Nonenzymatic Browning Reaction of Essential Amino Acids: Effect of pH on Caramelization and Maillard Reaction Kinetics

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The interaction between glucose and essential amino acids at 100 °C at pH values ranging from 4.0 to 12.0 was investigated by monitoring the disappearance of glucose and amino acids as well as the appearance of brown color. Lysine was the most strongly destroyed amino acid, followed by threonine which induced very little additional browning as compared with that undergone by glucose. Around neutrality, the nonenzymatic browning followed pseudo-zero-order kinetics after a lag time, while the glucose and amino acid losses did not follow first-order kinetics at any of the pH values tested. Glucose was more strongly destroyed than all of the essential amino acids, the losses of which are really small at pH values lower than 9.0. However, glucose was less susceptible to thermal degradation in the presence of amino acids, especially at pH 8.0 with threonine and at pH 10.0 with lysine. The contribution of the caramelization reaction to the overall nonenzymatic browning above neutrality should lead to an overestimation of the Maillard reaction in foods.

Keywords: *Maillard reaction; heat treatment; amino acid reactivity; caramelization*

INTRODUCTION

Several model systems have been used to study the nonenzymatic browning reaction. The formation of dark-colored products in glucose−lysine solutions at pH values ranging between 3.0 and 9.0 has been reported to be mainly due to the alkaline degradation of the sugar and secondarily to the interaction between lysine and glucose (Willits et al., 1958). Moreover, the effect of lysine cannot be attributed to its basic characteristics, since the two other basic amino acids, histidine and arginine, did not significantly affect the amount of color in heated glucose solutions. In 1979, Piloty and Baltes reported that the three basic amino acids, as well as the hydroxy amino acids serine and threonine, had the highest reactivity with α-dicarbonyl compounds, while the nonpolar and acidic amino acids had the lowest reactivity. In their study on the extent of Maillard product formation in parenteral alimentary solutions containing 25% glucose and 4.25% [^{14}C]-labeled amino acids, Fry and Stegink (1982) showed that proline and other hydrophobic amino acids were less reactive than the other amino acids except tryptophan and hydroxy amino acids, which reacted most rapidly. After heating each of the L-amino acids naturally present in proteins at 121 °C for 10 min in solution containing a common reducing sugar (D-ribose, D-glucose, D-fructose, α-lactose, or sucrose) at pH values ranging from 6.0 to 12.0, Ashoor and Zent (1984) observed that they fell into three categories, depending on the amount of color measured at 420 nm. The first group, which gave the most intense Maillard browning, included lysine, glycine, tryptophan, and tyrosine, while alanine, valine, leucine, isoleucine, phenylalanine, proline, methionine, asparagine, and glutamine were intermediate browning-producing amino acids. Seven amino acids remain, namely, the two basic amino acids histidine and arginine, the two acidic ones, aspartic acid and glutamic acid, the two hydroxy amino acids, serine and threonine, and the thiol-containing amino acid, cysteine, all of which belong to the low browning-producing group. More recently Hwang et al. (1995a,b) have determined the contribution of a number of amino acids to the formation of some volatile Maillard reaction products (pyrazines, pyridines, pyrroles, and oxazoles) from a ^{15}N-glycine−glucose mixture. While asparagine was found to have the highest contribution to the production of pyrazines and oxazoles, aspartic acid and lysine were mostly involved in the formation of pyridines and pyrroles. On the contrary, the contribution of glutamine and glutamic acid to the formation of these compounds was very low.

A number of kinetic studies have also been carried out on the Maillard reaction. Fox et al. (1983) and Petriella et al. (1985) investigated the velocity of Maillard browning in liquid model systems with high water activity containing glucose and alanine when heated at 85 and 95 °C and glucose and glycine when heated at 35, 45, and 55 °C, respectively. The browning rates at pH values below neutrality and water activity above 0.9 were all consistent with a zero-order kinetic reaction, except for the runs at 35 °C, where the reaction order was between 0 and 1, and increased with increasing pH and temperature values in the reaction medium. A fractional order reaction has also been reported to take place in nonenzymatic brown pigment formation in aqueous solutions containing glycine and a sugar (glucose, xylose, lactose, maltose, or sucrose) when heated to between 45 and 65 °C at pH 4.0−6.0 (Buera et al., 1987). In their study on the nonenzymic browning of aqueous model systems consisting of glucose and glycine at three different solid concentrations and three glucose−glycine molar ratios, which were heat-treated at 70, 80, and 90 °C, Lerici et al. (1990) established that, after

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various lag times, carbon dioxide formation via the Strecker degradation, release of volatiles at various steps in the Maillard browning reaction, and increase in the absorbance at 294 nm could be described by a zero-order kinetics.

Valuable kinetic information on the effect of initial reactant molar ratio on the Maillard browning reaction was provided by Baisier and Labuza (1992) in their study on the storage of liquid model systems containing varying concentrations of glucose and glycine at 37 °C and at pH 7.0. When the concentrations of both initial reactants were varied, the glycine loss initially followed a pseudo-first-order kinetics, and then a no-loss period occurred when about 25% of the amino acid was lost, while the glucose loss definitely followed a first-order kinetics. Moreover, whatever the glucose−glycine concentration ratio, the rate of brown pigment formation followed pseudo-zero-order kinetics after an induction period. Regarding the effect of the pH on the Maillard reaction, it is generally agreed that increasing the pH values of the reaction medium enhances the reaction (Fox et al., 1983; Ashoor and Zent, 1984; Petriella et al., 1985), although an opposite conclusion was drawn by O'Beirne (1986) in the case of an apple juice concentrate which was stored for forty weeks at 37 °C at pH values ranging from 2.0 to 4.0.

Although people have been heating sugars and amino acids at different pHs and temperatures for a long time, there is no precise study, as far as we know, which clearly distinguishes the respective contribution of the Maillard and caramelization reactions on both the development of browning and the loss of reactants, especially under alkaline conditions where both reactions are highly enhanced. The aim of the present study therefore was to determine the Maillard reactivity of the essential amino acids with regard to their destruction and involvement in brown pigment formation. Special attention was given to the contribution of glucose caramelization to the overall nonenzymatic browning. For this purpose, a solution of glucose alone or in the presence of a given essential amino acid at an equimolar concentration was heated to 100 °C at pH values ranging from 4.0 to 12.0.

