

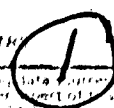
AD-A250 633



IDENTIFICATION PAGE

Form Approved

OMB No. 0704-0188



estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering the necessary information, and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE		3. REPORT TYPE AND DATES COVERED	
4. TITLE AND SUBTITLE Temperature influence on acetyllysine interaction with glucose in model systems due to Maillard reaction.				5. FUNDING NUMBERS	
6. AUTHOR(S) K. Ananth Narayan and Michael E. Cross					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Natick RD+E Center Attn: STENC-MIL Kansas St. Natick, MA. 01760-5040				10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) See reprint					
<div style="text-align: center;"> </div>					
14. SUBJECT TERMS BROWNING GLUCOSE CELLULOSE MAILLARD REACTION ACETYLlysine-GLUCOSE-CELLULOSE MODELS				15. NUMBER OF PAGES 7	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED		18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED		19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	
				20. LIMITATION OF ABSTRACT UNCLASSIFIED	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet optical scanning requirements.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (Maximum 200 words) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (NTIS only).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

Temperature Influence on Acetyllysine Interaction with Glucose in Model Systems due to Maillard Reaction

K. ANANTH NARAYAN and MICHAEL E. CROSS

ABSTRACT

The interaction between N- α -acetyllysine and glucose was investigated in a dry cellulose matrix (1.8% moisture) at 40, 50 and 60°C and a_w 0.19–0.21. The loss in acetyllysine in 5 days at those temperatures was 22, 54, and 79%, respectively. The degradation data more clearly fit a double exponential function ($Ae^{Bx} + Ce^{Dx}$) than the first order function, Ae^{Bx} . An estimate of the activation energy of 36 kcal/mol and a Q_{10} of 5.5 was computed from second order rate constants for acetyllysine and glucose degradation. The increase in color followed zero order kinetics and indicated an activation energy of 44 kcal/mol for the presumed third phase of the Maillard reaction in the solid state.

Key Words: browning, Maillard-reaction, glucose, acetyllysine, cellulose

INTRODUCTION

PROTEIN QUALITY losses are widely recognized to occur at moisture activities between 0.3 and 0.75 during processing and storage due to the Maillard reaction (Ben-Gara and Zimmerman, 1972; Burvall et al., 1978; Desrosiers et al., 1989; Kato et al., 1986, 1988; Labuza and Saltmarch, 1981; Loncin et al., 1968; Ringe and Love, 1988; Warmbier et al., 1976; Warren and Labuza, 1977). Because of the ease with which absorbance measurements can be made, Maillard browning rather than Maillard protein quality losses has been extensively investigated (Eichner and Karel, 1972; Flink et al., 1974; Hendel et al., 1955; Hsu and Fennema, 1989; Kim et al., 1981; Labuza and Saltmarch, 1981; Mizrahi et al., 1970a, 1970b; Petriella et al., 1988; Stamp and Labuza, 1983). Hence, there are many published reports for kinetic parameters such as Q_{10} and the energy of activation (E_a) for Maillard browning at low a_w (< 0.3) in food systems (Hendel et al., 1955; Flink et al., 1974; Kopelman et al., 1977; Mizrahi, et al., 1970a, 1970b). The water activity (a_w) region of 0.2 to 0.3 is generally considered to be a stable region for foods because the water is tightly bound and not available to facilitate nutrient interactions. Therefore, understandably much of the reported work on food systems has focused in the water activity region 0.33–0.75 to evaluate kinetic parameters of protein or lysine quality loss, particularly E_a and Q_{10} .

Compressed foods and uncompressed food powders used for military subsistence have low a_w (< 0.22) and may need to be stored for long periods of time. Not only are data on protein quality loss kinetics at given storage conditions important but also prediction of the temperature sensitivity of protein quality loss at low a_w (< 0.22) is useful. Hannan and Lea (1952) reacted N- α -acetyllysine and glucose at 37°C and 20, 40, and 60% relative humidities (R.H.) and showed that 25% of the lysine (as free amino nitrogen) was lost in 3 days at a R.H. of 20%. Burvall et al. (1978) investigated the storage stability of

ordinary and lactose-hydrolyzed milk powders at 25°C at a_w of 0.11, 0.22, 0.33 and 0.62. While ordinary milk powder was quite stable at $a_w \leq 0.22$, lactose-hydrolyzed milk powder indicated at 17% loss in lysine at 1 mo storage at 25°C. Similarly, valuable information on the kinetics of lysine loss have been obtained by several investigators (Eichner, 1975; Schmickels et al., 1976; Warmbier et al., 1976; Warren and Labuza, 1977) at one temperature (40, 35, 45, and 35°C, respectively) and at one or more a_w (0.23–0.82, 0.63–0.78, 0.52, and 0.3–0.7 respectively). Kaanane and Labuza (1985) have investigated the storage effect of three temperatures of 25, 38, and 45°C on lysine losses in fish flour at a_w 0.33, 0.44, and 0.65. They obtained an E_a of 13.1 kcal/mol for lysine loss at an a_w of 0.33. The data of Ben-Gara and Zimmerman (1972) were analyzed by Labuza and Saltmarch (1981) who have provided E_a values of 14.6 kcal/mol at a_w 0.4 and 19.4 at a_w 0.6 for nonfat dry milk stored at 20, 30 and 40°C. Jokinen et al. (1976) reacted a soybean protein concentrate, glucose, sucrose, starch and cellulose model to temperatures of 80–130°C and a_w 0.33–0.93 and obtained an average E_a of 28.5 kcal/mol for the degradation of lysine. Activation energies for lysine losses in egg noodles of 15.2 ± 9.1 and in pasta of 12.8 ± 5.2 kcal/mol have been reported at a_w 0.44 and 0.49 using storage temperatures of 30–55°C (Chen et al., 1983; Labuza et al., 1982).

