ELSEVIER

Food Chemistry 65 (1999) 427–432

Food Chemistry

Color formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems

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Received 15 September 1997; accepted 15 January 1998

Abstract

Polymeric matrix model systems (gelatinized starch, maltodextrin and polyvinylpyrrolidone (PVP) MW 40 000) containing a low concentration of added Maillard's reactants (glucose and L-lysine) were freeze dried, further desiccated (P_2O_5) to 'zero' % moisture, and heated for 48 h at 90°C. Under these conditions, all model systems were well below their measured glass transition temperature (T_g). Skim-milk powder samples (1.7 and 3.9% (dry basis) moisture) were also stored at 45 or 60°C, and browning was recorded. Browning was observed in the glassy polymeric matrices kept well below T_g and in the virtual absence of water. Browning developed in the milk powder samples stored at 60°C at a higher rate than at 45°C, even when both systems were in glassy conditions. This suggests that the T_g parameter should not be considered as an absolute threshold of stability with regard to non-enzymatic browning reactions. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

It is well known that the amorphous matrix of many dehydrated food polymers and other food components, such as sugars, may exist either as a very viscous glass or as a more liquid-like rubber. The change from the glassy to the rubbery state occurs as a second-order transition at a specific temperature for each material, which is known as the glass transition temperature, T_g. Water, the most common plasticizer in foods, decreases the glass transition temperature (Slade et al., 1989; Roos and Karel, 1990, 1991ab).

In recent years, mainly as a result of the pioneering work of Slade and Levine (Slade and Levine, 1987, 1991a,b, 1995), the concept of glassy and rubbery states was used to interpret the stability of low-moisture foods and biomaterials. This approach put emphasis on thermodynamically unstable states that achieve an apparent structural and chemical stability by virtue of their high viscosity. The formation of a glassy solid results in a significant arrest of translational molecular motion, and translational mobility and diffusion are considered to be virtually non-existent (Slade et al., 1989; Levine and Slade, 1992). Under these conditions (glassy state), chemical reactions that are dependent upon the diffusion of reactant molecules would be quite slow or would not occur at all, mainly well below T_g (Koster, 1990; Franks, 1993; Sun and Leopold, 1994). However, conflicts exist as to whether chemical reactions are governed by molecular mobility associated with the glass transition (Bell and Hageman, 1994). Karel (1993) summarized the theoretical reasons for the observation that chemical reaction rates may be unaffected by the glass transition.

The Maillard reaction is one of the reactions that might be affected by the glass transition phenomenon, since it can be, under certain circumstances, diffusion-limited (Buera and Karel, 1995). Non-enzymatic browning is an important cause of food quality loss, and it has also been reported as a cause of damage of pharmaceutical products, since a great majority of drugs have amine functionality, and they are mixed with reducing sugars and other carbonyl-containing pharmaceutical coadjuvants (Kumar and Banker, 1994).

The purpose of the present work was to ascertain whether or not non-enzymatic browning may occur in anhydrous glassy food models well below their glass transition temperature.

PII: S0308-8146(98)00041-7

2. Materials and methods

2.1. Model systems

Samples were prepared by making 20% w/w (in most cases) aqueous solutions (in phosphate buffer, 0.1 M, pH 7) containing a polymeric matrix material and the browning reactants, L-lysine (0.1% w/w) and glucose (0.1% w/w). L-lysine was obtained from Sigma Chemical Co. (St Louis, MO) and glucose from Merck (Darmstadt, Germany). These compounds were chosen because they are very reactive, and in all model systems, they were at very low concentrations (0.5% each on a dry basis), so that diffusional effects could be expected.