EXPERIMENTAL SECTION

Materials. L-Lysine, L-methionine, L-tryptophan, L-threonine, L-valine, L-isoleucine, L-leucine, L-phenylalanine, L-α-amino-*n*-butyric acid, D-glucose, and triethylamine (TEA) were purchased from Sigma Chemical Co. (St. Louis, MO). Phenylisothiocyanate (PITC) in 1 mL vacuum-sealed vials and the standard mixture of amino acids (2.5 μmol/mL each in 0.1 N HCl) were supplied by Pierce Chemical Co (St. Louis, Mo). HPLC grade acetonitrile from SDS (Peypin, France) and water purified with an Elgastat Prima 1-3 and Maxima system (Elga, UK) were used. All of the other chemicals were of the purest grade available commercially.

Heating Procedure. An equimolar (0.05 M) mixture of glucose and each of the essential amino acids (3 mL) was heated in a 0.05 M sodium phosphate buffer at pH 7.5 in screw-sealed tubes for various periods of time. After 5, 15, 30, 60, 90, and 120 min, the tubes were removed and immediately cooled in ice. Part of the heated solution was directly used to make browning measurements, and the rest was stored at −20 °C for glucose and amino acid loss determinations. Lysine, methionine, and threonine were selected for studying effects of the pH on the Maillard browning reactions, and as in the previous case, the same equimolar concentrations of the amino acid and glucose were heated for 5, 15, 30, 60, 90, and 120 min. All of the browning intensity determinations and extents of reactant losses were done at least in triplicate and the corresponding mean values used for the kinetic plots. The following buffers were used: 0.05 M sodium acetate adjusted to pH 4.0 with 1 M acetic acid, 0.05 M sodium phosphate adjusted at pH 6.0, 7.0, and 8.0 using either monobasic or dibasic sodium phosphate, 0.05 M Tris-carbonate adjusted to pH 9.0 with 1 M hydrochloric acid, 0.05 M sodium carbonate bicarbonate adjusted to pH 10.0 and pH 11.0 using either sodium carbonate or sodium bicarbonate, and 0.05 M sodium bicarbonate adjusted to pH 12.0 with 1 M sodium hydroxide. All of the buffer solutions and the HPLC solvants, except acetonitrile, were filtered through 0.45 μm Millipore HA filters before use.

Browning Measurements. The browning intensity of the cooled amino acid-containing glucose solutions was measured at 420 nm using a Beckman model DU 640 spectrophotometer (Beckman Instruments, Irwin, CA). When necessary, apropriate dilutions were made in order to have an optical density of less than 1.5.

Glucose Loss. The remaining nondegraded glucose was monitored using HPLC Waters Associates equipment connected to a DDL 21 light-scattering detector (Eurosep). Degassed water−acetonitrile mixture (20:80, v:v) was delivered by a Waters Associates model 510 pump under isocratic conditions at a 1.5 mL min^{-1} flow rate. A 10-fold diluted heated glucose solution (20 μL) was injected using a WISP 710 autosampler (Waters Associates) in the solvant running through the Nucleosil-NH2-Macherey Nagel column (250 × 4 mm, 5 μm particle size) equipped with a guard column (11 × 4 mm, 5 μm particle size). The light-scattering detection of glucose was achieved at an evaporation temperature of 60 °C, under 2.5 bar of air pressure. The integration of the area under the eluted peak was performed with an Olivetti Pentium P 75i integrator using the Borwin chromatography Software program with Windows 3.11, based on a 10−200 nmol range glucose calibration table. The detector response, for all of the heating periods used, was checked by concomitant monitoring of 100 nmol of sucrose.

Amino Acid Loss. The unreacted amino acids were monitored by performing reverse-phase high-performance liquid chromatography after precolumn derivatization with phenylisothiocyanate as described by Bidlingmeyer et al. (1984). The 250-fold diluted amino acid solutions (10 μL) were first dried under vacuum in the presence of 2 nmol of α-amino butyric acid. The dried mixture was then incubated in 20 μL of an ethanol−water mixture (7:1, v:v) containing TEA and PITC (1:1, v:v) for 20 min and dried again in a vacuum. The resulting amino acid samples were further diluted in 200 μL of a 5 mM phosphate buffer containing 5% acetonitrile (v:v), and 20 μL was analyzed using a Pico Tag column (3.9 × 300 mm, 5 μm particle size, Waters Associates). The elution was performed with a 10 min convex gradient from 5% B (60% acetonitrile in water) and 95% A (0.14 M sodium acetate, pH 6.4, containing 0.5 mL of TEA/L) to 54% B and 46% A, running at 1.0 mL min^{-1}. The eluted amino acids were detected at 254 nm using a model 486 variable wavelength detector (Waters), and the resulting peaks were integrated as indicated in the case of glucose.

RESULTS AND DISCUSSION

Browning at pH 7.5. The complexity of nonenzymatic browning reactions is known to be at least partly due to the sugar caramelization processes (Greenshields, 1973; Myers and Howell, 1992). It therefore seemed to be of interest to determine the contribution of caramelization and that resulting from the interaction between amino acids and reducing sugars to the overall nonenzymatic browning. As shown in Figure 1, the browning of a glucose solution at 100 °C definitely increased in the presence of any of the essential amino acids except threonine, whereas none of these amino acids, with the exception of tryptophan, gave rise to any