In an earlier study, Narayan and Andreotti (1989) investigated the interaction between lysine and glucose in an inert cellulose matrix. Interpretation of the data was complicated by the presence of two reactive groups, the alpha and the epsilon amino groups. In our present investigation, N- α -acetyllysine was reacted with glucose in a cellulose matrix at low a_w in the range 0.19–0.21. The low a_w was selected to simulate the environment in compressed foods and uncompressed food powders used for military subsistence. Determination of acetyllysine was achieved using a reverse phase Pico.Tag[®] column in a High Performance Liquid Chromatography (HPLC) system.

The specific objectives of this investigation were to investigate the quantitative aspects of acetyllysine availability in a low moisture model, to determine the kinetics of acetyllysine degradation at 40, 50 and 60°C, and to determine E_a and Q_{10} of acetyllysine degradation due to Maillard reaction between acetyllysine and glucose.

MATERIALS & METHODS

Sample preparation

Separate solutions of N- α -acetyllysine (Sigma Chemical Co., St. Louis, MO) and anhydrous glucose (Fisher Scientific Co., Medford, MA) in highly purified deionized Milli Q water (Millipore Corp., Bedford, MA) were prepared. Ten mL volumes of each containing 5.31 mmol were pipetted into several freeze-drying flasks. Dispersions in water containing 15.2g of cellulose (Sigmacell, Type 70, Sigma Chemical Co., St. Louis, MO) were added to each flask, mixed, immediately shell frozen in an alcohol-dry ice bath, and freeze-dried. The dry powders were mixed in a blender. For equilibration, 1g samples of the powder mixture in petri dishes (52 \times 15 mm) placed over 50 mL beakers were incubated in separate 237mL Mason jars containing saturated potassium acetate slush at room temperature for 6 days (zero hr samples). They were then stressed at 40, 50, and 60°C over saturated potassium acetate solutions in the Mason jars for various times (see below) in mechanical convection electrical ovens.

Authors Narayan is with the Technology Acquisition Division, Food Engineering Directorate, U.S. Army Natick Research, Development & Engineering Center, Natick, MA. 01760. Author Cross is with the Blood Research Division, Letterman Army Institute of Research, Presidio of San Francisco, San Francisco, CA 94129. Address inquiries to Dr. Narayan.

Table 1—Estimates of parameters for first order mathematical model relating changes in acetyllysine and glucose with time^a

Temp (°C)	A	[10 ⁻³]B	t _{1/2} hr		Deviation ^c %
			OSB ^b	CALC ^b	
Mathematical Model ^d					
Y = Ae ^{BX}					
Acetyllysine					
40	92.0	-0.527	1000	1300	19
50	93.2	-4.88	100	140	8.6
60	79.7	-18.0	14.5	38	20
Glucose					
40	90.4	-0.600	950	1200	22
50	89.2	-4.18	120	170	12
60	77.6	-9.58	22	72	23

^a Acetyllysine-glucose-cellulose powders incubated over saturated potassium acetate solution. X = time in hours, Y = acetyllysine or glucose values (in mg/g powder) expressed as percent of zero time values.

^b OBS = Observed; CALC = Calculated.

^c Expressed as (Sum of squared residuals) × 100 / (Corrected sum of squares)

^d First order rate constant equals negative B in Y = Ae^{BX} expressions.

At periodic intervals, 0, 2, 4, 8, 12, 24, 48, 96, 144, and 288 hr, the 50 and 60°C samples were withdrawn, cooled, and analyzed for acetyllysine, glucose, Amadori compound and color. For the 40°C incubation, the withdrawal times were 0, 4, 8, 24, 72, 144, 288, 576, 1156, and 4848 hr because of the expected slower overall rate of reaction at 40°C compared with the other temperatures. The 40°C samples were analyzed for reactants and products as were the 50 and 60°C samples. The cellulose had an initial a_w of 0.37 and an initial moisture content of 5.0%. Copper, iron and cobalt were not detected by the Perkin Elmer emission spectrometer. The cellulose was 98% pure (dry weight basis). The bulk (98.9%) of the cellulose particles were < 400 mesh.

Water activity measurement

Water activities were measured using a Beckman Model EEJA3 Instrument. Water activities of the saturated potassium acetate solution were measured at 25, 40 and 50°C. The a_w of the powders was measured at 30°C.

Moisture determination

Drying at 80°C or higher temperature was not possible because of continuous release of Maillard second stage degradation components. Hence, moisture was determined in duplicate for each temperature model by initially desiccating at room temperature over Drierite[®], later over phosphorus pentoxide to constant weight.

Glucose

The assay employed was the highly specific coupled enzyme reaction catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. Reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Triplicate analyses of the powders were conducted with 3 separate Milli Q water extracts using 40 mg powder and 2 mL water. Centrifugation at 2000 rpm for 15 min was performed to sediment cellulose. The supernatants were clear and free of any cellulose particles. The reduction of NAD to NADH was determined using a Bausch and Lomb Spectrophotometer at 340 nm. Suitable volumes of 20–60 μ L were used for the assays.

Color

Absorbance at 410 nm using a Bausch and Lomb Spectrophotometer of Milli Q extracts of the powders (300 mg and 3 mL water) gave a good indication of the third stage Maillard reaction products, i.e. the brown melanoidin pigments.

Acetyllysine

A Waters Pico.Tag[®] column was used to quantify acetyllysine after derivatization with phenylisothiocyanate. Triplicate analyses of the powders were conducted using 30 mg of powder and 4 mL of Milli Q as in the procedure used for glucose. The solutions were filtered through 0.45 μ m Millex filters (Waters Associates, Milford, MA) to prevent clogging of HPLC column during subsequent analysis. Suit-

Table 2—Estimates of parameters for double exponential mathematical model relating changes in acetyllysine and glucose with time^a

Temp (°C)	A	[10 ⁻³]B	C	[10 ⁻³]D	t _{1/2} hr		Deviation ^c %
					OSB ^b	CALC ^b	
Mathematical Model							
Y = Ae ^{BX} + Ce ^{DX}							
Acetyllysine							
40	57.1	-0.129	41.8	-4.17	1000	1100	0.27
50	43.1	-26.6	56.3	-1.60	102	105	0.24
60	37.5	-3.28	58.6	-114	14.5	12.5	1.1
Glucose							
40	58.8	-0.136	41.1	-6.24	950	1200	0.98
50	36.2	-46.2	62.7	-1.83	120	125	0.16
60	48.6	-3.5	50.3	-143	22	17.5	0.64

^a Acetyllysine-glucose-cellulose powders incubated over saturated potassium acetate solution. X = time in hours, Y = acetyllysine or glucose values (in mg/g powder) expressed as percent of zero time values.

