Evaluation of Kinetic Parameters for a Glucose–Lysine Maillard Reaction

Chong M. Lee,* Bonni Sherr, and Young-Nam Koh

Glucose–lysine Amadori compounds were prepared by eluting refluxed reaction products through a Dowex 50W-X4 column by using 0.2 M pyridine–acetic acid buffer of pH 4.25 and confirmed by an Avicel TLC. The kinetics of the formation of Amadori compounds (monofructosyllysine, MFL; difructosyllysine, DFL) and brown pigment were studied with respect to temperature and pH under closed and isothermal conditions. Amadori compounds were formed at an exponential rate while showing a pseudo-first-order degradation of lysine in the presence of excess glucose. One-hour refluxing in metanol reduced 50% of lysine without an apparent pigment formation. A breakpoint for the pH-dependent reaction rate for the formation of MFL, DFL, and pigment was found to be at pH 6 at all temperatures studied. The pH dependency of the reaction rate was decreased with an increase in reaction temperature. Computed E_a ranged from 8.5 to 5.7, 11.5 to 6.3, and 18.0 to 5.1 kcal mol^{-1} for MFL, DFL, and pigment, respectively, at pH 4–7.

During thermal processes, losses of lysine frequently occur in most proteinaceous products (Adrian, 1967; Finot et al., 1979; Hurrell, 1980). These losses are generally believed to result mainly from the formation of sugar–lysine Amadori compounds during the Maillard browing reaction. The extent of loss is mainly determined by the type of thermal process where temperature and holding time appear to be important process parameters. Kinetic data based on such parameters need to be generated for a better prediction of kinetics of lysine losses during thermal process. This study was thus designed using a model glucose–lysine system to determine various kinetic parameters that concern the formation of lysine Amadori compounds and pigment and temperature and pH-dependent reaction rates. The results of this study will not only supplement the previous kinetic study (Warmbier et al., 1976) but also help assess the significance of the physiological implication of the glucose–lysine Amadori compounds that were previously reported (Sherr et al., 1979). The importance of this study is further signified by the facts that lysine is most susceptible to the Maillard reaction and yet it is the most limiting essential amino acid.

EXPERIMENTAL SECTION

Synthesis of Glucose–Lysine Amadori Compounds. Amadori compounds formed during Maillard browning of a glucose–lysine mixture were synthesized by using the method of Finot and Mauron (1969) with some modification. Fifteen grams of glucose and 2.5 g of lysine were dissolved in 250 mL of 100% methanol and refluxed for $4^1/_2$ h. In our preliminary work, the 4-h refluxing resulted in a maximum formation of monofructosyl-L-lysine (MFL) and difructosyl-L-lysine (DFL) as shown from the TLC separation. Twenty milliliters of the reaction mixture was concentrated in a rotor evaporator, dissolved in a minimum amount of elution buffer, and applied to a Dowex 50W-X4 column ($1^1/_2$ in. × 18 in.; 200-mesh resin). The column had previously been equilibrated at pH 4.25 with a 0.2 M pyridine–acetic acid buffer. The loaded column was then eluted with the same buffer.

Fractions were collected by a fraction collector (Buchler Instruments, Inc., Fort Lee, NJ) allowing 7 mL for each fraction. A sample of every third fraction was reacted with freshly prepared ninhydrin solution (0.04 g of stannous chloride in 25 mL of 0.2 M citrate buffer, pH 5.0, mixed with 1 g of ninhydrin in 15 mL of methylcellosolve), and readings of color intensity were taken on a spectrophotometer at 570 nm to prepare a chromatogram. Fractions corresponding to each peak in the chromatogram were spotted on a TLC plate coated with cellulose (Avicel, 250-μm layer thickness; Analtech, Inc., Newark, DE). The original refluxed sample was also spotted for reference. The plate was developed with a solvent system consisting of pyridine, acetic acid, and water (9:1:2). Spots of lysine, MFL, and DFL were developed by spraying a cupric nitrate–ninhydrin solution (Moffat and Lytle, 1959) and heating for 2 min at 105 °C in an oven and identified following the reported chromatogram of Finot and Mauron (1969).