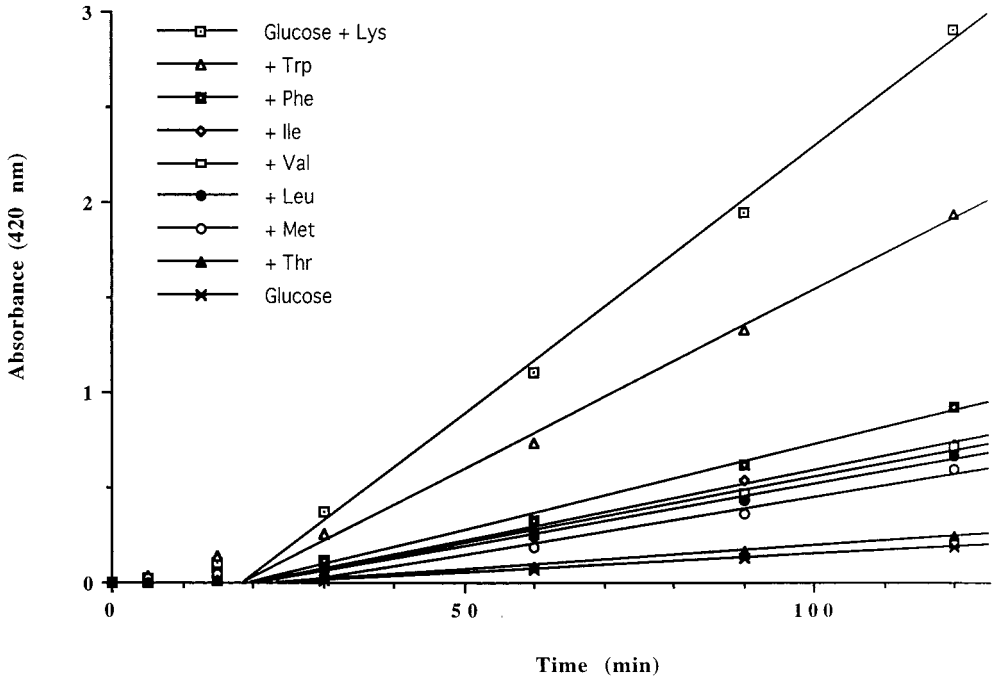

Figure 1. Brown color development in aqueous solutions containing glucose alone or in the presence of an essential amino acid when heated to 100 °C at pH 7.5 as a function of time.

Table 1. Lag Time of the Nonenzymic Browning Reaction of a Glucose Solution Alone and in the Presence of Various Amino Acids

amino acid	lag time (min)	regression coefficient (R^2)
	24.2	0.999
Lys	18.8	0.997
Trp	18.5	0.997
Phe	18.7	0.995
Ile	20.7	0.994
Val	20.6	0.996
Leu	21.2	0.996
Met	27.0	0.991
Thr	24.4	0.994

browning in the absence of glucose. Lysine and to a lesser extent tryptophan were the most reactive while phenylalanine, isoleucine, valine, leucine, methionine, and threonine were distinctly less reactive and exhibited decreasing reactivities. It is therefore rather difficult to determine any direct relationships between the type of amino acid side chain and its ability to induce browning. The basic side chain does not necessarily give rise to much browning, as it was observed in Ashoor and Zent's studies (1984) where lysine and arginine were found to belong to the amino acid groups which induce the highest and lowest nonenzymatic browning, respectively. It seems nevertheless that acidic, SH-containing, and hydroxy amino acids contribute very little to the nonenzymatic browning reaction (Nafisi and Markakis, 1982; Ashoor and Zent, 1984; Friedman and Molnar-Perl, 1990).

As shown in Table 1, the lag time of the browning reaction, which was determined by extrapolating the linear part of the curves (intercept between the regression line and the abscissa), was found to increase significantly as the browning reactivity of the amino acid-containing glucose solution decreased. The long methionine lag time is probably due to the previously observed ability of sulfur amino acids to inhibit the nonenzymatic browning of amino acid-containing glucose solutions (Friedman and Molnar-Perl, 1990) as well as that of fruit juices and protein-containing foods (Molnar-Perl and Friedman, 1990). No adverse effect is likely to occur during the lag period prior to the browning reaction, which takes place in heated reducing sugar solutions in the presence of amino acids.

Essential Amino Acid and Glucose Losses at pH 7.5. Although a large number of studies have been devoted to the Maillard reaction during the past decades, little attention has been paid to the kinetics of the concomitant disappearance of glucose and essential amino acids in heated solutions. Figure 2 gives the essential amino acid losses in glucose-containing solutions heated to 100 °C at pH 7.5 as a function of time. Lysine is definitely the most easily degraded amino acid, due to the presence of two reactive amino groups, followed by threonine and phenylalanine, and surprisingly, methionine was found to be the least reactive essential amino acid. In the absence of glucose, no significant loss of any amino acid was observed except for tryptophan, the loss of which reached 6% after a 2 h heating period. The reactivity of lysine has been reported to be 5−15 times higher than that of the other amino acids (Adrian, 1974), and that of threonine has also been observed to be high, with regard to its degradation at temperatures ranging from 4 to 60 °C (Fry and Stegink, 1982) and from 60 to 200 °C (Piloty and Baltes, 1979). The Maillard reactivity of some amino acids, such as threonine, may therefore be over- or underestimated if one considers separately their loss extent and browning-inducing ability. This observation may also be true for proteins since, for example, the storage of ovalbumin at 50 °C and 65% relative humidity for a week in the presence of disaccharide (maltose, cellobiose, isomaltose, lactose, or mellibiose) resulted in an overall loss of the amino groups of more than 80%, while only mellibiose and isomaltose were found to give rise to some browning (Kato et al., 1989).

