Journal of Thermal Analysis, Vol. 47 (1996) 1437-1450

NON-ENZYMATIC BROWNING-INDUCED WATER PLASTICIZATION

Glass transition temperature depression and reaction kinetics determination using DSC

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Abstract

An exotherm, observed in differential scanning calorimetry (DSC) scans of amorphous food materials above their glass transition temperature, T_g, may occur due to sugar crystallization, nonenzymatic browning, or both. In the present study, this exothermal phenomenon in initially anhydrous skim milk and lactose-hydrolyzed skim milk was considered to occur due to browning during isothermal holding at various temperatures above the initial T_g. The nonenzymatic, Maillard browning reaction produces water that in amorphous foods, may plasticize the material and reduce T_g. The assumption was that quantification of formation of water from the T_g depression, which should not be observed as a result of crystallization under anhydrous conditions, can be used to determine kinetics of the nonenzymatic browning reaction. The formation of water was found to be substantial, and the amount formed could be quantified from the T_g measured after isothermal treatment at various temperatures using DSC. The rate of water formation followed zero-order kinetics, and its temperature dependence well above T_g was Arrhenius-type. Although water plasticization of the material occurred during the reaction, and there was a dynamic change in the temperature difference $T-T_g$, the browning reaction was probably diffusion-controlled in anhydrous skim milk in the vicinity of the T_g of lactose. This could be observed from a significant increase in activation energy. The kinetics and temperature dependence of the Maillard reaction in skim milk and lactose-hydrolyzed skim milk were of similar type well above the initial T_g. The difference in temperature dependence in the T_g region of lactose, but above that of lactose-hydrolyzed skim milk, became significant, as the rate in skim milk, but not in lactose-hydrolyzed skim milk, became diffusion-controlled. The results showed that rates of diffusion-controlled reactions may follow the Williams-Landel-Ferry (WLF) equation, as kinetic restrictions become apparent within amorphous materials in reactions exhibiting high rates at the same temperature under non-diffusion-controlled conditions.

Keywords: browning, crystallization, glass transition, kinetics, milk powder, water

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0368–4466/96/ $ 5.00

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Introduction

Nonenzymatic browning reactions in biological and food materials are associated with the formation of brown discoloration, off-flavors and colors, loss of nutritional value, and textural changes [1, 2]. As classified by Hodge [1], there are three basic types of nonenzymatic browning reactions: 1) carbonyl-amino reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, and proteins, i.e., Maillard reaction; 2) caramelization; and 3) oxidative browning reactions, such as oxidation of ascorbic acid in citrus fruits. The Maillard-type browning reaction involves changes in flavor that are often advantageous in food preparation and the flavor industry, but may be detrimental in production and storage of low- and intermediate-moisture foods. Especially foods containing reducing sugars and proteins, e.g., dairy powders, are highly susceptible to the browning reaction, and the decreased solubility, discoloration, and loss of nutritional value of dairy powders, due to the Maillard reaction, have been well established [3].

A number of studies have related the rate of browning in low-moisture foods to water content and water activity. Labuza *et al.* [4] proposed that the rate of browning in carbonyl-containing systems increases with increasing water content, up to a maximum that depends on specific conditions. The acceleration of the reaction by water was accounted for by the increased availability and mobility of reactants. Duckworth [5] stated that mobilization of solutes occurs above the Brunauer-Emmett-Teller (BET)-monolayer water content, resulting in increased reaction rates, until full mobilization at the rate maximum is followed by dilution and decreased reaction rate. Simatos and Karel [6] pointed out that solubilizing or plasticizing water promotes the browning reaction. The low rates of the Maillard reaction at low water contents suggest that the reaction may become diffusion-controlled rather than limited by dissolution of reactant solutes in sorbed water, as proposed by Duckworth [5], in highly concentrated, viscous foods [6, 7]. It has also been recognized that most low- and intermediate-moisture foods are amorphous materials that exist either as solid glasses or supercooled liquids [6, 8]. Presumably, the rate of a chemical reaction is affected by the glass transition of the food matrix, as reactant and product mobility is required, although browning has been observed to occur also in food systems below the glass transition temperature, T_g [9, 10]. Moreover, other glass transition-controlled changes, e.g., crystallization of amorphous sugars, above T_g may affect reaction rates.