^b OBS = Observed; CALC = Calculated.

^c Expressed as (Sum of squared residuals) × 100 / (Corrected sum of squares)

able volumes (25–75 μ L) were used for derivatization. Two Model 510 pumps were used to obtain the required gradient with Eluent A (pH 5.4 acetate buffer in 6% (v/v) acetonitrile-water) - Eluent B (60% (v/v) acetonitrile-water). A system interface module (SIM) provided microprocessor-control of the pumps and the Waters autosampler (WISP model 710B). It also served as an A/D converter for the detector (Kratos Spectroflow 773) output and was connected with a DEC 350 computer through an input/output (I/O) interface.

The procedure of Bidlingmeyer, Cohen and Tarvin (1984) for amino acids was used. Under these conditions it was possible to separate the reaction product from the unreacted acetyllysine in a Waters HPLC system (Waters Associates, Milford, MA) with detection at 254 nm.

Curve fitting of data and kinetic analysis

A Hewlett Packard, HP 9000, model 236 desk-top computer was used to perform standard nonlinear regression analysis. As one simple means of judging the overall goodness of fit of these nonlinear mathematical models to the data, the sum of squares of the residuals were expressed as percent of the total sum of squares corrected for the mean (Tables 1 and 2). The rate constants and activation energies were determined by standard procedures (Atkins, 1978; Espenson, 1981; Frost and Pearson, 1953; Jencks, 1969). These values were obtained from separate regression analysis and not by graphical methods. The probable order of the reaction was determined from $\ln p$ vs t plots (Wilkinson, 1961) where p = cumulative fraction of acetyllysine or glucose degraded in time t .

RESULTS & DISCUSSION

Application of a standard nonlinear model to fit the kinetic data

The degradation data for acetyllysine and glucose fit a double exponential expression ($Y = Ae^{BX} + Ce^{DX}$) better than the first order expression ($Y = Ae^{BX}$) where X = time and Y = concentration of reactant expressed as a percent of the zero hr value (Fig. 1 and Tables 1 and 2). Figure 1A suggests that the data did not fit the first-order models. The fit appeared to be better using a double exponential expression (see Fig. 1B and 1C). This was further indicated by the much lower percent deviation for the double (0.2 to 1.1) exponential functions (Table 2) compared with the single (9 to 23) exponential functions (Table 1). From the first order expression (Table 1), the first-order rate constant could be derived; it represents the negative value of the coefficient, B. Unlike linear regression, there are no simple means of judging goodness of fit for standard nonlinear models.

Goodness of fit was based upon: comparison of the sum of squared residuals with the total sum of squares corrected for the mean; examination of residuals and residual plots; and correlation coefficients between observed and computed Y values.

Residual plots revealed no indication of notable distortion. Correlation coefficients between observed and computed Y