As shown in Figure 3, under the same experimental conditions, glucose was degraded more rapidly than in the presence of amino acids, which apparently exerted a protective effect on the thermal degradation of this

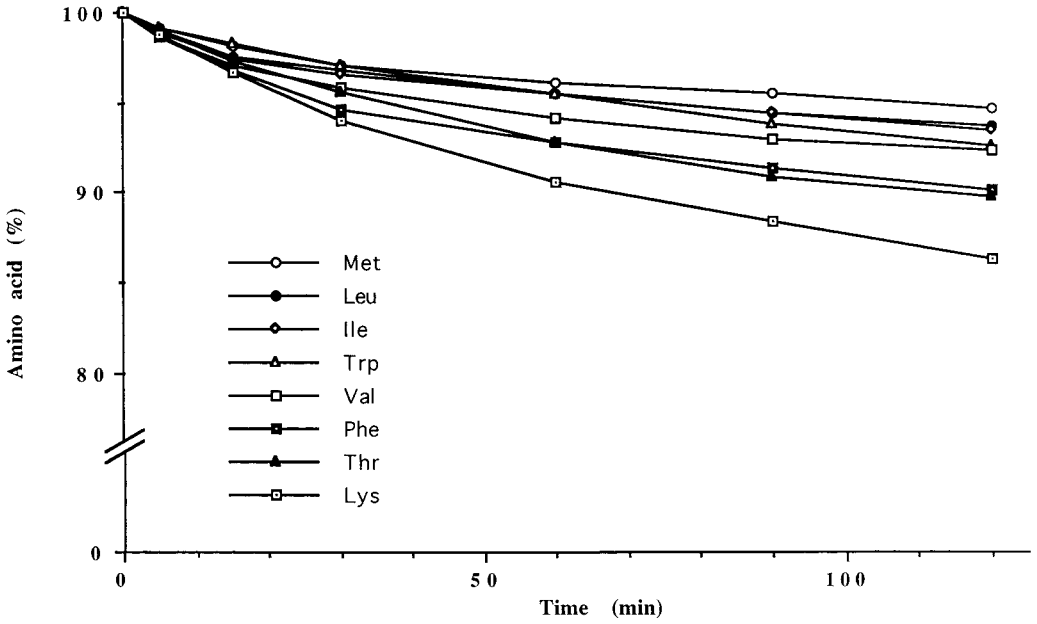

Figure 2. Time course of essential amino acid loss in the presence of glucose at 100 °C and at pH 7.5.

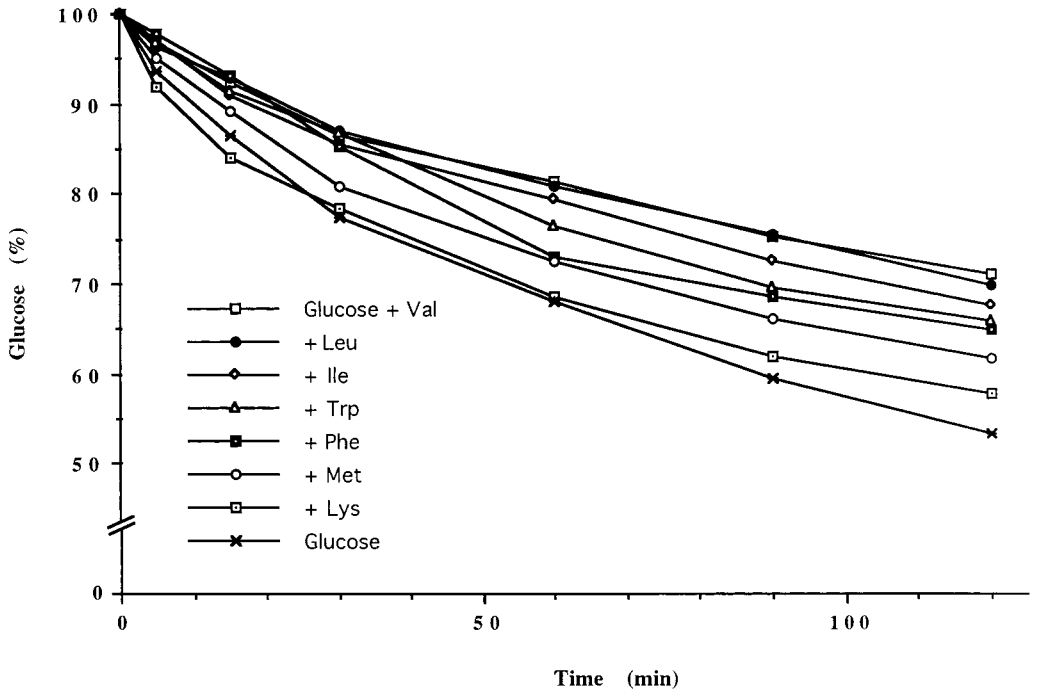

Figure 3. Time course of glucose loss in the presence or absence of essential amino acids when solutions were heated to 100 °C at pH 7.5 for increasing lengths of time.

hexose. After a 2-h heating period, the extent of glucose retention was as low as 53% but increased up to 58%, 60%, 62%, 65%, 66%, 68%, 70%, and 71% when lysine, threonine, methionine, phenylalanine, tryptophan, isoleucine, leucine, and valine were added to the glucose solution, respectively. This protective effect probably resulted from a displacement of the equilibrium in the first step in the Maillard reaction, leading to the formation of glycosylamines which subsequently underwent Amadori rearrangement, as recently suggested by Ge and Lee (1997). In their study on the glucose–phenylalanine system, these authors observed that the kinetic constant of the reverse reaction in Schiff's base formation was 10^3 times higher than that of the so-called glycosylamine formation itself, which consequently is the rate-limiting step in the overall reaction.

All in all, our data (Figures 2 and 3) are not consistent with a first-order kinetic model, however, in sharp contrast to those published by Baisier and Labuza (1992), who obtained comparable first-order constant values for both glucose and glycine disappearance from a solution which was stored for a 3 month period at 37 °C and pH 7.0. A fixed concentration of glycine and decreasing concentrations of glucose below that of glycine were used by these authors and resulted in a comparable decrease in the first-order rate constant of both glycine and glucose disappearance. In our glucose–lysine system, for example, 45% of glucose was destroyed after a 2 h heating period, whereas the loss of reactive lysine was only 14%. It is therefore quite conceivable that the glucose–amino acid molar ratio progressively decreased during heat treatment under