Milk powders often contain lactose in the amorphous form. Amorphous sugars are highly hygroscopic and they may sorb large amounts of water from surroundings, resulting in crystallization during storage above a critical, temperature-dependent relative humidity [8, 11]. Crystallization of lactose may occur above T_g, with a rate dependent on the temperature difference, $T–T_g$ [12].

The crystalline material may not sorb water, and in closed containers, nonhydrate water remains in the amorphous phase and causes a significant depression of the T_g. Therefore, crystallization may also increase the rate of diffusion-controlled reactions. An exotherm, occurring in differential scanning calorimetry (DSC) scans of dairy powders above T_g, has been attributed to instant crystallization of lactose [13, 14]. Roos and Karel [15] observed that the instant crystallization temperature, T_{cr}, of amorphous lactose decreased as T_g decreased similarly with increasing water content. However, an exotherm above T_g in materials, such as strawberries [16] and lactose-hydrolyzed skim milk [14], in which instant sugar crystallization is unlikely has also been observed. In anhydrous materials, these exotherms occurred well above 100°C, where the Maillard reaction is known to become more rapid.

The onset temperature of the exotherm for skim milk and lactose-hydrolyzed skim milk powders, observed by Jouppila and Roos [14], did not decrease with increasing water content at low water contents. Although the exotherm was considered to be due to sugar crystallization, it could also have been due to nonenzymatic browning [16]. In an anhydrous milk powder, sugar crystallization should not affect water content of the remaining amorphous material, unlike the Maillard reaction, which, even at the early stages of the reaction, is known to produce water [1, 2]. However, browning studies that have considered the enhancement of the reaction by water released from a crystallizing sugar [3, 9] have not reported on the effects of the water produced in the reaction, itself, on the browning rate. It would be expected that the water produced acts as a plasticizer in a material sealed in a container, such as a DSC pan or food package. The purpose of the present study was to determine the effect of the exothermal phenomenon, considered here to be due to browning during storage at various temperatures above T_g, on the T_g of initially anhydrous skim milk and lactose-hydrolyzed skim milk powders. The assumption was that quantification of water formation from the T_g depression, which should not be observed as a result of crystallization under anhydrous conditions, can be used to determine kinetics of the non-enzymatic browning reaction.

Materials and methods

Milk powders

Fresh skim milk (Valio Oy, Finland) and ultrapasteurized lactose-hydrolyzed skim milk (Valio Oy, Finland) were purchased from a local store. According to the manufacturer, the materials contained 4.9% w/w carbohydrates and 3.4% w/w protein. The total amount of solids was 9.14 and 9.49% w/w for skim milk and lactose-hydrolyzed skim milk, respectively [17]. In skim milk, the carbohydrate fraction was composed of lactose, and in the lactose-hydrolyzed skim milk, of 1:1 mixture of the lactose-hydrolysis products, galactose and glucose. Samples of the materials were prepared on petri dishes (glass), frozen at –80°C, and freeze-dried (Lyovac GT2, Amsco Finn-Aqua GmbH, Germany) at room temperature and pressure <0.1 mbar for at least 48 h. The freeze-dried materials were stored in evacuated vacuum desiccators over P_2O_5 for at least one week prior to the experiments, to ensure full dehydration of the materials.

DSC

The DSC used was a Mettler 4000 TA instrument equipped with a DSC 30 low-temperature measuring cell, personal computer, and TA72AT.2 thermal analysis software. The instrument was calibrated, over the temperature range used, with *n*-hexane (*m.p.* –95.0°C), water (*m.p.* 0.0°C), and indium (*m.p.* 156.6°C). The heat-flow calibration was based on the latent heat of melting of indium, $\Delta H_m=28.5$ J g^{-1}.