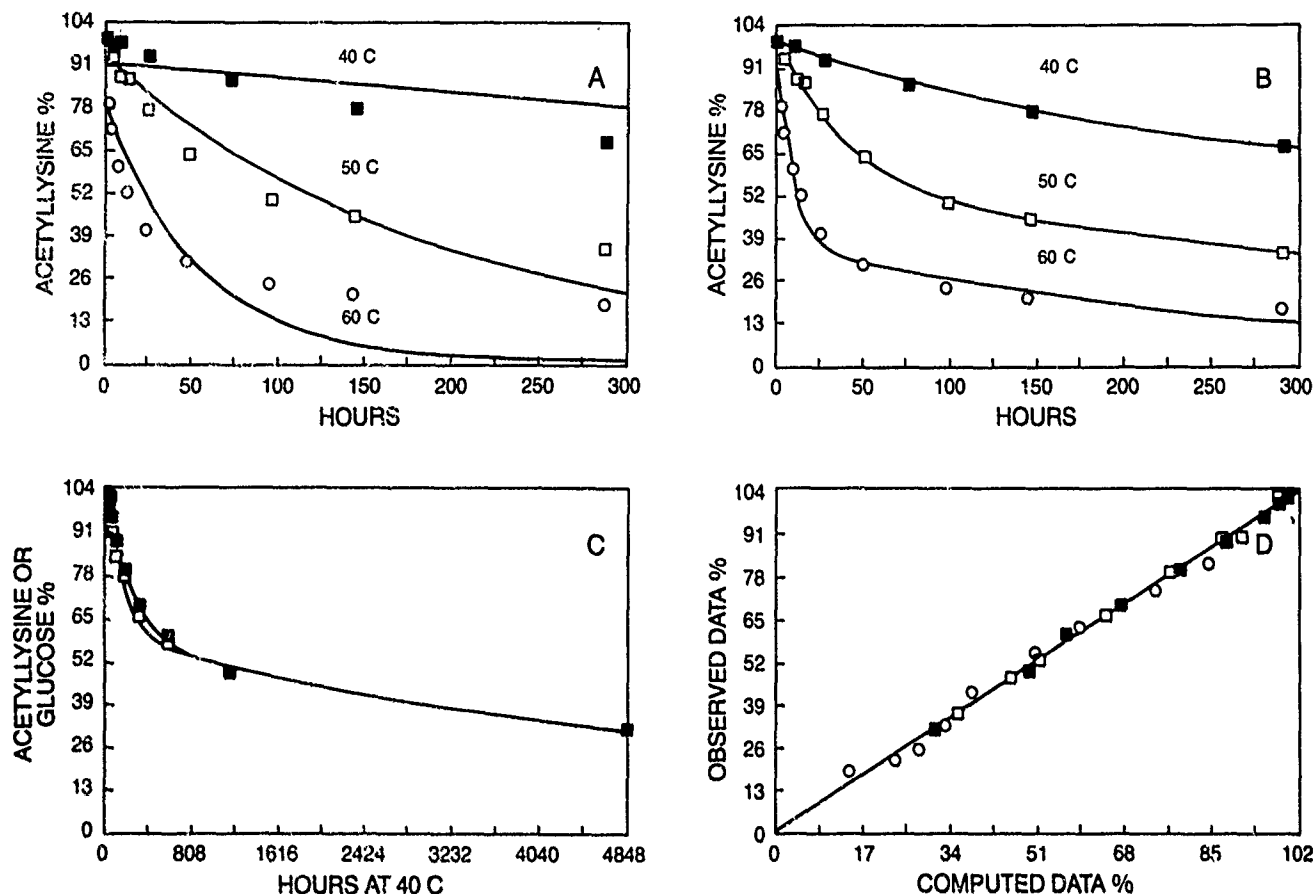


Fig. 1—Curve fitting of acetyllysine and glucose degradation data to exponential functions. Averages of triplicate determinations. The acetyllysine and glucose values (in mg/g powder) are expressed as percent of zero time values. (A) Data fit to the first order exponential equation, $Y = Ae^{kx}$. (B) Data fit to double exponential equations, $Y = Ae^{kx} + Ce^{lx}$. (C) Data fit to double exponential equations, $Y = Ae^{kx} + Ce^{lx}$. (D) Correlation between observed and computed data for acetyllysine loss percent using the double exponential functions at 40 (dark squares), 50 (open squares) and 60°C (open circles).

values provided a third means of testing the goodness of fit. Figure 1D shows excellent correlation ($r > 0.99$) between observed and computed values at all temperatures. These data suggest that there was no notable lack of fit of the data to the tentative mathematical models. However, note that the double exponential expression was developed solely to curve fit the data and thus provide empirical predictive information on long term stability and that it was not developed as a mechanistic tool.

Water activity and equilibration

The water activities (measured at 30°C) of the initial freeze-dried powders were in the range 0.043–0.082 and the moisture contents 0.63 to 0.80%. After incubation at room temperature for 6 days, the a_w were in the range 0.242–0.247 and the moisture contents 1.7–1.9% consistent with previous data using a compressed lysine-glucose-cellulose model (Narayan and Andreotti, 1989).

It is always difficult to strike a balance between absolute equilibration (constant weight to ± 1 mg/g sample) and limiting the degradation at zero time. In published studies a variety of equilibration methods have been used. These included 5°C and a_w 0.33–0.65 for 2 wk with a large quantity of whey powder (Saltmarch et al., 1981). Room temperature (ca 21°C) and a_w 0.11–0.65 for 1 wk was used with 200g of whey powder (Kim et al., 1981). At 50°C no equilibration at a_w 0.65 was used for ovalbumin-aldohehexose models (Kato et al., 1986, 1988). At 25, 30 or 45°C and a_w 0.11–0.85 for 2 to 3 wk was used for fish flour (Labuza et al., 1985). At 40°C, and a_w of 0.23

to 0.82 apparently no equilibration was used for a lysine-glucose-Avicel model system (Eichner, 1975). Infusion of humidified air at 40°C was used with subsequent blending of dry and moistened whey protein concentrates (Hsu and Fennema, 1989). Temperature 25°C and a_w of 0.11 to 0.62 were used with regular and lactose-hydrolyzed milk powder for 1 to 4 days (Burvall et al., 1978).

The attainment of a_w in the range 0.242–0.247 in our experiments suggested that equilibration had been reached. Further, the low initial a_w and the low moisture content of the initial freeze-dried models suggested that equilibration occurred along the adsorption path rather than the desorption path of the hysteresis loop.

The water activity of saturated potassium acetate solution is influenced by temperature. The observed a_w values at 40 and 50°C were both 0.21. Since the instrument could not be operated above 50°C, the a_w at 60°C was estimated in four different ways. Using Raoult's Law and a computed activity coefficient at a_w 40 and 50°C gave a value of 0.19. Extrapolation of the data of Greenspan (1977) from 5 to 30°C gave a_w values of 0.21, 0.20 and 0.19 at 40, 50 and 60°C respectively for a saturated potassium acetate solution. Regression analysis relating $\ln a_w$ with $1/T$ (where T is °K) of our data at 30, 40, and 50°C gave an a_w of 0.20 at 60°C. This a_w was somewhat higher than predicted ($a_w = 0.175$ at 60°C) by the regression equation for potassium acetate by Labuza et al. (1985). In our experiments, the assumption was made that the models equilibrated to the a_w of the salt solutions at the three temperatures following 6 day incubation at room temperature.