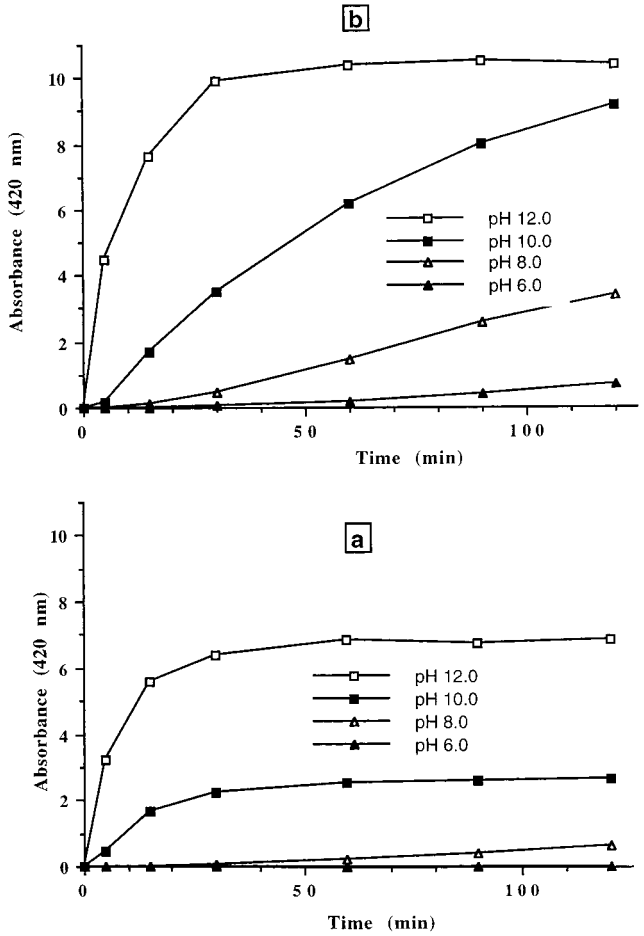

Figure 4. Brown color development in an aqueous solution of glucose (a) and of lysine-containing glucose solution (b) heated to 100 °C at pH values ranging from 4.0 to 12.0 for increasing periods of time.

our conditions, which may in turn result in a decrease in the rates of both amino acids and glucose losses, then deviating the plots from first-order kinetics.

Effect of pH on Glucose and Glucose−Amino Acid Browning. As can be seen from Figure 4a, almost no browning occurred in the glucose solution at pH 6.0, whereas some browning was measured at pH 8.0 after a 20 min lag phase. As the pH of the reaction medium increased, the lag time decreased and the rate of the browning reaction increased. In agreement with the studies by Willits et al. (1958) and Buera et al. (1987), in which glucose browning was monitored at pH values ranging from 3.0 to 9.0 and 4.0 to 6.0, respectively, at different sugar concentrations and temperature values from those used in the present study, the higher the basicity of the sugar solution, the higher the browning intensity. As was to be expected, Figure 4b shows that the browning intensity increased in the lysine-containing solution as compared with that containing glucose alone, at pH values ranging from 6.0 to 12, as well as at pH 4.0 (not shown). It is worth noting that no plateau was reached in the browning of lysine-containing glucose solution at pH 10.0, as compared to that of glucose alone. This strongly suggests the existence of high interactions between the amino acid and the sugar under these pH conditions.

The kinetics of the Maillard browning produced by methionine and threonine (data not shown), two other essential amino acids which are variably limiting in some plant proteins, was comparable to that of lysine. As shown in Figure 5, the browning intensity of the glucose solution was not significantly affected by the presence of either methionine or threonine when heated for 2 h at 100 °C at pH values ranging from 4.0 to 8.0, in sharp contrast with lysine, which was found to have quite a positive effect. In the pH range 8.0−12.0, the browning effect of the first two amino acids increased up to pH 12.0 but still remained lower than that of lysine, the maximum effect of which was observed at pH 11.0. The greatest increase in the browning of the amino acid-containing glucose solution was observed between pH 9.0 and 10.0, as the result of the complete deprotonation of the α-amino group, which in turn accelerated the carbonylamine reaction between the sugar and each amino acid.

The net contribution of each of the three above-mentioned amino acids to the browning was calculated by deducing the browning intensity of glucose alone from that of glucose in their presence (data not shown). The browning-promoting effect of lysine was observed at pH levels as low as 6.0 and increased up to pH 10.0−11.0, whereas no significant contribution of methionine and threonine was observed below pH 9.0, and the maximum browning effect was reached with these two amino acids at pH 10.0. A slightly inhibitory effect of theonine on glucose browning was observed at pH values ranging from 7.0 to 9.0 and might be due to the rather nucleophilic character of the threonine side chain, which might be able to trap the highly reactive electrophilic intermediates of glucose degradation, thus preventing the further polymerization necessary for browning to occur. The decrease in browning observed at the highest pH values tested here might be due to the small number of protons required for the first steps in the Maillard reaction to occur. The supply of an additional proton from the ϵ-amino group of lysine at about pH 10 may explain the shift of the maximum pH toward a higher value in the browning reaction of a lysine-containing glucose solution. A similar high pH value has been reported by Ashoor and Zent (1984) to give maximum color production when common amino acids were heated for 10 min at 121 °C with either the monosaccharides glucose and fructose or the disaccharide lactose. It is rather difficult to compare the extent of browning reactions when the experimental conditions were not really identical, since both the type and the amount of the final products may differ, depending on the temperature and heating time (Adrian and Favier, 1961), or the pH values (Ames et al., 1997), as well as whether the pH is controlled when the reaction proceeds (Ames et al., 1993).

Effect of pH on Glucose and Amino Acid Losses. The kinetics of glucose loss in a solution boiled at pH values ranging from 4.0 to 12.0 is given in Figure 6. Although glucose was quite stable for 2 h at pH 4.0, its retention decreased to 85% and 79% at pH 6.0 and 7.0, respectively. The retention was as low as 36% at pH 8.0, which is roughly 3-fold lower than that observed at one pH unit lower. High levels of destruction of glucose occurred during the first 5 min of boiling at pH values above 9.0 (60% at pH 10.0, 79% at pH 11.0, and 88% at pH 12.0). This is in agreement with the reported high glucose degradation extents at 100 °C, under alkaline conditions (Yang and Montgomery, 1992).