The fractions, which were identified to contain MFL and DFL, were respectively pooled and extracted with 10 volumes of ether in a separatory funnel to remove dissolved pyridine and acetic acid. The resulting solutions were rotor-evaporated and freeze-dried. The freeze-dried samples were dissolved in 20 mL of distilled water and extracted again 3 times with 5 volumes of ether to remove residual pyridine and acetic acid. After removal of the remaining either by a rotor evaporator, the resulting solution was freeze-dried. The purity of each freeze-dried Amadori compound was further confirmed by a TLC separation following the same procedure previously described (Finot and Mauron, 1969).

For the next run, the column was regenerated by cleaning with 0.2 N NaOH and distilled water and subsequently equilibrating at pH 4.25 with a pyridine–acetic acid buffer.

Preparation of Reaction Mixture for Kinetic Study. The mixture was prepared by dissolving L-lysine and D-glucose (1:6 w/w) in 80% methanol solution (v/v in water) at the respective concentration of 0.054 M for lysine and 0.33 M for glucose. An 80% methanol solution was used to allow a complete solubilization of reactants prior to reaction. For the study of the effect of reaction time, 100% methanol (Mallinkrodt, ACS reagent grade) was used instead of 80%. The reason was that when 80% methanol was used the reaction rate at a refluxed temperature was too slow to run a kinetic study.

Effect of Reaction Time. A 250-mL reaction mixture in 100% methanol (pH 5.7) was refluxed over a $4^1/_2$-h period. Samples were taken at every $^1/_2$-h interval for the measurement of color intensity, as well as for the quantitation of lysine, MFL, and DFL. The color intensity of the reaction solution was measured at 420 nm (λ_{max}) after diluted 10 times with methanol. For the quantitation of lysine, MFL, and DFL, a 5-μL aliquot was spotted on a

Department of Food Science and Nutrition, University of Rhode Island, Kingston, Rhode Island 02881.

0021-8561/84/1432-0379$01.50/0

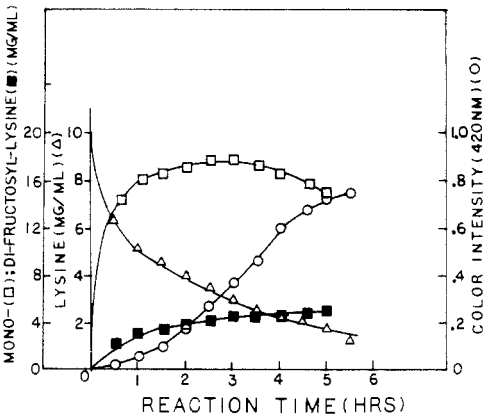

Figure 1. Formation of MFL, DFL, and pigment and degradation of lysine as a function of reaction time. (□) MFL; (■) DFL; (○) pigment; (△) lysine.

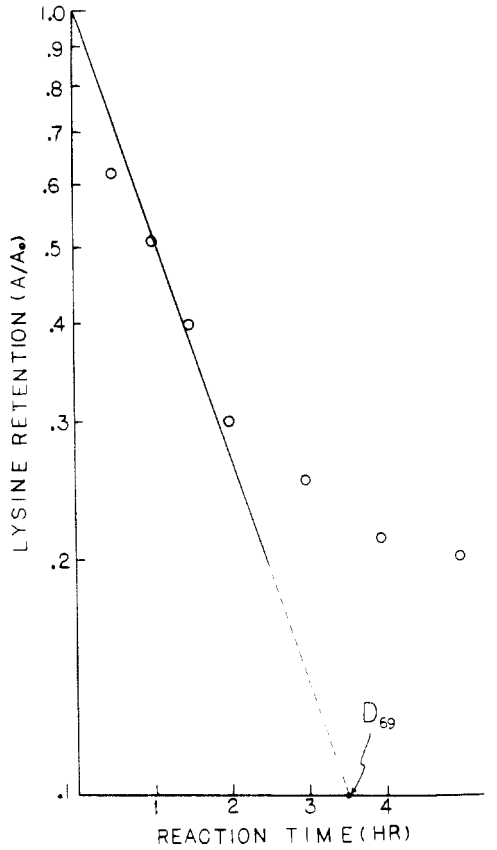

Figure 2. Lysine retention as a function of reaction time. D_{69} = the time required for a 90% reduction in the concentration of lysine at 69 °C.

cellulose plate along with the reference of known concentration that was synthesized previously. The plate was eluted and developed as described previously. The concentration of each substance was quantitated by measuring the color intensity of the corresponding spot with a scanning densitometer (E-C Apparatus Corp., Petersburg, FL).