The use of small Mason Jars appeared to provide rapid equil-

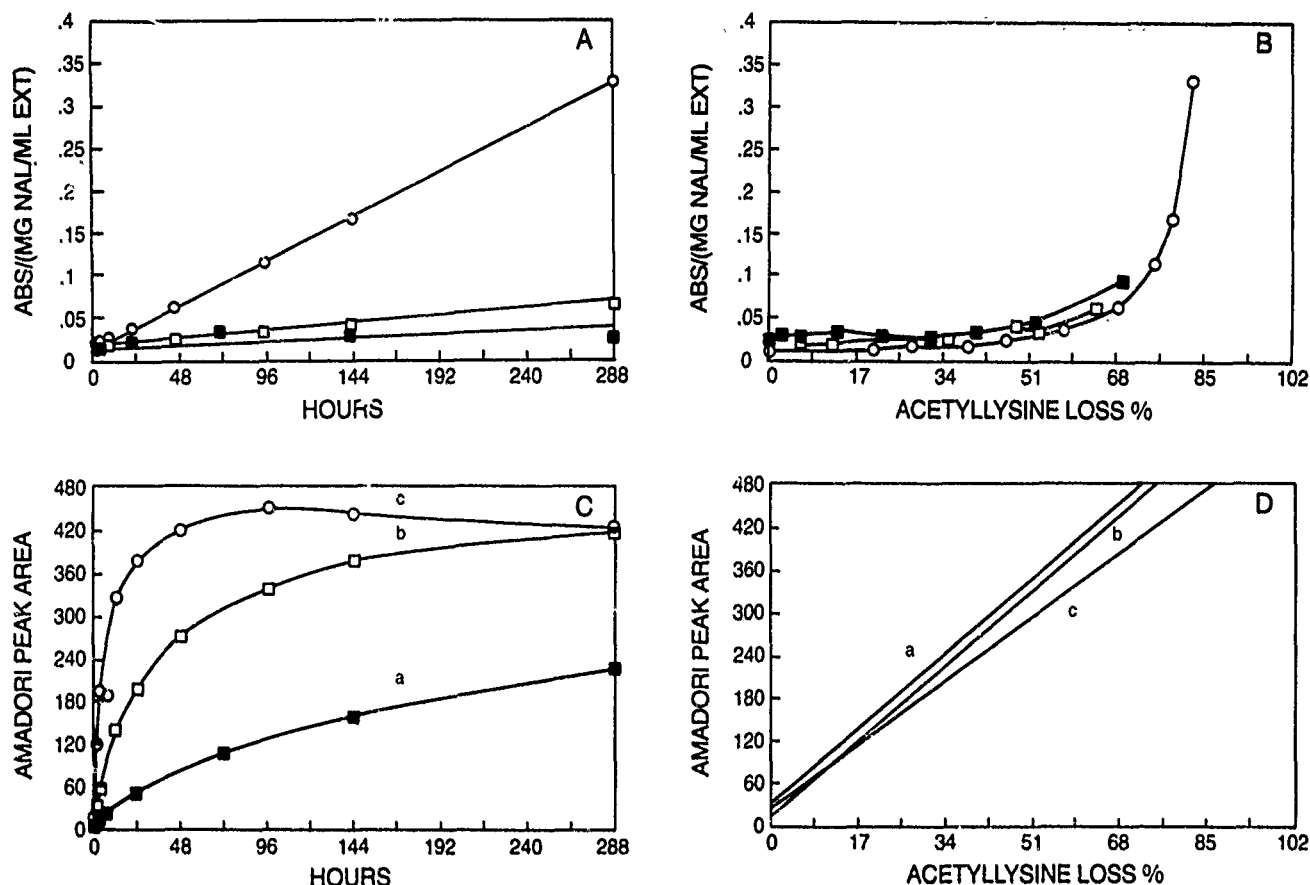


Fig. 2—Quantitative aspects of the formation of brown pigments and of Amadori compound. The data at 40, 50, and 60°C are represented by either dark squares and/or curve a; open squares and/or curve b; and open circles and/or curve c, respectively. (A) The influence of time and temperature on the formation of brown pigments (absorbance at 410 nm/mg acetyllysine per mL of extract at zero hr). (B) Correlation between color and the loss in acetyllysine. (C) Influence of time and temperature on the formation of Amadori compounds expressed as [10²] peak area units/mg acetyllysine per mL of extract at zero hr. (D) Correlation between the increase in peak area of the Amadori compound and the decrease in acetyllysine.

ibration for both powders and compressed bars (Narayan and Andreotti, 1989) because of the greatly reduced headspace as compared with the conventional desiccator method. Further, it was possible to withdraw each sample (i.e. each Jar) at a precise time without affecting the a_w or temperature of other samples.

Brown pigments

The changes in absorbance at 410 nm were linear with time at all temperatures (Fig. 2A). As with the lysine-glucose model reported earlier (Narayan and Andreotti, 1989), no simple linear relationship was found between color and loss in acetyllysine (Fig. 2B). The large effect of temperature seen here (Fig. 2A) and reported before (Narayan and Andreotti, 1989) may have been due to the low a_w of the model. Activation energies for Maillard browning are known to increase with a decrease in a_w (Mizrahi et al., 1970b; Hendel et al., 1955).

Amadori compounds

A peak with retention time 3.4 min was observed during reactions at 40, 50 and 60°C. During the HPLC analysis for acetyllysine, the area of the peak was recorded and related to changes in peak areas for acetyllysine at different reaction times. In order for the unknown compound (peak at 3.4 min) to be considered as an Amadori compound, it had to meet several minimum requirements. The compound should have a reactive group capable of coupling with phenylisothiocyanate. The phenylthiocarbonyl derivative should be significantly polar and

should elute early in the run on a reversed phase C-18 column. The compound should be present in insignificant amounts in the early stages of the reaction. Its concentration should increase with time coincident with the decrease in concentration of the reactant. Since the compound which eluted at 3.4 min met these criteria, it was tentatively identified as the Amadori compound.

The concentration of the compound increased exponentially with time (Fig 2C) and correlated well ($r=0.98$, at all temperatures) with the decrease in acetyllysine, as would be expected with formation of an Amadori compound (Fig. 2D). These data are similar to the high degree of correlation observed in a lysine-glucose-cellulose model (Narayan and Andreotti, 1989) between lysine loss and the formation of Amadori compounds estimated indirectly by furosine.

Order of the reaction

Applicability of kinetic theory to low a_w solid systems. Evidently the reaction in low a_w solid matrices takes place under totally nonideal conditions which are far different from reactions in homogeneous solutions (Frost and Pearson, 1953). In our present study, we have a heterogeneous system and the reactants may not always be in close proximity, especially during the terminal stages of the reaction. High diffusional resistance associated with a low a_w low moisture system, can seriously impede mobility of the reactants. Although here we are primarily concerned with the disappearance of acetyllysine or glucose during the first phase, the Maillard reaction is complex and multistage. As a result, there could be selective in-

hibition spots. While the straight-forward application of the classical chemical kinetic theory to our model may be subject to question, it has been applied in the food area (Labuza and Saltmarsh, 1981; Lea and Hannan, 1949; Saguy and Karel, 1980; Wolf et al., 1977, 1981) and has provided considerable information on shelf life of foods.