As mentioned in the first part of this study, the thermal degradation of glucose was variably reduced in

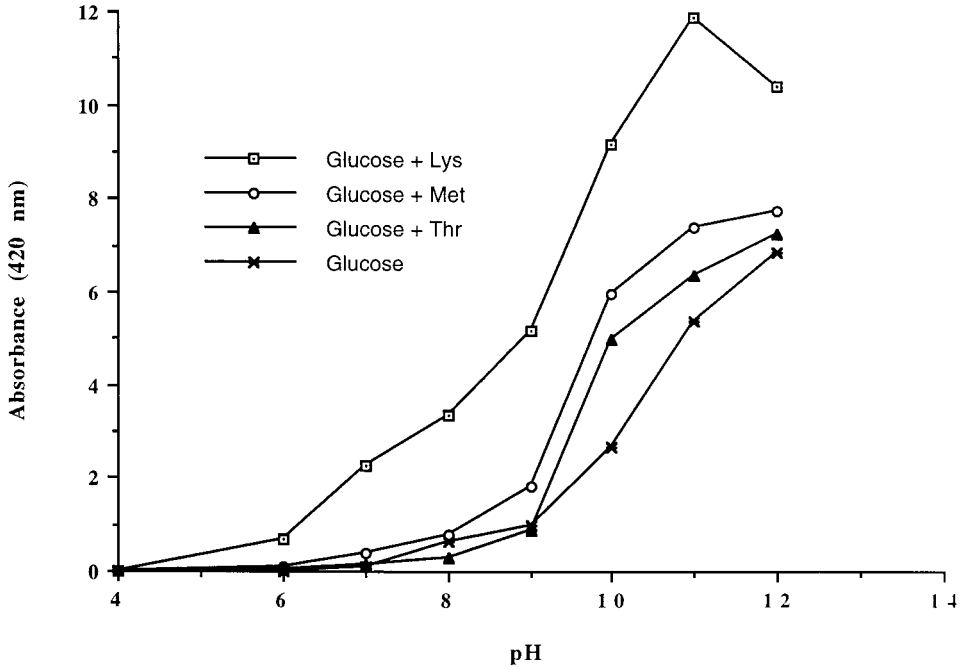

Figure 5. Effect of pH on browning development in an aqueous solution of glucose heated for 120 min at 100 °C in the presence or absence of lysine, methionine, and threonine.

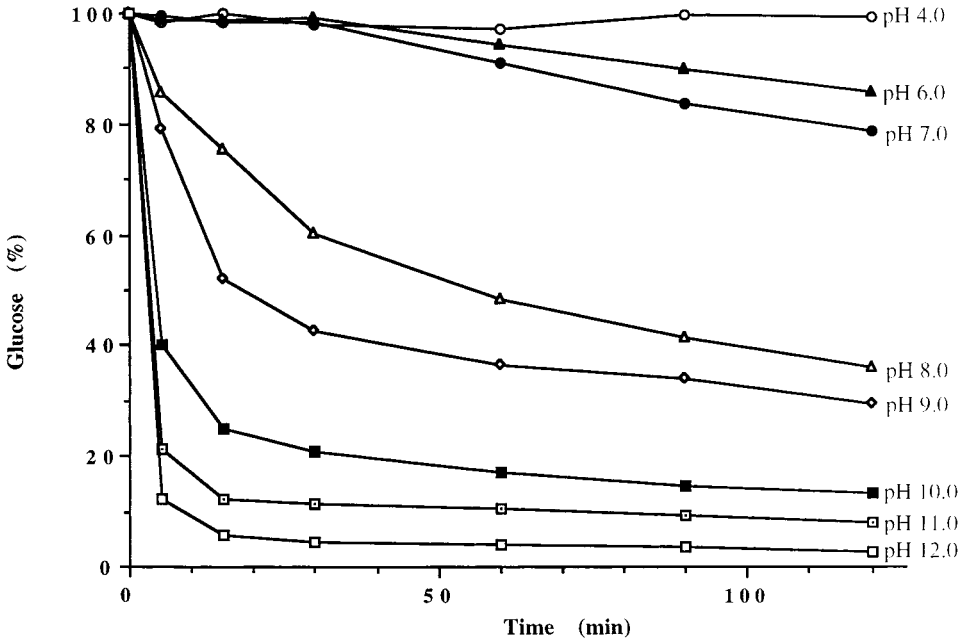

Figure 6. Time course of glucose loss in an aqueous solution heated to 100 °C at pH values ranging from 4.0 to 12.0.

the presence of amino acids, depending on the pH of the solution. At pH 4.0−7.0, lysine, methionine, and threonine had almost no significant protective effects, whereas at pH 8.0−10.0, conspicuous effects of this kind were observed. As shown in Figure 7, the degradation of glucose decreased when the solution was heated in the presence of each of these three amino acids, and threonine was found to protect glucose more efficiently than the other two amino acids at pH 8.0. After the solutions had been boiled for 2 h, the percentage of remaining glucose was 38%, 43%, 58%, and 66% when glucose was tested alone and in the presence of lysine, methionine, and threonine, respectively. At pH 9.0 (not shown), all three amino acids exerted comparable protective effects, whereas at pH 10.0, only lysine was found to have any protective effect, especially during the first 30 min of heating (Table 2). Lysine was still efficient at pH 11.0, although to a lesser extent (not shown). The protective effects of these three amino acids, especially at pH 9.0, may be attributable to the interaction between glucose and their amino groups during the first steps in the Maillard browning reaction, since the protection of glucose was found to be the most efficient at pH values corresponding to the pK_a of the α-amino group and to that of the ϵ-amino group of lysine.