The dehydrated powders were prepared in aluminum, 40 μl DSC pans. The sample size was 2–8 mg. Filled sample pans were stored in evacuated desiccators over P_2O_5 for at least 24 h, before the pans were hermetically sealed after breaking the vacuum with dry air. Duplicate samples were loaded in the DSC cell at 25°C, and the temperature was increased at the maximum rate (about 100°C min^{-1}) to a predetermined holding temperature. The samples were kept isothermally at the holding temperature for various time periods (Fig. 2) and then cooled at the maximum cooling rate (about 30°C min^{-1}) to –50°C. After cooling, skim milk and lactose-hydrolyzed skim milk samples were scanned at 5°C min^{-1} from –50 to 140 and 110°C, respectively. An empty aluminum pan was used as a reference in all experiments. The DSC curves were analyzed for glass transition, and the average onset temperature of the T_g range for duplicate samples was taken as T_g. Examples of DSC curves for skim milk and lactose-hydrolyzed skim milk, obtained after isothermal holding at 120 and 110°C, respectively, and determination of T_g are shown in Fig. 1.

Water plasticization

Water plasticization was assumed to depress the T_g of the materials according to Eq. (1), as reported by Jouppila and Roos [14].

$$T_g = \frac{w_1 T_{g1} + k_G w_2 T_{g2}}{w_1 + k_G w_2} \tag{1}$$

$$w_2 = 1 - \frac{k_G(T_g - T_{g2})}{k_G(T_g - T_{g2}) + T_{g1} - T_g} \tag{2}$$

Equation (1), known as the Gordon-Taylor equation [18], defines T_g of a plasticized material as a function of T_g of the component compounds, T_{g1} and T_{g2}, their respective weight-fractions, w_1 and w_2, and a constant, k_G. The T_{g1} and k_G values were obtained from Jouppila and Roos [14]. The values reported for lactose (T_g=101°C and k_G=6.7) and lactose-hydrolyzed skim milk (T_g=49°C and k_G=8.0) were used for skim milk and lactose-hydrolyzed skim milk, respectively, to obtain the weight-fraction of water, w_2, using Eq. (2). A T_{g2} of –135°C [19] was used for amorphous water. However, it should be pointed out that although the onset T_g for lactose and skim-milk powder were the same, the transition in skim-milk powder occurred over a broader temperature range [14], probably due to the presence of proteins.

Browning rate and temperature dependence

The rate of formation of water in the Maillard reaction can be considered to follow Eq. (3), which states that the rate of water formation, via a bimolecular reaction, is defined by the concentrations of amino compounds, [A], and reducing sugars, [B], and the rate constant, *k*. Although the initial browning reaction, which produces brown-pigment precursors and water, is considered as a second-order reaction according to Eq. (3) [20], the approximate overall rate of the nonenzymatic browning reaction is likely to follow either zero- or first-order kinetics [2]. Therefore, a plot of either water content or logarithm of water content against time of the reaction at a constant temperature should show linearity, and *k* can be obtained from the slope of the straight line obtained. In the present study, zero-order kinetics gave a better fit, and Eq. (3) was reduced to Eq. (4), which was used in the kinetic analysis.

$$\frac{d[H_2O]}{dt} = k[A][B] \tag{3}$$

$$\frac{d[H_2O]}{dt} = k \tag{4}$$

The temperature dependence of rate constants of many reactions follows the Arrhenius equation (5), which states that the rate constant is a function of the pre- exponential factor, *A*, activation energy, E_a, gas constant, R= 8.31451 J(K mol)$^{-1}$, and absolute temperature, *T*. According to the Arrhenius equation, a plot of ln*k* *vs.* $1/T$ is linear with a slope equivalent to E_a/R.

$$k = Ae^{-(E_a/RT)} \tag{5}$$

In supercooled amorphous liquids, reactions may become diffusion-controlled, and therefore the plot of ln*k* *vs.* $1/T$ can exhibit nonlinearity. In such cases, the temperature dependence may deviate from the Arrhenius-type, with E_a changing as the material approaches the glassy state [9], or it may follow Williams-Landel-Ferry (WLF)-type behavior above T_g, according to Eq. (6) [21–23]. Equation (6) defines the ratio of the rate constant, k_s, at a reference temperature, T_s, to the rate constant, k, at temperature, T, as a function of the WLF constants, C_1 and C_2, and the temperature difference, $T–T_s$ [22, 23].