Previous studies on lysine loss kinetics. Lenz and Lund (1980) have stated that a first order model adequately described degradation of food components. It is widely accepted that lysine loss in food systems undergoing Maillard degradation follows first order kinetics (Labuza and Saltmarsh, 1981). That is based upon numerous studies where, at least up to 50% loss, the semilog plots of concentration vs time were linear (Lee et al., 1984; Labuza and Massaro, 1990; Warmbier et al., 1976; Warren and Labuza, 1977; Wolf et al., 1977).

Wolf et al. (1977) concluded that the hypothesis for a first order assumption for the lysine loss in a soybean protein concentrate, glucose, Avicel model could not be rejected even at 50% confidence level. Additional studies by Wolf et al. (1981) have further confirmed that postulate. The precise ratio of glucose/lysine was not reported in those experiments. Lee et al. (1984) evaluated the kinetics of lysine degradation for the lysine-glucose Maillard reaction in 100% methanol using a very large excess of glucose and observed pseudo-first order kinetics for lysine loss up to only 70%. A noticeable curvature of the semilog plot was noticed after 70% loss. Lea and Hannan (1949) assumed zero order kinetics for the initial reaction between casein and glucose at 70% relative humidity. Jokinen et al. (1976) have noted that zero order kinetics was applicable for the initial lysine loss in a model system containing soybean protein, glucose, sucrose, starch and Avicel processed between 80 and 130°C.

Labuza and Massaro (1990) investigated lysine loss in a model total parenteral nutrition (TPN) solution at a pH of about 3.8. They have suggested that the complex kinetics they observed could be explained on the basis of a Bodenstein steady state approximation. They proposed a balance between the rate of lysine regenerated from the Amadori product and the rate of degradation of lysine through the normal first phase Maillard reaction between lysine and glucose. This is an interesting possibility and would certainly account for anomalies in the first order plots observed by previous investigators. While it is well known that up to 50% of the lysine is recovered upon hydrolysis of the Amadori compound using concentrated acids, (Hodge, 1953; Hurrell and Carpenter, 1981; Finot et al., 1981) data on regeneration of lysine at normal food pH (5-6) are not available. Investigations on the influence of time, temperature, pH and water activity on freshly synthesized fructose-lysine may help to test and extend this hypothesis.

Reaction kinetics and molecularity. In a first order reaction, the rate is proportional to concentration of a reactant. In a second order reaction, the rate is proportional to the product of the concentrations of two reactants. A second order reaction may appear to behave like a first order reaction when there is a very large excess of one of the reactants. Although molecularity and order are not synonymous, they are often coincidental (Glasstone, 1961). Further, a bimolecular reaction is usually second order (Frost and Pearson, 1953). Thus, it is easy to conceive that the thermal degradation of thiamin would follow first order kinetics. For the first phase of the Maillard reaction between lysine and glucose, teleologically we would theorize that the reaction rate would be dependent upon the concentration of the 2 reacting species. In the past, Tsao et al. (1978) made the explicit assumption that lysine loss followed a monomolecular reaction. On the basis of partial linearities of the first order plots, other investigators have concluded that lysine loss followed first order kinetics.

Determination of the kinetic order of the reaction. Our study is one of the few investigations to follow kinetics of the lysine-glucose reaction where the glucose/acetyllysine ratio at zero time was set at 1. The semilog plot of concentration vs

time was a straight line only up to $\leq 50\%$ loss and confirmed numerous other published data described above. In the studies where the glucose/lysine ratio was kept at 2.3, nonlinearity of the first order plots was also observed (Narayan and Andreotti, 1989).

In our present study, the second-order rate constants were obtained by plotting the reciprocal of the concentrations vs time at the three temperatures (Fig. 3A). The correlation coefficients of the linear expressions were 0.95, 0.98 and 0.93, at 40, 50, and 60°C, respectively. The lack of better linearity, particularly at 40, and 60°C, of these reciprocal plots posed the question whether the reaction followed second order kinetics or some other kinetics at all temperatures. Wilkinson plots (1961) were therefore constructed for all temperatures by plotting t/p vs t where p = cumulative fraction of reactant degraded at time = t . Wilkinson has shown that the order of a reaction (zero, first, second or third) could be determined from the slope of a single plot. On the basis of these plots (Fig. 3B), a reaction order of 2.2 to 2.6 was estimated for acetyllysine degradation at the three temperatures. This led to the conclusion that the reaction was more appropriately described by a second order rate expression than by a first order rate expression at all three temperatures.

The advantage of a Wilkinson plot is that it permits a simple differentiation between first-order and second-order reactions on the basis of initial data points in an experiment (Espenson, 1981). Furthermore, Espenson has convincingly demonstrated that if the data from a second order reaction are plotted as a first order plot (\ln concentration vs time) noticeable curvature would be observed beyond approximately 50% reaction. It would be imprudent to conclude that every nonlinear first order plot implies a second order kinetics. However, further tests such as fractional time approach, Wilkinson plots and statistical testing of rate constants to determine the order of the reaction should not be excluded (Espenson, 1981; Frost and Pearson, 1953; Jencks, 1969; Wilkinson, 1961; Wolf et al., 1981).

Estimation of activation energy and Q_{10}

In a previous study, Narayan and Andreotti (1989) evaluated the interaction between lysine and glucose in a cellulose matrix. The present model was less complex since acetyllysine has only one free amino group and is therefore comparable to a protein-reducing sugar model. Using second order rate constants, the E_a and Q_{10} were coincidentally 36 kcal/mol and 5.5 respectively for the degradation of both acetyllysine and glucose (Fig. 3C).

The estimated E_a of 36 kcal/mol from the present study was lower than the value of 57 kcal/mol observed for the lysine-glucose-cellulose model and is attributable to the greater sensitivity to temperature of the lysine-glucose model. The first order rate constants of $0.52 \times 10^{-3} \text{ hr}^{-1}$ and $1.89 \times 10^{-3} \text{ hr}^{-1}$ at 40°C for the acetyllysine and lysine degradation, respectively, gave an indication of the greater reactivity of lysine at 40°C. Similarly, the first-order rate constants at 50 and 60°C for the acetyllysine-glucose model were considerably lower than those observed for the lysine-glucose model ($5.0 \times 10^{-3} \text{ hr}^{-1}$ and $23 \times 10^{-3} \text{ hr}^{-1}$, respectively, vs $54 \times 10^{-3} \text{ hr}^{-1}$ and $472 \times 10^{-3} \text{ hr}^{-1}$, respectively).