Matrix materials consisted of polyvinylpyrrolidone (PVP, molecular weight 40 000 or 10 000, obtained from Sigma); maltodextrin (DE 10.9, obtained from Refinaçoes de Milho, Sao Paulo, Brazil); wheat starch (obtained from Sigma) previously gelatinized by heating an aqueous suspension for 15 min at 80°C; and mixtures of maltodextrin (DE 10.9) plus gelatinized wheat starch at different ratios of maltodextrin:starch. One milliliter of each model solution was placed in a 4 ml vial and frozen using liquid air. A Stokes freeze-dryer (model 21; F. J. Stokes Co., Equipment Div., Pennsalt Chem., Corp., Philadelphia, PA) was used; it was operated at a condenser plate temperature of −40°C and a chamber pressure of less than 100 μmHg. After freeze-drying (48 h) the model systems were further dehydrated in vacuum desiccators over P_2O_5 for 1 week at 35°C. The samples were considered to be close to 'zero' % moisture content (actual moisture contents were determined to be below 1%).

2.2. Milk powder

Aliquots of approximately 1 g of skim-milk powder (Molico, Nestlé; moisture content 3.9%, fat 1.2%, lactose 51.7% and protein 35.3%, all on total weight basis) were placed in 4 ml vials, used as is or further desiccated in a vacuum desiccator over magnesium perchlorate (desiccant), and stored for 4 days at 35°C until the moisture content was reduced to 1.7% (dry basis).

2.3. Determination of moisture content

The moisture contents of different systems were determined by drying in a vacuum oven at 70°C for 48 h over a desiccant (magnesium perchlorate).

2.4. Heat treatment

After drying over P_2O_5, the vials containing the dried model systems were hermetically sealed and placed in forced air ovens operated at 90°C for 48 h; some model systems were also heated at 110°C for 48 h. The vials containing the skim-milk powder were hermetically sealed and placed in forced air ovens at 45 and 60°C, and at suitable intervals, two samples were removed for colorimetric measurements.

2.5. Determination of browning

2.5.1. Model systems

The heated, dried model systems were reconstituted with distilled water to their original concentration, and a photograph was immediately taken, for comparison with a control sample (unheated). Spectrophotometric measurements were performed in a Carl Zeiss Spekol UV–Vis spectrophotometer at four wavelengths, and the X, Y, Z tristimulus values calculated as reported by Huidobro and Simal (1985) according to the following equation:

$$X = T_{625} \times 0.42 + T_{550} \times 0.35 + T_{445} \times 0.21$$
$$Y = T_{625} \times 0.2 + T_{550} \times 0.63 + T_{495} \times 0.17Z$$

where T_{625}, T_{550}, T_{495} and T_{445} are the transmittance values at 625, 550, 495, and 445 nm, respectively.

2.5.2. Skim-milk powder

Colorimetric measurements were performed with a HunterLab 5100 Color Difference Meter (Hunter Associates Laboratory, USA) using a 1.6 cm diameter aperture and an illumination mode that illuminated the aperture area from the top of the sample. The CIE tristimulus values, X, Y, Z were obtained directly from the instrument. The average of five determinations was reported for each sample.

For the skim-milk powder, color functions were calculated for illuminant C and a secondary observer.

The browning index ($BR = 100(x - 0.31)/0.172$, where $x = X/(X + Y + Z)$), was found to be an adequate measure of non-enzymatic browning reactions (Buera and Resnik, 1990).

2.6. Glass transition temperatures of anhydrous polymeric models and skim-milk powder

Glass transition temperatures of the anhydrous polymeric sytems (desiccated for 1 week over P_2O_5 at 35°C) were determined by differential scanning calorimetry (DSC; onset values) using a Shimadzu Corp. DSC-50 (Kyoto, Japan) with a workstation TA-50, 50WSI at a scanning rate of 5°C min^{-1}. The instrument was calibrated with indium and the anhydrous samples were placed in hermetically sealed aluminum pans. Samples were scanned twice to eliminate hysteresis effects of thermal relaxations, typical of glass transitions (Roos and Karel, 1991a,b).