Reaction Temperature. The pH of reaction in 80% methanol was adjusted to 7 by using 0.1 N NaOH. The reason behind the use of pH 7 instead of pH 5.7 was that at pH 5.7, the rate of reaction was not appreciable enough to cause detectable changes in an initial velocity. Seven milliliters of the solution was transferred into a respective ampule (Kimax, 10-mL capacity) by using a hypodermic syringe and flame sealed. This reaction system thus allowed a closed condition. Reaction was allowed at 90 °C for 80, 100, and 120 min, at 100 °C for 50, 70, and 90 min, and 110 °C for 40, 50, and 60 min, respectively, in a water

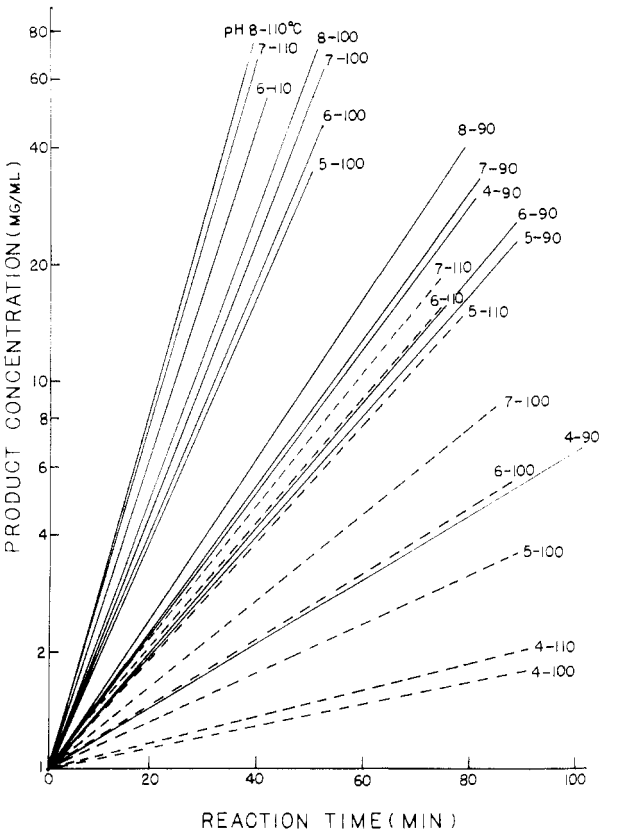

Figure 3. Formation of MFL and DFL as a function of reaction time at various temperatures and pHs. (—) MFL; (---) DFL. All lines were drawn in the manner which the data points fit best.

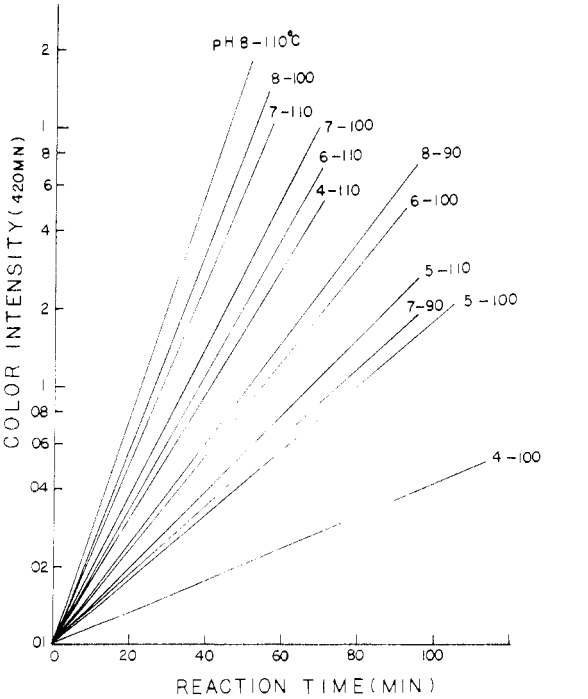

Figure 4. Formation of pigment as a function of reaction time at various temperatures and pHs. All lines were drawn in the manner which the data points fit best.