Activation energy values have also been computed from first order fitted equations (Table 1) for acetyllysine and glucose degradations. Those values were for acetyllysine 37 and glucose 29 kcal/mol. The values are close to that obtained from acetyllysine loss using second order rate constants. For glucose loss, the E_a value was not substantially different from that computed with second order rate constants.

Comparison of computed E_a value with published values. With respect to losses in protein quality and available lysine, Lea and Hannan (1949) have obtained an E_a of 29 kcal/mol in a casein-glucose food system equilibrated to 70% R.H. and stressed between 0 to 70°C. Jokinen et al. (1976) reported an

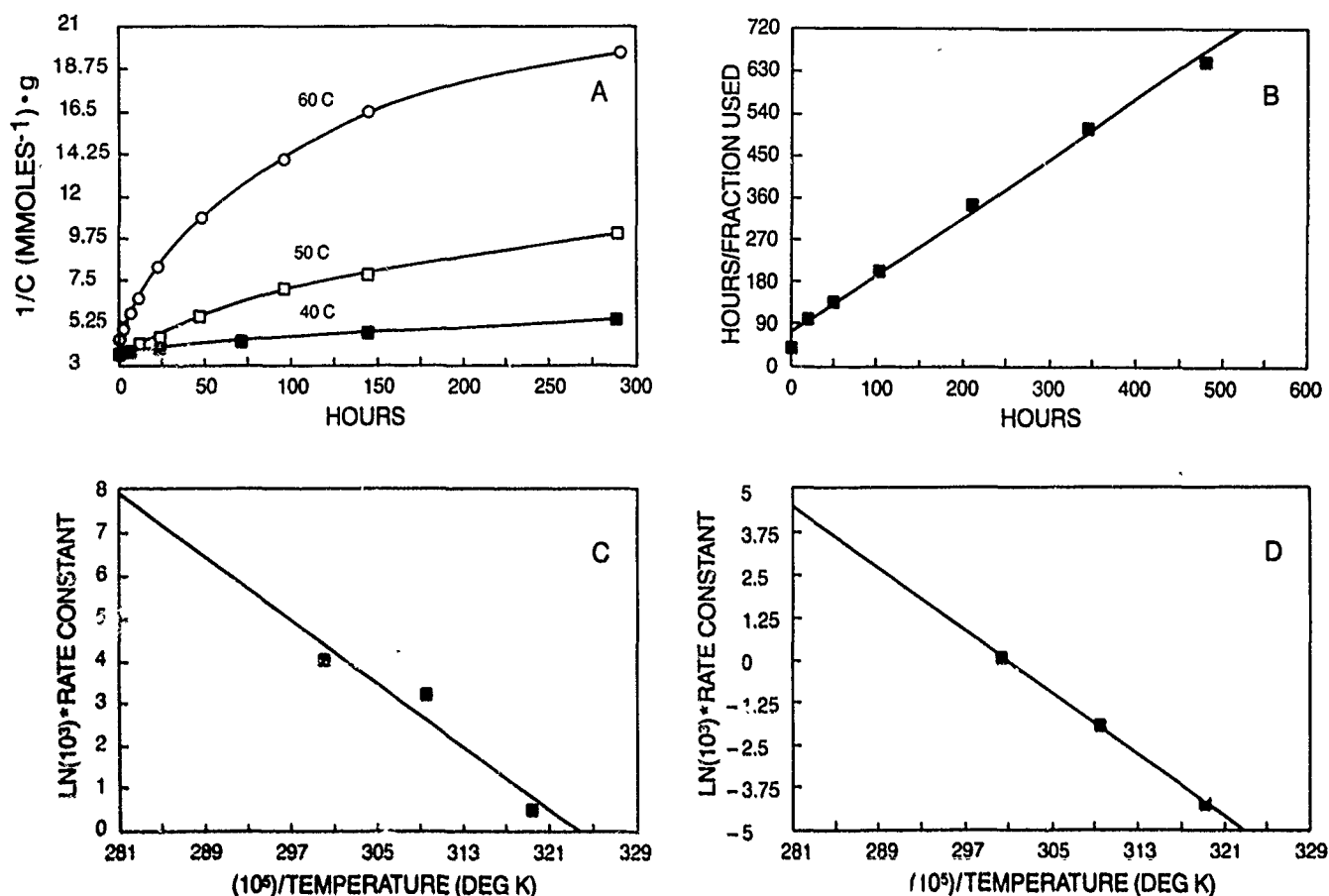


Fig. 3--Determination of order of the reaction between acetyllysine and glucose, and the determination of the E_a for acetyllysine degradation and Maillard browning. (A) Second order plots for acetyllysine degradation at 40, 50, and 60°C: $1/c$ plotted against time. (B) Wilkinson plot t/p vs t of data obtained at 50°C for lysine degradation. p = fraction completed in time t . (C) Arrhenius plot for acetyllysine loss at 40, 50, and 60°C. (D) Arrhenius plot for Maillard browning at 40, 50, and 60°C.

E_a of 28.5 kcal/mol for a soybean-glucose model system stressed at 30 to 80°C and a_w of 0.33 to 0.93. Similar E_a values in the range 25–37.5 kcal/mol have been reported by other workers (Carpenter et al., 1962; Taira et al., 1966) in two food systems (herring press cake and soybean meal, respectively). Our value of 36 kcal/mol (Fig. 3C) was in the range of those studies. However, low E_a values in the range of 5 to 19 kcal/mol have also been reported (Ben-Gara and Zimmerman, 1972; Chen et al., 1983; Kaanane and Labuza, 1985; Labuza et al., 1982; Tsao et al., 1978) in several food systems (nonfat mild powder, pasta, fish flour, egg noodles, and fortified rice meal respectively). The low E_a values in the range 5–13 kcal/mol for fish flour stored in open and sealed systems at 25, 38 and 45°C at three a_w of 0.33, 0.44, and 0.65 have been attributed to a free radical induced reaction, rather than to the Maillard reaction (Kaanane and Labuza, 1985).