Figure 8 shows the time course of the destruction of lysine in the presence of glucose when solutions with increasing alkalinity were boiled. There was almost no loss of lysine at pH 4.0 and 6.0, in agreement with the statement that the more acid the medium, the greater the stability of the free amino acids heated in the

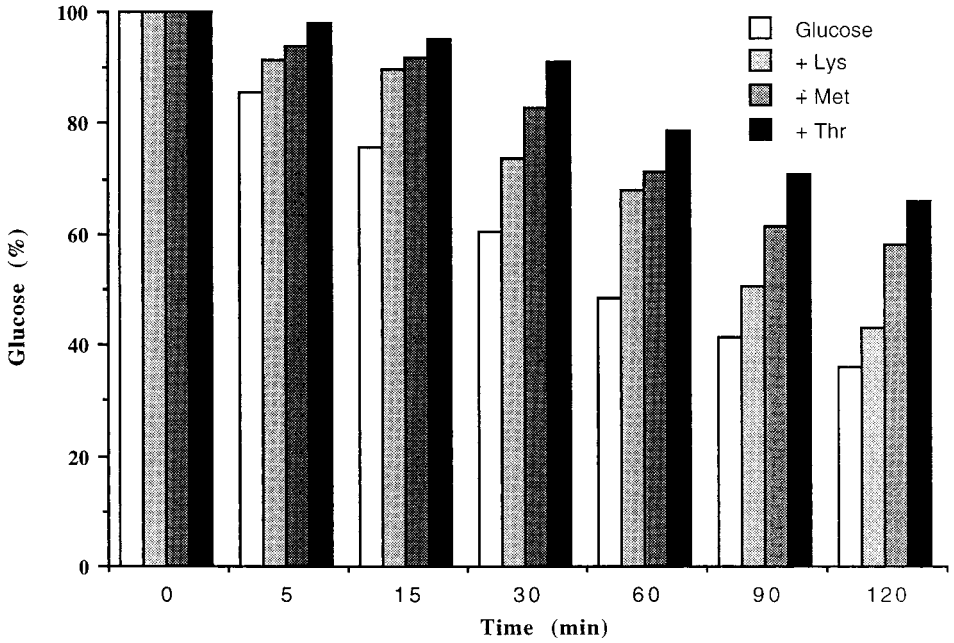

Figure 7. Remaining glucose in essential amino acid-containing glucose solutions maintained for 2 h at a temperature of 100 °C and at pH 8.0.

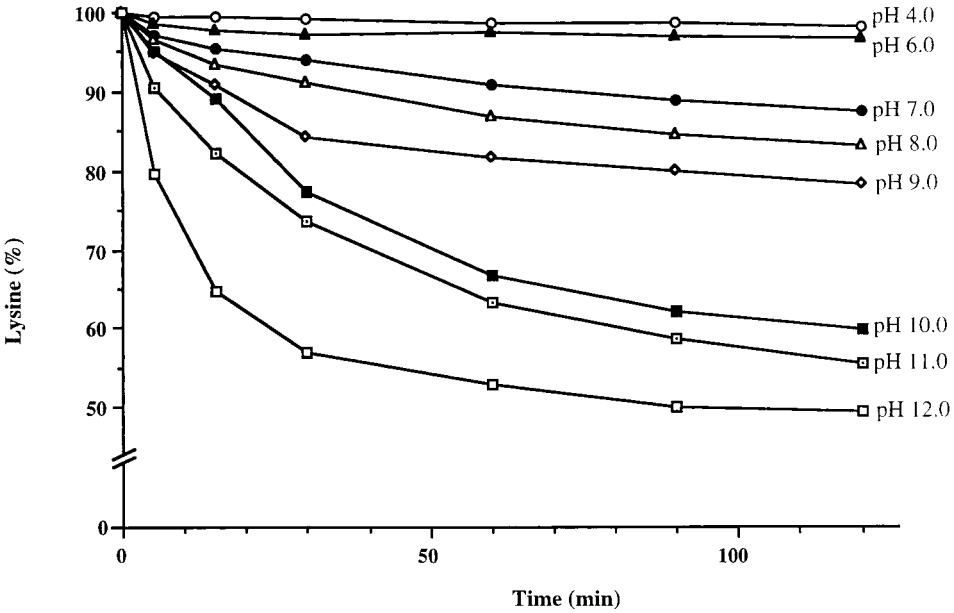

Figure 8. Time course of lysine loss in glucose solutions heated to 100 °C at pH values ranging from 4.0 to 12.0.

Table 2. Remaining Glucose in the Presence or Absence of Lysine, Methionine, and Threonine in Solutions Heated to 100 °C at pH 10.0

	remaining glucose (%)			
		glucose +		
heating time (min)	glucose	lysine	methionine	threonine
0	100	100	100	100
5	40	79	51	50
30	21	41	21	23
120	14	18	10	14

presence of glucose (Adrian, 1974). Lysine was still fairly stable in the 7.0−9.0 pH range. The accelerated destruction of lysine at higher pH values might be linked to that of glucose under the same pH conditions and suggests that strong interactions take place between the amino acids and the sugar under these pH conditions. Despite the existence of some conflicting data (Jokinen et al., 1976), it is generally assumed that alkalinity favors the Maillard browning reaction. Figure 8 also shows that the lysine loss did not follow first-order kinetics, since the lysine degradation was rapid in the early stages of heating, and then slowed and tended toward a no-loss period, which has also been observed at lower temperature values (Baisier and Labuza, 1992). The deviation from first-order kinetics of the lysine loss and glucose loss is probably due to the differences between their rates of destruction, since the hexose was destroyed more rapidly than the amino acid at all of the pH values tested. This finding is consistent with that obtained in a study by Mohr et al. (1971), in which glucose and fructose were completely destroyed, whereas only 50% of the free amino acids disappeared from cocoa bean extracts roasted at 121 °C for 8 min. The decrease in pH during the heating of glucose and amino acids containing glucose solutions, especially above neutrality,

may also contribute to the lowering of the rates of reactant losses. Indeed, for example, the pH of glucose and lysine-containing glucose solutions was almost unchanged after 2 h of heating at initial pH 4.0, but decreased by 0.25 and 0.5 pH units at initial pH 8.0 and by 2.7 and 1.8 pH units at initial pH 12, respectively.