$$\ln\frac{k_s}{k}=\frac{-C_1(T-T_s)}{C_2+(T-T_s)} \tag{6}$$

$$\frac{1}{\ln\frac{k_s}{k}}=\frac{1}{-C_1}-\frac{C_2}{C_1(T-T_s)} \tag{7}$$

$$C_1'=\frac{C_1C_2}{C_2-\delta} \tag{8}$$

$$C_2'=C_2-\delta \tag{9}$$

We fitted the Arrhenius equation to the water-formation data of lactose-hydrolyzed skim milk and both the Arrhenius and WLF equations to the data obtained for skim milk. In the WLF equation, the rate constant at $T_s=110°C$ was taken as k_s, and the WLF constants were determined from a plot of $1/(\ln k_s/k)$ *vs.* $1/(T–T_s)$, according to Eq.(7). Equations (8) and (9), where C_1' and C_2' are the WLF constants at a reference temperature $T_s'=T_s–\delta$, were used to detemine the WLF constants when T_g was taken as the reference temperature, i.e., $T_g=T_s'$. Thus, the rate constant at T_g could be predicted and the WLF equation fitted to the data with T_g as the reference temperature, as described by Ferry [24] and Peleg [25].

Results and discussion

Browning and glass transition

Dehydrated skim milk and lactose-hydrolyzed skim milk are amorphous materials that show a clear glass transition in DSC curves when scanned over the T_g range [14]. The T_g decreases with increasing water content, as can be predicted from the Gordon-Taylor equation. Heating and isothermal holding of anhydrous skim milk and lactose-hydrolyzed skim milk at temperatures at and above 100°C resulted in significant discoloration and browning due to the Maillard reaction. The Maillard reaction caused a significant decrease in T_g (Fig. 1). As shown in Fig. 2, the T_g depression occurred rapidly with an increase in temperature, and it was accounted for by an increase in water content resulting from water produced in the reaction. It should be emphasized that the amount of water produced in the reaction was substantial, being several percent of the sample weight, and presumably caused significant plasticization. The Maillard reaction, especially in its later stages, also results in the formation of polymeric compounds [2] that are likely to increase T_g of the material. However, the possible increase in T_g was considered to be insignificant in comparison to the T_g depression caused by water plasticization, although the broadness of the transition seemed to increase with increasing isothermal holding time (Fig. 1). The increase in water content could also be detected by evaporation of water and weight loss during heating in nonhermetically sealed DSC pans. Such loss of water caused an increase in T_g, nearly to the initial T_g of the anhydrous milk powder.

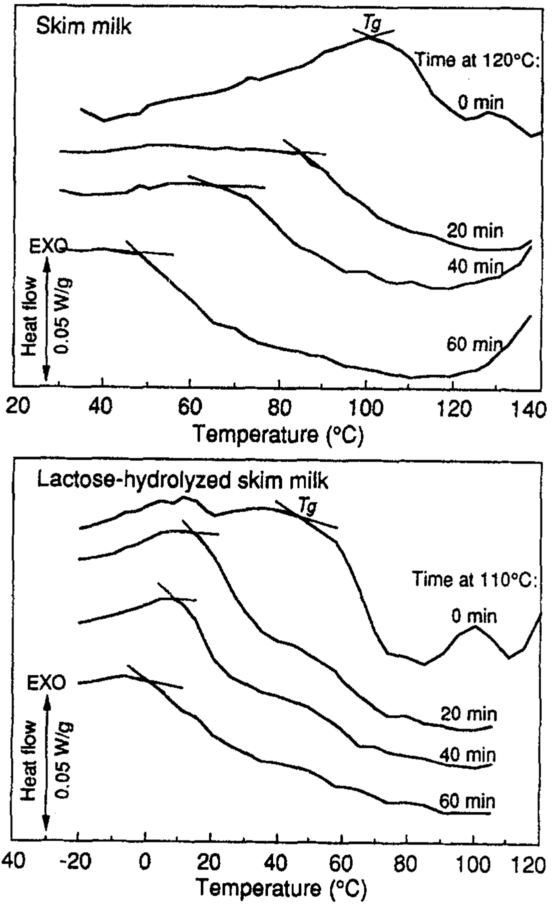

Fig. 1 Typical DSC curves obtained for skim milk and lactose-hydrolyzed skim milk, before and after isothermal holding at and above 100°C. The glass transition temperature, T_g, taken as the onset temperature of the glass transition temperature range, decreased with increasing holding time