The T_g values obtained were, 170, 96 and 156°C, for maltodextrin DE 10.9, PVP-10 and PVP-40, respectively. These values are approximately 4–11% higher than those of 160, 92 and 140°C reported by Roos and

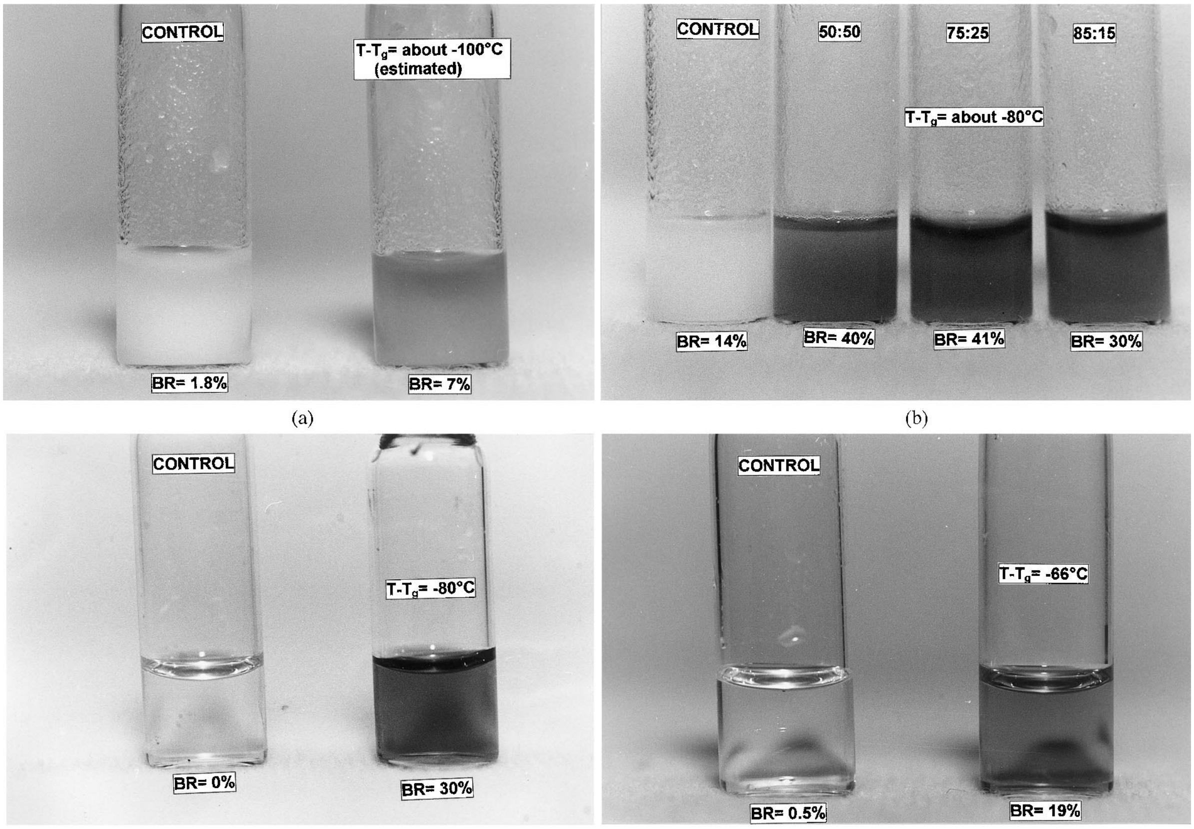
Karel (1991b) and Buera et al. (1992) for maltodextrin DE 10, PVP-10 and PVP-40, respectively. Perhaps, this was due to small differences in the amount of residual water left in the desiccated samples after P_2O_5 treatment. Bonelli et al. (1997) recently showed that the amount of residual moisture in freeze-dried sugars decreased by increasing the desiccation (P_2O_5) temperature from 25 to 45°C. Roos and Karel (1991b) and Buera et al. (1992) desiccated their samples at 20°C as compared with 35°C used in the present samples.

For anhydrous mixtures of maltodextrin DE 10.9 plus gelatinized wheat starch (50:50 and 85:15), measured T_g values were 176 and 173°C, respectively. We did not attempt to measure the glass transition temperature of anhydrous gelatinized starch, because literature reports indicated it would be very high and difficult to determine. Roos and Karel (1991a) modeled the T_g values reported by Zeleznak and Hoseney (1987) for native wheat starch, using the Gordon and Taylor (1952) equation, and obtained an extrapolated T_g value of 243°C for anhydrous starch.