bath except for 110 °C, which was brought up in a propylene glycol bath. Only those ampules having a constant weight before and after the reaction were used for the analysis in order to exclude the ampules having a leak that erroneously caused changes in concentration. The amounts of MFL, DFL, and brown pigment formed after reaction were quantitated as described in the previous section. The

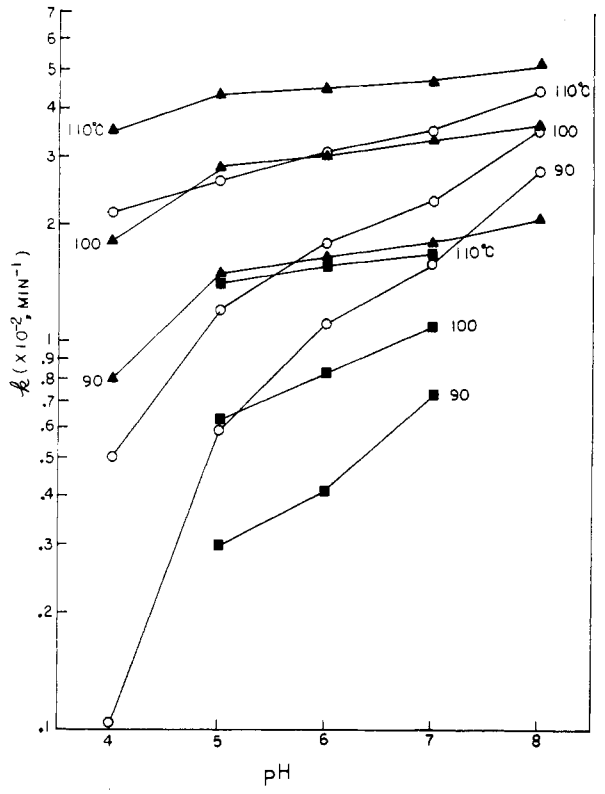

Figure 5. Arrhenius plots for the formation of MFL and DFL at various pHs. (●) MFL; (▲) DFL. All lines were drawn in the manner which the data points fit best.

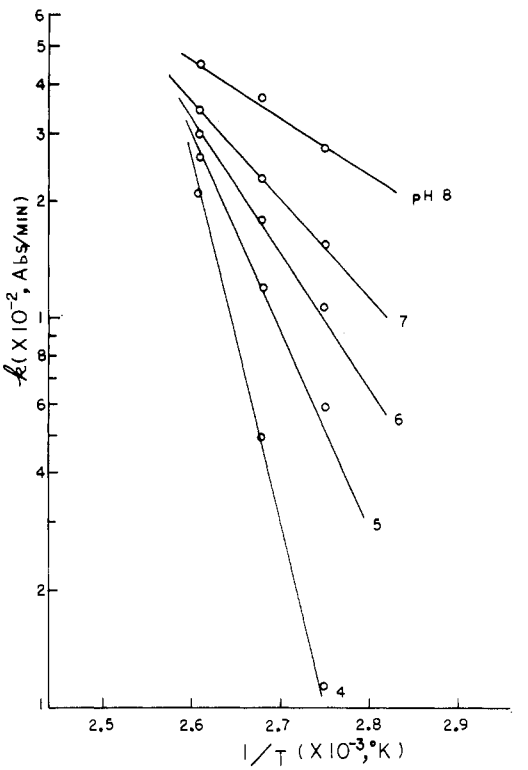

Figure 7. Arrhenius plots for the formation of pigment at various pHs. All lines were drawn in the manner which the data points fit best.

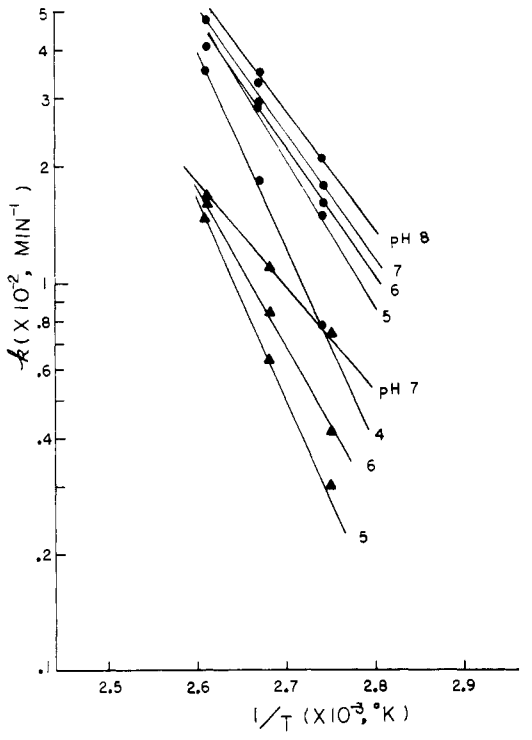

Figure 6. Changes in reaction rate constant (k) as a function of pH. (▲) MFL; (■) DFL; (●) pigment.