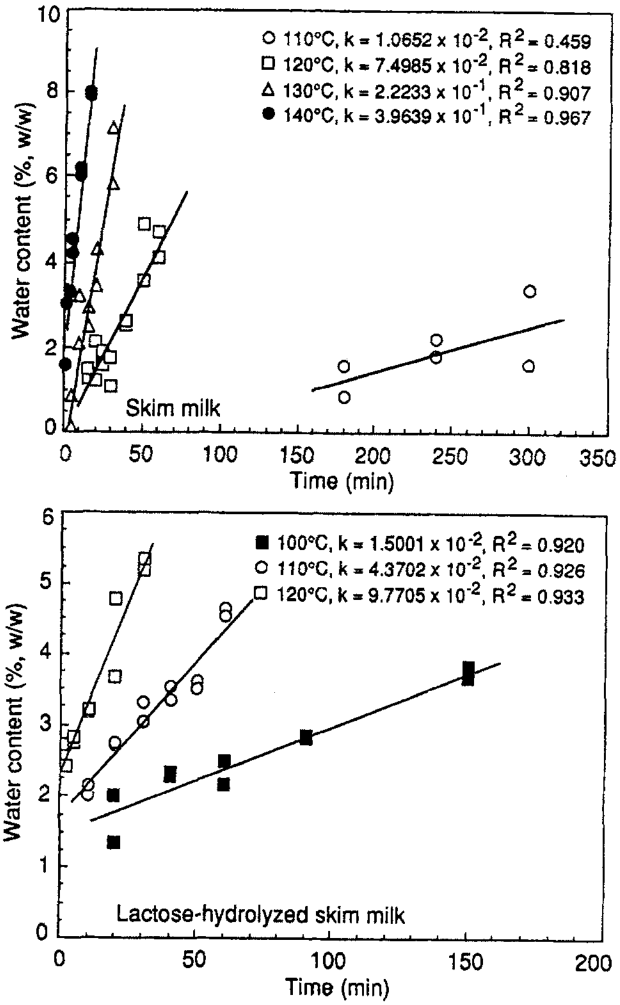

Fig. 2 Glass transition temperatures of initially anhydrous skim milk and lactose-hydrolyzed skim milk, after isothermal holding at 100 to 140°C for various times

Water content and reaction rate

The use of the Gordon-Taylor equation allowed prediction of the amount of water produced during isothermal holding of skim milk and lactose-hydrolyzed skim milk samples at various temperatures. The water content was predicted as a function of holding time from the T_g depression shown in Fig. 2. The estimated water contents of samples after holding at various temperatures were plotted against holding time (Fig. 3), which suggested that the increase in water content at each temperature was linear with time. Therefore, the formation of water was assumed to follow zero-order kinetics, which is often found to apply to the nonenzymatic browning reaction in food materials [10, 26]. However, it should be noted that the true reaction kinetics are more complicated, as described by Labuza and Baisier [2]. The apparent rate constants, *k*, at various temperatures for the increase in water content were obtained by linear regression and are shown in Fig. 4.