Jouppila and Roos (1994) recently determined (by DSC) the effect of moisture content on the T_g of different milk powders, including skim-milk containing 57.9% lactose on a non-fat dry solids basis. The standard deviation of their T_g values for the anhydrous (1 week over P_2O_5) skim-milk powder was 2°C. On the basis of their results, we estimate that the T_g values for the present skim-milk powders were approximately 76 and 56°C for the samples with 1.7 and 3.9% (dry basis) moisture, respectively. A confirmatory DSC measurement of the T_g for the present anhydrous skim-milk powder was performed to be sure of being below T_g for the particular conditions of the present experiments. In this case, a Perkin Elmer DSC 7 at a scanning rate of $5°C\,min^{-1}$. was used, and an average value (duplicate) of 90.5°C was obtained, which compares well with that of 92°C reported by Jouppila and Roos (1994).

3. Results and discussion

Darkening (brown color formation) could easily be observed upon reconstitution of the heated (90°C, 48 h) anhydrous polymeric matrices containing browning reactants, as shown for gelatinized wheat starch (Fig. 1a), mixtures of maltodextrin plus gelatinized starch (Fig. 1b), maltodextrin (Fig. 1c) and PVP-40



(a) (b) (c) (d)

Fig. 1. Browning in heated (90°C, 48 h) polymeric model matrices freeze-dried and desiccated ('zero' % moisture), containing added Maillard reactants (see Materials and Methods). In all cases, photographs were taken immediately after reconstitution of the heated/dried matrices with water to their original weight. *BR* is the browning index (see Materials and Methods). (a) Gelatinized wheat starch; (b) maltodextrin (DE 10.9) plus gelatinized wheat starch (numbers indicate the maltodextrin:starch dry ratio); (c) maltodextrin DE 10.9; (d) PVP-40.

(Fig. 1d). The browning index (*BR*; see Materials and Methods) is also indicated. Some control systems (PVP plus L-lysine alone and PVP plus glucose alone) were also heated in identical solutions, and the results (not shown) suggested that the browning in the anhydrous polymeric matrices was mainly due to a Maillard-type reaction, although L-lysine alone also produced some color.

According to the measured anhydrous T_g values, all different model system matrices (starch, maltodextrin, mixtures maltodextrin plus gelatinized starch and PVP-40) were in the glassy state at 90°C, and well below their corresponding glass transition temperatures, since calculated/estimated ($T - T_g$) values for the various anhydrous model matrices were approximately −60 to −110°C. As expected, collapse was not observed upon heating (48 h) of any of the matrices, since glassy materials are stable to collapse (Slade and Levine, 1991b; Roos, 1995).

The present obervations suggest that browning does occur in glassy matrices kept well below T_g and also in the virtual absence of water. Various recent studies (Karmas et al., 1992; Nelson, 1993; Bell and Hageman, 1994; Bell, 1996) have indicated that some chemical reactions (non-enzymatic browning, aspartame degradation, ascorbic acid degradation), within amorphous polymeric systems, were still observed to occur at reasonable rates within glassy systems, and thus failed to show the stability to chemical changes often cited for glassy systems (Koster, 1990; Levine and Slade, 1992; Slade and Levine, 1995).

Fig. 2 shows the physical appearance of anhydrous model system matrices of PVP-10 and PVP-40 heated at 110°C for 48 h. Collapse is observed as a dramatic visible shrinkage of the PVP-10 matrix (measured T_g is 96°C), while that of PVP-40 (measured T_g is 156°C) shows no evidence of collapse. This is in agreement with the well-known fact that collapse is observed only within systems in the rubbery state (e.g. PVP-10) above T_g, but glassy systems (PVP-40) are stable to collapse (Slade and Levine, 1991b; Nelson, 1993; Roos, 1995). This agreement, although qualitative, because collapse is a time-dependent phenomenon, also corroborates the reliability of the T_g values used in the present work to describe the behavior of the PVP systems. It can be seen that browning increased in the collapsed matrix (PVP-10) as compared with the non-collapsed one (PVP-40). Karmas et al. (1992) also noted that browning in model systems increased simultaneously with the occurrence of a visually observed collapse, and Buera and Karel (1995) showed that browning rates were affected by the initial collapse of the stored samples.