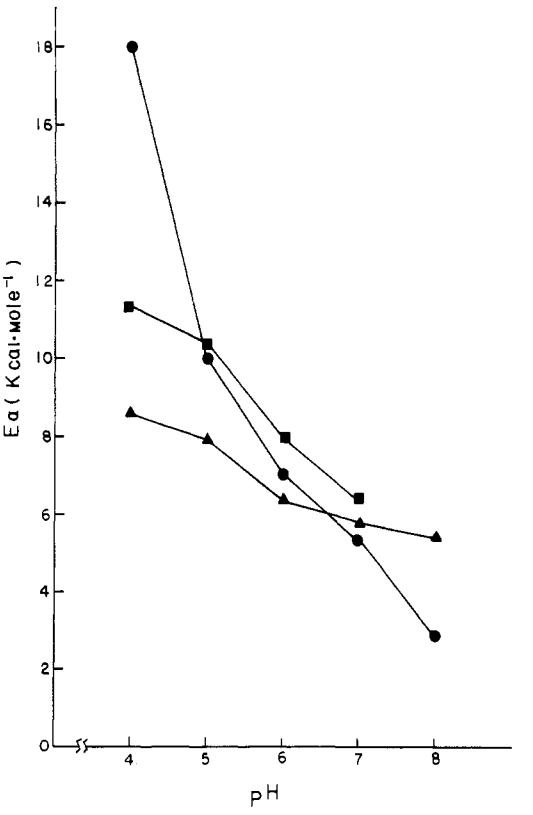

Figure 8. Changes in activation energy values as a function of pH. (▲) MFL; (■) DFL; (●) pigment.

slope of log product concentration vs. time was used to determine the rate constant (k) at a given temperature. The respective activation energy (E_a) for the formation of MFL, DFL, and brown pigment was computed from the slope of the Arrhenius plot.

Effect of pH. The pH of reaction mixture in 80% methanol was varied from 4 to 8 by adding either 0.1 N HCl or 0.1 N NaOH. The idea of using a phosphate buffer was abandoned because it produced a considerable cloudy precipitation when added to methanol. Reaction was allowed at 90, 100, and 110 °C for various periods as specified previously, and the quantitation of reaction products was done in the same manner as described previously. Changes in pH at the end of the reaction were +2.9%, +1.3%,

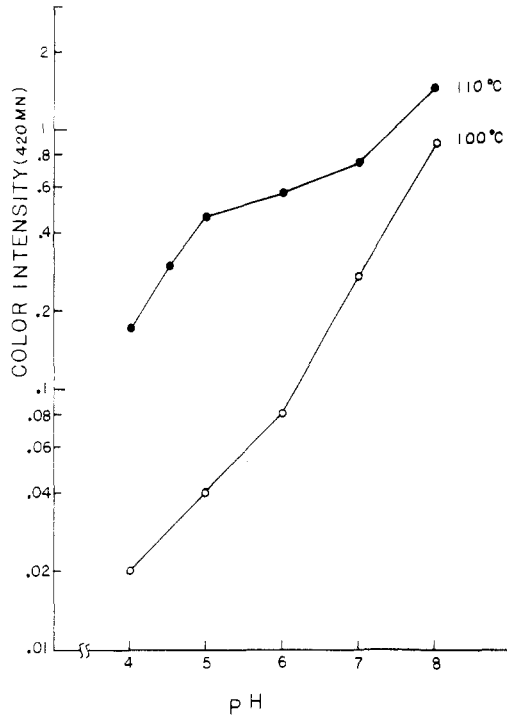

Figure 9. Changes in pigment formation as a function of pH. (○) 100 °C; (●) 110 °C.