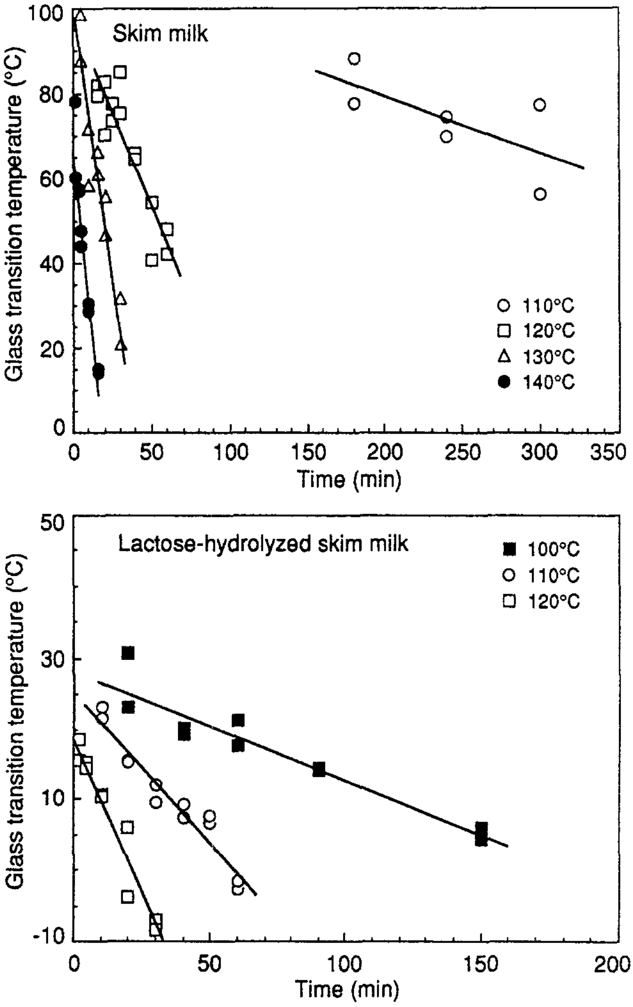

Fig. 3 Increase in water content, as determined from the T_g depression using the Gordon-Taylor equation, for skim milk and lactose-hydrolyzed skim milk as a function of isothermal holding time at 100 to 140°C. The apparent rate constants, *k*, of water formation in the Maillard reaction were obtained from the slopes of the regression lines shown

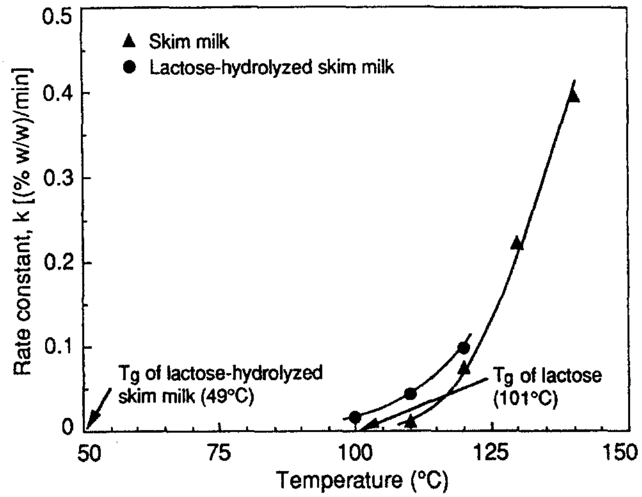

Fig. 4 Apparent rate constant, *k*, for water formation in the Maillard reaction for skim milk and lactose-hydrolyzed skim milk, as a function of the isothermal holding temperature

Physical state and temperature dependence

The nonenzymatic browning reaction is unlikely to occur at room temperature in anhydrous skim milk and lactose-hydrolyzed skim-milk powders. As discussed by Jouppila and Roos [14], the T_g values of the anhydrous materials are well above room temperature. However, fairly small amounts of water are sufficient to depress the T_g to below 24°C. These critical water contents were 7.1 and 2.0% w/w for skim milk and lactose-hydrolyzed skim milk, respectively [14]. These water contents correspond to those reported as extreme limits for safe storage of skim-milk powders at normal storage conditions [14, 27]. It is also known that the rate of the nonenzymatic browning reaction in dairy powders increases significantly, when the materials are stored above 44% relative humidity (RH) [3], which corresponds to steady-state water adsorption by amorphous lactose that is more than sufficient to depress T_g to below room temperature. Therefore, it may be assumed that the nonenzymatic browning reaction at low water contents may become diffusion-controlled and extremely slow at temperatures below T_g of the material [7, 8]. However, Karmas *et al.* [9] have pointed out that the rate of the reaction in various food systems is affected by T_g, but it is also dependent on water content, among other additional factors including sugar crystallization.

In the present study, the nonenzymatic browning kinetics were determined for skim milk and lactose-hydrolyzed skim milk at and above the initial $T-T_g$ of 9 and 51°C, respectively. As shown in Fig. 4, the apparent rate constant for formation of water as a product of the reaction was lower for skim milk than for lactose-hydrolyzed skim milk at all temperatures studied. However, the apparent rate constant for skim milk increased significantly above T_g of amorphous lactose and approached that found for lactose-hydrolyzed skim milk at 120°C. It is also interesting to note that the apparent rate constant for lactose-hydrolyzed skim milk was relatively low, even at 51°C above T_g. Obviously, in both systems, concentrations of the reducing sugars and reactive amino compounds were high. The main difference was in the sugar concentration, as the hydrolysis of one mole of the reducing sugar, lactose, produced two moles of reducing sugars, i.e., 1 mol galactose and 1 mol glucose. It is possible that the rate of the reaction was not significantly dependent on sugar concentration, as the reactive amino compounds and their concentration should have been the same in both systems. If the reactivity of the amino compounds in the skim-milk powders with glucose, galactose, and lactose was similar, the lower apparent rate constant for skim milk in the vicinity of the T_g of lactose can be explained by diffusion-controlled kinetics, due to the low $T-T_g$ of the system.