Fig. 3 shows browning development during storage, at 45 or 60°C, of skim-milk powder adjusted to two different moisture contents (1.7 and 3.9% non-fat dry basis). Both samples were probably in the glassy state, as indicated by their estimated ($T - T_g$) values. It is noteworthy that after 6 months of storage at either 60 or 45°C, the milk powder samples remained free-flowing; e.g. stickiness/caking was not observed. The absence of these physical changes is assumed to corroborate (although qualitatively, because stickiness/caking are time-dependent phenomena) the glassy nature of these milk samples, since it is likely that stickiness/caking would have occurred if the samples had been in the rubbery state for such a long time (6 months) (Roos, 1995). As shown in Fig. 3, browning developed in the glassy sample stored at the higher temperature (60°C) while no browning was apparent in the glassy sample stored at the lower temperature (45°C). These results suggest that the term ($T - T_g$) does not completely account for the various effects of temperature, which are important to the browning reaction. Similar results were reported by Karmas (1994) for rates of non-enzymatic

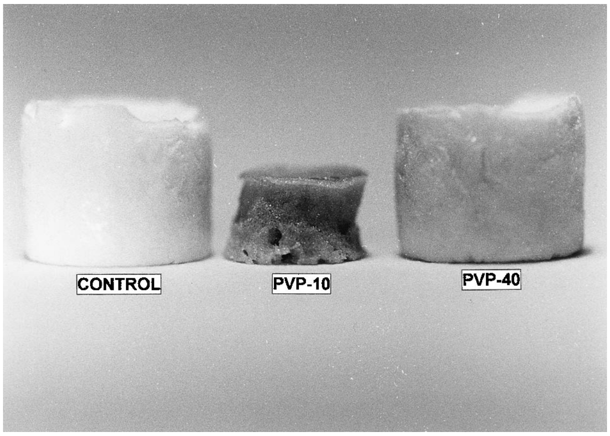


Fig. 2. Browning and collapse in heated (110°C, 48 h) polyvinylpyrrolidone (PVP) model matrices freeze-dried and desiccated ('zero' % moisture) containing added Maillard reactants (see Materials and Methods).

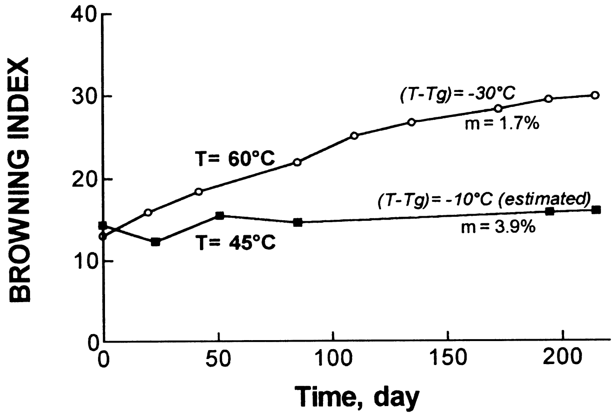


Fig. 3. Non-enzymatic browning in glassy skim-milk powder stored at 45 and 60°C. The T_g values were estimated from data reported by Jouppila and Roos (1994). m, moisture content on dry basis (d.b.); Browning index is defined in Materials and Methods.

browning in low-moisture (glassy/rubbery) model matrices containing browning reactants.

Many steps of the non-enzymatic browning reaction, including the initial reaction of the amino group and reducing sugar, are bimolecular condensations, where two molecular species must encounter each other for the reaction to occur. Mobility of the reactants within a food matrix may be necessary for a bimolecular reaction to be initiated. However, present results for polymeric model system matrices suggest that the relationship between non-enzymatic browning and the glass transition is not clear cut. It is usually assumed that the formation of a glassy solid results in greatly reduced molecular translational mobility, and translational diffusion is considered to be virtually non-existent by some workers (Slade et al., 1989; Levine and Slade, 1992). If these assumptions are correct, then in the glassy state, those chemical reactions that are dependent on diffusion of reactant molecules (i.e. diffusion-controlled reactions) would not be observed at temperatures well below T_g (Koster, 1990; Franks, 1993; Sun and Leopold, 1994). The underlying assumption of these assertions is that reactions approach the diffusion-control limit as the temperature drops below T_g.