−4.6%, −6.2%, and −8.0% from the initial pH of 4, 5, 6, 7, and 8, respectively.

The average value from triplicate measurements was used for each datum point in the analysis of kinetic parameters.

RESULTS AND DISCUSSION

Figure 1 shows the reaction kinetics relating to the formation of MFL and DFL and the loss of lysine at the concentration of 0.33 M glucose and 0.054 M lysine in methanol at refluxing temperature (69 °C). Both MFL and DFL were formed at an exponential rate. Fifty percent of lysine was already lost after 1-h refluxing, while the pigment formation was not visible until after 2-h refluxing at which the absorbance reached 0.2.

The linear portion of the semilog plot suggested pseudo-first-order kinetics during the early stage of the reaction, which requires an excess glucose and is lysine concentration dependent (Figure 2). After extrapolation of the degradation curve, a decimal reduction value of lysine at refluxing temperature (D_{69}) was found to be $3^1/_2$ h at the prescribed concentration.

The pseudo-first-order plots for the formation of MFL, DFL, and brown pigment at various pH are shown in Figures 3 and 4, respectively. The slope at each respective reaction temperature was used as k, and these values were fitted to the Arrhenius equation (Figure 5). The increases in reaction rate constant (k) were marked from pH 4 to 5 and thereafter were steady up to pH 8 for MFL, DFL, and pigment at all temperatures studied, except for pigment at 110 °C where no breakpoint was observed (Figure 6). It was also noted that the pH dependency of the reaction rate was decreased with an increase in reaction temperature.

Effect of reaction temperature on the formation of MFL, DFL, and pigment at various pH values is shown in Arrhenius plots (Figures 5 and 7). The computed activation energy values for MFL, DFL, and pigment at various pH are compared in Figure 8. The kinetic data indicate that DFL requires greater activation energy than MFL in the pH range studied. Generally, activation energy values for the formation of MFL, DFL, and pigment were reduced with an increase of pH (Figure 8). The breakpoint for activation energy was found at pH 5 and 6 for pigment formation and MFL, respectively. The activation energy, 7.9 and 10.4 kcal mol^{-1} at pH 5, for the formation of MFL and DFL, respectively, appears much smaller than that for the formation of fructose–L-tryptophan, 19.0 kcal mol^{-1} at pH 5.2, which was previously reported by Lee et al. (1979).

Effect of pH on the formation of pigment at 100 and 110 °C for 50 min is depicted in Figure 9. Changes in pigment formation with an increase in pH followed a parabolic curve. In the same figure, it can be noticed that breakpoints in pigment formation occurred at pH 6 and 5 for 100 and 110 °C, respectively.

ACKNOWLEDGMENT

We express our special thanks to Margaret Unterwald for her technical assistance.

Registry No. MFL, 88495-97-0; DFL, 23931-61-5; D-glucose, 50-99-7; L-lysine, 56-87-1.

LITERATURE CITED

Adrian, J. *Ann. Nutr. Aliment.* **1967**, *21*, 129.
Finot, P. A.; Deutsch, R.; Bujard, E. "Proceedings, International Symposium on the Maillard Reactions in Food"; International Union of Food Science and Technology: Sweden, 1979.
Finot, P. A.; Mauron, J. *Helv. Chim. Acta* **1969**, *52*, 1488.
Hurrell, R. F. In "Food and health: science and technology"; Birch, G. G., Parker, K. J., Eds.; Applied Science Publishers: Essex, England, 1980; p 369.
Lee, C. M.; Lee, T.-C.; Chichester, C. O. *J. Agric. Food Chem.* **1979**, *27*, 478.
Moffat, E. D.; Lytle, R. I. *Anal. Chem.* **1959**, *31*, 427.
Sherr, B.; Lee, C. M.; Jelesiewize, C. "Abstracts of Papers", 39th National Meeting of the Institute of Food Technologists, St. Louis, MO, June 1979; Institute of Food Technologists: St. Louis, MO, 1979.
Warmbier, H. C.; Schnickels, R. A.; Labuza, T. P. *J. Food Sci.* **1976**, *41*, 981.

Received for review July 18, 1983. Accepted November 21, 1983. A part was presented at the 39th IFT Annual Meeting of the Institute of Food Technologists, St. Louis, MO, June 1979. Rhode Island Agricultural Experiment Station Contribution No. 2159.