The Arrhenius plots showing the apparent rate constant of water formation in browning of skim milk and lactose-hydrolyzed skim milk are presented in Fig. 5. The temperature dependence of the reaction rate in skim milk was found to deviate from Arrhenius kinetics, as was similarly found for the browning rate in model systems by Karmas *et al.* [9]. Therefore, the equation was fitted to the experimental data in two parts, which suggested that there was a significant increase in activation energy, as the temperature approached the T_g of lactose. This agreed with the results of Karmas *et al.* [9], which showed that the activa-

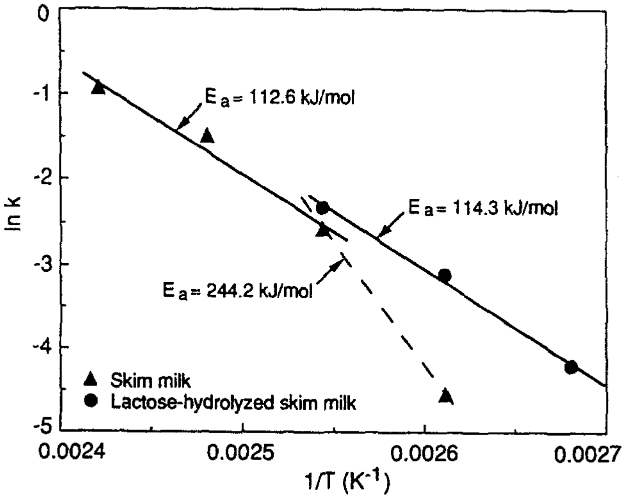

Fig. 5 Arrhenius plots for the rate of water formation in the Maillard reaction for skim milk and lactose-hydrolyzed skim milk. The activation energy, E_a, for the reaction in skim milk increased significantly, as the isothermal holding temperature approached the initial T_g of lactose

tion energy, close to T_g of food models, may have a value more than double that observed well above T_g. The activation energy for water formation in anhydrous skim-milk powder was found to be 244.2 kJ mol^{-1} and 112.6 kJ mol^{-1} at 9 to 19°C and 19 to 39°C above T_g of lactose, respectively. In general, the lower value corresponds to those reported for the nonenzymatic browning reaction occurring in dairy powders [28], but the higher value is well above those generally reported for nonenzymatic browning in foods [2]. Interestingly, the activation energy for water formation in lactose-hydrolyzed skim milk (114.3 kJ mol^{-1}) was almost the same as that in skim milk above 120°C. It may be assumed that above 120°C, water plasticization due to water formation occurred rapidly neglecting the diffusion-controlling effects of T_g in skim milk, although the initial T–T_g values were considerably different. This can be considered as a further indication of about equal reactivity of the amino compounds with galactose, glucose, and lactose, and that the temperature dependence of the Maillard reaction in skim-milk powders is similar, unless the reaction becomes diffusion-controlled in the vicinity of T_g.

The deviation of the rate of water formation in skim milk from Arrhenius kinetics at temperatures fairly close to T_g suggested that the reaction probably became diffusion-controlled. In particular, the facts that the rates of water formation in skim milk and lactose-hydrolyzed skim milk were almost the same well above T_g, but at significantly different initial T–T_g, and that the activation