The above assertions may not be valid, because of the following potential deviations from the underlying assumption. (1) It has been well documented that in synthetic and natural polymers, the translational diffusion coefficients, especially of small molecules, may remain at quite a high level in the glassy state (Comyns, 1985). This is also particularly true when the glassy matrix is relatively 'non-dense'. Hydrogels, and other polymeric matrices, may consist of structures that show glass-like behavior with respect to thermal and mechanical properties, without substantially hindering diffusion of molecules smaller than the distance between chain segments of the matrix. (2) The assumption of diffusional limitations is of consequence only when reactants are initially sufficiently separated so that translational diffusion is in fact necessary for the reaction to occur. In the case of dehydrated materials, especially those prepared by freeze-drying, but also by other methods of dehydration, the removal of water may create local concentrations of intermixed reactants, so that some extent of chemical reaction may occur, even in the absence of translational diffusion.

In the case of skim-milk powder in the glassy state, browning may have been facilitated because of the 'proximity' of reactant molecules (lactose and amino grups) brought about by their relatively high concentration. Flink (1974) also reported browning of freeze-dried non-fat milk desiccated to 0% RH and then heated to relatively high temperatures (90–110°C). He also observed that rates of browning increased sharply at a temperature of about 100°C; interestingly, this value is close to the anhydrous T_g for non-fat milk (92°C), as reported by Jouppila and Roos (1994).

The present results suggest that non-enzymatic browning in anhydrous amorphous polymeric matrices is not entirely governed by molecular mobility associated with the glass transition. However, another explanation could be based on the possibility that diffusion and rotational mobility in these solid polymers below T_g might still occur through pre-existing holes (pores), due to defects and porosity in the structure (Kovarskii et al., 1978; Hori et al., 1986). Ageing of glasses involves some relaxations of the material in the glassy state (Hay, 1993).

The 'solution' scheme (Duckworth, 1981) has also been proposed to explain the effect of water on non-enzymatic browning in dried foods. This theory states that the reactants must first be dissolved for browning to be observed. Obviously, in the present study, the reactants were able to produce browning when heated at 90°C, and apparently without being dissolved, since the amount of water was negligible ('zero' % moisture). Evidently, browning occurred under conditions in which the solution theory clearly would not apply. The assumption of cessation of reaction below T_g appears also not to be valid here. It seems that at high temperatures, in at least some food systems, it is the temperature *per se*, rather than the value of $(T - T_g)$ that determines the possibility of a significant extent of browning. Perhaps, $(T - T_g)$ would be a better predictor of browning, when the storage temperature of the system is somewhat closer to 'room' conditions. Roos and Himberg (1994) studied non-enzymatic browning at low temperatures (5, 10 and 20°C), in a food model composed of maltodextrin, lysine and xylose, and reported that the rate was very low below T_g, although the temperature *per se* was also a dominant parameter.

In summary, the overall picture that appears to emerge from the present browning experiments in glassy amorphous model matrices is one in which there may be significantly more molecular mobility (as reflected in color formation) in glassy materials than might previously have been expected. Such results would be in agreement with Simatos et al. (1995), who stated that T_g cannot be considered as an absolute threshold of stability. Rotational mobility, the ageing of the glassy material and the diffusion through pores or defects of the glasses may explain the occurrence of chemical reactions in the glassy state.

Acknowledgements

The authors acknowledge financial support from the Universidad de Buenos Aires (Secretaría de Ciencia y Técnica), the Agencia Nacional de Promoción Científica y Tecnológica (Proyecto 344, Préstamo BID 802/OC-AR), and from the International Foundation for Science (Sweden). The authors are also grateful to Dr.

Fabio Cukiernik (Dpto. de Química Inorgánica FCEyN, UBA) for providing the DSC equipment.

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