The stability of the hydroxy amino acid, threonine, and that of the S-containing methionine in solutions boiled for 2 h at different pH values were similar to that of lysine. The relative stabilities of the three amino acids in the 4.0−12.0 pH range remained unchanged and were in agreement with the results of Mohr et al. (1971), where in the roasted extracts of cocoa beans, the percentage destruction of lysine, threonine, and methionine was 69%, 60%, and 53%, respectively. When lysine, methionine, and threonine were heated in the absence of glucose, no significant loss of these amino acids was observed, even at high pH values. It is worth noting that, at high alkalinity levels, these amino acids may undergo some racemization (Friedman, 1991). However, since the detection method used in this study was not appropriate for distinguishing D- from L-amino acids, racemization cannot be taken into account here in the observed loss of amino acids.

From the nutritional point of view, the destruction of essential amino acids is one of the most unfavorable effects of the Maillard reaction. The present study shows that the losses of essential amino acids are not so drastic in dilute amino acid-containing glucose solutions when they are boiled at pHs found in foods. Still, some food proteins such as those of soybean are sometimes exposed to high alcalinity levels during their processing, and more intense destruction of the limiting amino acid lysine may occur. In addition, the study of nonenzymatic browning reactions under alkaline conditions where both Maillard and caramelization reactions are greatly accelerated is expected to bring useful information about the mechanisms whereby these reactions take place.

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LITERATURE CITED

Adrian, J.; Favier, J. C. La reaction de Maillard. 1. Etude du comportement de la lysine pure. *Ann. Nutr. Aliment.* **1961**, *15*, 181−225.

Adrian, J. Nutritional and physiological consequences of the Maillard reaction. *World Rev. Nutr. Diet.* **1974**, *9*, 71−122.

Ames, J. M.; Apriyantono, A.; Arnoldi, A. Low molecular weight coloured compounds formed in xylose-lysine model system. *Food Chem.* **1993**, *46*, 121−127.

Ames, J. M.; Arnoldi, A.; Bates, L.; Negroni, M. Analysis of the methanol-extractable of a model extrusion-cooked cereal product. *J. Agric. Food Chem.* **1997**, *45*, 1256−1263.

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

Baisier, W. M.; Labuza, T. P. Maillard browning kinetics in liquid model system. *J. Agric. Food Chem.* **1992**, *40*, 707−713.

Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid analysis of amino acids using precolumn derivatization. *J. Chromatogr.* **1984**, *336*, 93−104.

Buera, M. D. P.; Chirife, F.; Resnik, S. L.; 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.

Fox, M.; Loncin, M.; Weiss, M. Investigations into the influence of water activity, pH and heat treatment on the velocity of the Maillard reaction in foods. *J. Food Qual.* **1983**, *6*, 103−118.

Friedman, M. Formation, nutritional value, and safety of D-amino acids. *Adv. Exp. Med. Biol.* **1991**, *289*, 447−481.

Friedman, M.; Molnar-Perl, I. Inhibition of browning by sulfur amino acids. Heated amino acid-glucose systems. *J. Agric. Food Chem.* **1990**, *38*, 1942−1947.

Fry, L. K.; Stegink, L. D. Formation of Maillard reaction products in parenteral alimentation solutions. *J. Nutr.* **1982**, *112*, 1631−1637.

Ge, S.-J.; Lee, T.-C. Kinetic significance of the Schiff base reversion in the early-stage of Maillard reaction of phenylalanine-glucose aqueous model system. *J. Agric. Food Chem.* **1997**, *45*, 1619−1623.

Greenshields, R. N. Caramel-part 2. Manufacture, composition and properties. *Process Biochem.* **1973**, *4*, 17−20.

Hwang, H.-I.; Hartman, T. G.; Ho, C.-T. Relative reactivities of amino acids in pyrazines formation. *J. Agric. Food Chem.* **1995a**, *43*, 179−184.

Hwang, H.-I.; Hartman, T. G.; Ho, C.-T. Relative reactivities of amino acids in the formation of pyridines, pyrroles, and oxazoles. *J. Agric. Food Chem.* **1995b**, *43*, 2917−2921.

Jokinen, J. E.; Reineccius, G. A.; Thompson, D. C. Loss in available lysine during thermal processing of soy protein model systems. *J. Food Sci.* **1976**, *41*, 816−819.

Kato, Y.; Matsuda, T.; Kato, N.; Nakamura, R. Maillard reaction of disaccharides with protein: suppressive effect of non reducing end pyranoside groups on browning and protein polymerization. *J. Agric. Food Chem.* **1989**, *37*, 1077−1081.

Lerici, C. R.; Barbanti, D.; Manzano, M.; Cherubin, S. Early indicators of chemical changes in foods due to enzymic or nonenzymic browning reaction.1: study on heat treated model systems. *Lebensm.-Wiss. Technol.* **1990**, *23*, 289−294.

Mohr, W.; Roehrle, D.; Severin, T. Die bildung von kakaoaroma ans den vorstufen. *Fette, Seifen, Anstrichm.* **1971**, *73*, 515−521.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *J. Agric. Food Chem.* **1990**, *38*, 1648−1651.

Myers, D. V.; Howell, J. C. Characterization and specification of caramel colours: an over view. *Food Chem. Toxicol.* **1992**, *30*, 356−363.

Nafisi, K.; Markakis, P. Inhibition of sugar-amine browning by aspartic and glutamic acids. *J. Agric. Food Chem.* **1983**, *31*, 1115−1117.

O'Beirne, D. Effect of pH on non enzymatic browning during storage in apple juice concentrate prepared from bramley's seedling apples. *J. Food Sci.* **1986**, *51*, 1073−1074.

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 non enzymatic browning. *J. Food Sci.* **1985**, *50*, 622−625.

Piloty, M.; Baltes, W. Investigations on the reaction of amino acids with α-dicarbonyl compounds. I. Reactivity of amino acids in the reaction with α-dicarbonyl compounds. *Z. Lebensm.-Unters.-Forsch.* **1979**, *168*, 368−373.

Willits, C. O.; Underwood, J. C.; Lento, H. G., Jr.; Ricciuti, C. Browning of sugar solutions. Effect of pH and type of amino acid in dilute sugar solutions. *C. Food Res.* **1958**, *23*, 61−67.

Yang, B. Y.; Montgomery, R. Alkaline degradation of glucose: effect of initial concentration of reactants. *Carbohydr. Res.* **1996**, *280*, 27−45.

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