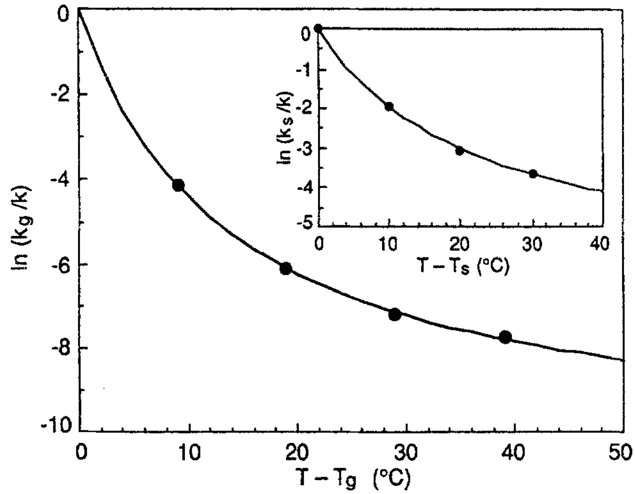

Fig. 6 WLF plot of the apparent rate constant, k, for water formation in skim milk, as a function of the initial temperature difference between the isothermal holding temperature, T, and the T_g of anhydrous lactose. The apparent rate constant, k_g, at T_g was obtained after fitting the WLF equation to the experimental data using another reference temperature, T_s, and an experimental value for the apparent rate constant, k_s, at that temperature, which gave the relationship shown in the inset figure

energies did not differ significantly were indicators of similar browning kinetics and temperature dependence of the reaction in the two materials. Since the lower reaction rate in skim milk at low $T-T_g$ was probably caused by restricted diffusion of the reactants, although there was a change in T_g during the reaction, the WLF equation was fitted to the data. The WLF constants, $C_1=6.47$ and $C_2=23.08$, were obtained from the relationship $1/(\ln k_s/k)=-0.155-3.568[1/(T-T_s)]$, with $R^2=0.999$. The constants, $C_1'=10.60$ and $C_2'=14.08$, were determined to apply when $T_s'=T_g$, which gave the rate constant, k_g, at T_g of 1.704×10^{-4} (% w/w)/min. These WLF constants differ from the "universal" constants, and the equation is probably valid over a temperature range much smaller than the common range from T_g to $T_g+100°C$ [21]. These differences can be accounted for, at least partly, by water formation and plasticization occurring within the material. As shown in Fig.6, the WLF equation gave a better fit than did the Arrhenius equation over the experimental temperature range. In a previous study, Nelson and Labuza [22] found that the WLF equation could be fitted to browning data above T_g, reported for a carbohydrate model system by Karmas *et al.* [9]. It may be assumed that the rate constant for a diffusion-controlled reaction may follow WLF kinetics above T_g, provided that the reaction would occur with a significantly higher rate at the same temperature in a non-diffusion-controlled situation. We conclude that under conditions of the Maillard reaction applicable to the present study, the reaction in lactose-hydrolyzed skim milk was not diffusion-controlled, while the reaction in skim milk was diffusion-controlled, due to the much higher initial T_g of lactose. Therefore, the temperature dependence of the apparent rate constant in skim milk probably followed WLF kinetics. However, at normal food-storage temperatures, the nonenzymatic browning reaction rates are often much lower, and the observation of diffusion-controlled situations under hermetic conditions may become more difficult, due to the rapid depression of T_g and concurrent, dynamic increase in diffusion.

Conclusions

The nonenzymatic browning occurring during heating of amorphous skim-milk powders is an exothermal phenomenon that affects the T_g of the material, due to formation of water and consequent plasticization. The formation of water can be quantified from the T_g depression, which allows kinetic analysis of the reaction. The rate of water formation in skim-milk powders followed zero-order kinetics, and its temperature dependence well above T_g was Arrhenius-type. Although water plasticization occurred during the reaction, the reaction was probably diffusion-controlled in anhydrous skim milk in the vicinity of the T_g of lactose. This could be observed from a significant change in activation energy. The temperature dependence close to T_g may follow WLF rather than Arrhenius kinetics, as a result of restricted diffusion. The kinetics and temperature dependence of the Maillard reaction were similar for skim milk and lactose-hydrolyzed skim milk. The difference in temperature dependence, below T_g of lactose but above that of lactose-hydrolyzed skim milk, becomes significant as the rate in skim milk, but not in lactose-hydrolyzed skim milk, becomes diffusion-controlled. The results showed that diffusion-controlled reaction kinetics may become apparent within amorphous materials, for reactions exhibiting higher rates at the same temperature under non-diffusion-controlled conditions.

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