.; Laaksonen, T. J.; Roos, Y. H. Glass transition and reaction rates: nonenzymatic browning in glassy and liquid systems. *J. Agric. Food Chem.* **1998**, *46*, 2778−2784.

(12) Schebor, C.; Buera, M. P.; Karel, M.; Chirife, J. Color formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems. *Food Chem.* **1999**, *65*, 427−432.

(13) Burin, L.; Jouppila, K.; Roos, Y.; Kansikas, J.; Buera, M. P. Color formation in dehydrated modified whey powder systems as affected by compression and T_g. *J. Agric. Food Chem.* **2000**, *48*, 5263−5268.

(14) Craig, I. D.; Parker, R.; Rigby, N. M.; Cairns, P.; Ring, S. Maillard reaction kinetics in model preservation systems in the vicinity of the glass transition: experiment and theory. *J. Agric. Food Chem.* **2001**, *49*, 4706−4712.

(15) Burin, L.; Buera, M. P.; Hough, G.; Chirife, J. Thermal resistance of β-galactosidase in dehydrated dairy model systems as affected by physical and chemical changes. *Food Chem.* **2002**, *76*, 423−430.

(16) Bell, L. N. Kinetics of non-enzymatic browning in amorphous solid systems: distinguishing the effects of water activity and the glass transition. *Food Res. Int.* **1996**, *28*, 591−597.

(17) Bell, L. N.; White, K. L.; Chen, Y.-H. Maillard reaction in glassy low-moisture solids as affected by buffer type and concentration. *J. Food Sci.* **1998**, *63*, 785−788.

(18) Bell, L. N.; Touma, D. E.; White, K. L.; Chen, Y.-H. Glycine loss and Maillard browning as related to the glass transition in a model food system. *J. Food Sci.* **1998**, *63*, 625−628.

(19) White, K. L.; Bell, L. N. Glucose loss and Maillard browning in solids as affected by porosity and collapse. *J. Food Sci.* **1999**, *64*, 1010−1014.

(20) O'Brien, J. Reaction chemistry of lactose: non-enzymatic degradation pathways and their significance in dairy products. In *Advanced Dairy Chemistry*; Fox, P. F., Ed.; Chapman and Hall: London, U.K., 1997; pp 155−231.

(21) Shallenberger, R. S.; Birch, G. G. *Sugar Chemistry*; AVI Publishing: Westport, CT, 1975; p 221.

(22) Bunn, H. F.; Higgins, P. J. Reaction of monosaccharides with proteins: possible evolutionary significance. *Science* **1981**, *213*, 222−224.

(23) Reyes, F. G. R.; Poocharoen, B.; Wrolstad, R. E. Maillard browning reaction of sugar-glycine model systems: changes in sugar concentration, color and appearance. *J. Food Sci.* **1982**, *47*, 1376−1377.

NEB Reaction: Effects of T_g and Sugars

J. Agric. Food Chem., Vol. 50, No. 24, 2002 **7041**

(24) Ashoor, S. H.; Zent, J. B. Maillard browning of common amino acids and sugars. *J. Food Sci.* **1984**, *49*, 1206−1207.

(25) Petriella, C.; Resnik, S. L.; Lozano, R. D.; Chirife, J. Kinetics of deteriorative reactions in model food systems of high water activity: color changes due to nonenzymatic browning. *J. Food Sci.* **1985**, *50*, 622−626.

(26) Buera, M. P.; Chirife, J.; Resnik, S.; Lozano, R. D. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to caramelization of various single sugars. *J. Food Sci.* **1987**, *52*, 1059−1062, 1073.

(27) Buera, M. P.; Chirife, J.; Resnik, S.; Wetzler, G. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. *J. Food Sci.* **1987**, *52*, 1063−1067.

(28) Lee, H. S.; Nagy, S. Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. *J. Food Process. Preserv.* **1990**, *14*, 171−178.

(29) Buera, M. P.; Resnik, S.; Petriella, C. Relative browning rates of fructose and glucose in high water activity systems as a function of amino compound concentration. *Lebensm. Wiss. Technol.* **1992**, *25*, 35−37.

(30) Gögüs, F.; Bozkurt, H.; Eren, S. Kinetics of Maillard reactions between the major sugars and amino acids of boiled grape juice. *Lebensm. Wiss. Technol.* **1998**, *31*, 196−200.

(31) Roos, Y. H.; Karel, M. Phase transitions of mixtures of amorphous polysaccharides and sugars. *Biotechnol. Prog.* **1991**, *7*, 49−53.

(32) Buera, M. P.; Levi, G.; Karel, M. Glass transition in poly-(vinylpyrrolidone): Effect of molecular weight and diluents. *Biotechnol. Prog.* **1992**, *8*, 144−148.

(33) Bell, L. N.; Hageman, M. J. Water activity and glass transition effects on solid state chemical reactions. *J. Food Qual.* **1995**, *18*, 141−147.

(34) Roos, Y. Melting and glass transition of low molecular weight carbohydrates. *Carbohydr. Res.* **1993**, *238*, 39−48.

(35) Baxter, J. H. Free amino acid stability in reducing sugar systems. *J. Food Sci.* **1995**, *60*, 405−408.

(36) Naranjo, G. B.; Malec, L. S.; Vigo, M. S. Reducing sugars effect on available lysine loss of casein by moderate heat treatment. *Food Chem.* **1998**, *62*, 309−313.

(37) Yeboah, F. K.; Alli, I.; Yaylayan, V. A. Reactivities of D-glucose and D-fructose during glycation of bovine serum albumin. *J. Agric. Food Chem.* **1999**, *47*, 3164−3172.

(38) Karel, M.; Buera, M. P. Glass transition and its potential effects on kinetics of condensation reactions and in particular on nonenzymatic browning. In *Maillard Reactions in Chemistry, Food, and Health*; Labuza, T. P., Reineccius, G. A., Monnier, V., O'Brien, J., Baynes, J., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1994; pp 164−169.

(39) Nelson, K. A.; Labuza, T. P. Water activity and food polymer science: implications of state on Arrhenius and WLF models in predicting shelf life. *J. Food Eng.* **1994**, *22*, 271−289.

(40) Lievonen, S. M.; Roos, Y. H. Water sorption of food models for studies of glass transition and reaction kinetics. *J. Food Sci.* **2002**, *67*, 1758−1766.

(41) van Boekel, M. A. J. S. Kinetic aspects of the Maillard reaction: a critical review. *Nahrung* **2001**, *45*, 150−159.

(42) Williams, M. L.; Landel, R. F.; Ferry, J. D. The temperature dependence of relaxation mechanisms in amorphous polymers and other glass-forming liquids. *J. Am. Chem. Soc.* **1955**, *77*, 3701−3707.

(43) Buera, M. P.; Karel, M. Application of the WLF equation to describe the combined effects of moisture and temperature on nonenzymatic browning rates in food systems. *J. Food Process. Preserv.* **1993**, *17*, 31−45.

(44) Roos, Y. H.; Jouppila, K.; Zielasko, B. Non-enzymatic browning-induced water plasticization. Glass transition temperature depression and reaction kinetics determination using DSC. *J. Therm. Anal.* **1996**, *47*, 1437−1450.

(45) Sá, M. M.; Sereno, A. M. The kinetics of browning measured during the storage of onion and strawberry. *Int. J. Food Sci. Technol.* **1999**, *34*, 343−349.

(46) Peleg, M. On the use of the WLF model in polymers and foods. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 59−66.