The low E_a value of 12.5 kcal/mol obtained by Tsao et al. (1978) for fortified rice meal has been rationalized on the basis of the physical structure and the high temperatures used (Labuza and Saltmarch, 1981). Further, it is recognized the trinitrobenzene sulfonic acid method used vastly overestimates available lysine (Hurrell and Carpenter, 1981; Mauron, 1981). The low E_a values for lysine losses in pasta and egg noodles may, in part, be related to the reliability of the rate constants at low temperatures where less than 25% lysine loss occurred.

Maillard browning. The linear responses for color with time ($r=0.98$ to 0.999) in our present experiments have permitted us to compute an E_a of 44 kcal/mol from zero order rate constants for Maillard browning (Fig. 3D). As an explanation for the wide difference in kinetics between lysine loss and browning, note that pig. .ant formation is not part of the

main reaction between acetyllysine and glucose but essentially takes place during the third phase of the Maillard reaction.

Stamp and Labuza (1983) estimated an E_a of 15.5 kcal/mol for Maillard browning in solution due to reaction of glycine with glucose at a_w 0.80. Published values of E_a for Maillard browning vary over a wide range from 16 to 40 kcal/mol and appear to be dependent upon a_w . The high E_a of 44 kcal/mol we observed was consistent with previous observations (H udel et al., 1955; Mizrahi et al., 1970b) that E_a for browning is inversely related to the a_w .

CONCLUSIONS

THE ACETYLlysine-GLUCOSE MODEL is comparable to a protein-glucose model because in both cases, only the ϵ -amino groups are free for interaction. The protein model requires enzymatic digestion to liberate the amino acids. In the acetyllysine model, a simple extraction followed by derivatization at room temperature for 20 min is all that is necessary to follow the degradation of acetyllysine. It is of considerable interest that 22 and 32% of the acetyllysine were lost in 6 and 12 days, respectively, in this model under relatively moderate conditions ($a_w = 0.21$ and 40°C). Additionally, as much as 69% was lost in less than 7 mo. Note, however that the reducing sugar was present at an equimol level with respect to acetyllysine, a situation that may not prevail in most foods. Dry foods (such as milk chocolate, yogurt products, dried fruits, milk powders, dairy cream products, egg powders, pancake mixes, cake mixes and biscuit mixes) containing proteins and/or amino acids and reducing sugars may be particularly vulnerable to adverse changes in protein quality and consequently

adversely influence shelf life and consumer acceptance. Further, our observations suggest that these protein quality losses may occur at a greater rate than recognized on the basis of generally accepted water activity-quality loss curves.

REFERENCES

- Atkins, P.W. 1978. The rates of chemical reactions. Ch. 26. In *Physical Chemistry*, P.W. Atkins (Ed.), p. 851. Oxford University Press, U.K.
- Ben-Gara, I., and Zimmerman, G. 1972. Changes in the Nitrogenous Constituents of Staple Foods and Feeds During Storage. 1. Decrease in the chemical availability of lysine. *J. Food Sci. Technol.* 9: 113.
- Bidlingmeyer, B.A., Cohen, S.A., and Tarvin, T.L. 1984. Rapid analysis of amino acids using precolumn derivatization. *J. Chromatog.* 336: 93.
- Burvall, A., ASP, N.-G., Bosson, A., San José, C., and Dahlqvist, A. 1978. Storage of Lactose-hydrolysed dried milk: Effect of water activity on the protein nutritional value. *J. Dairy Res.* 45: 381.
- Carpenter, K.J., Morgan, C.B., Lea, C.H., and Parr, L.J. 1962. Chemical and nutritional changes in stored herring meal. 3. Effect of heating at controlled moisture contents on the binding of amino acids in freeze-dried herring press cake and related model systems. *Brit. J. Nutr.* 16: 451.
- Chen, J.Y., Bohnsack, K., and Labuza, T.P. 1983. Kinetics of protein quality loss in enriched pasta stored in a sine wave temperature condition. *J. Food Sci.* 48: 460.
- Desrosiers, T., Savoie, L., Bergeron, G., and Parent, G. 1989. Estimation of lysine damage in heated whey proteins by furosine determinations in conjunction with the digestion cell technique. *J. Agric. Food Chem.* 37: 1385.
- Eichner, K. 1975. The influence of water content on nonenzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. Ch. 23. In *Water Relations of Food*, R.B. Duckworth (Ed.), p. 417. Academic Press, New York.
- Eichner, K. and Karel, M. 1972. The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J. Agr. Food Chem.* 20: 218.
- Espenson, J.H. 1981. Reactions with a simple kinetic form. Ch 2. In *Chemical Kinetics and Reaction Mechanisms*, J.H. Espenson (Ed.), p. 12. McGraw-Hill Book Company, New York.
- Finot, P. A., Deutsch, R., and Eujard, E. 1981. The extent of Maillard reaction during the processing of milk. In *Progress in Food and Nutrition Science. Maillard Reactions in Food*, C. Erickson (Ed.), p. 345. Pergamon Press Ltd., Great Britain.
- Flink, J.M., Hawkes, J., Chen, H., and Wong, E. 1974. Properties of the freeze drying "scorch" temperature. *J. Food Sci.* 39: 1244.
- Frost, A.F. and Pearson, R.G. 1953. Empirical treatment of reaction rates. Ch. 2. Experimental methods and treatment of data. Ch. 3. In *Kinetics and Mechanism. A study of homogeneous chemical reaction*, A.F. Frost and R.G. Pearson (Ed.), p. 9, 27. John Wiley & Sons, New York.
- Glasstone, S. 1981. Chemical kinetics. Ch. 13. In *Textbook of Physical Chemistry*, S. Glasstone (Ed.), p. 1044, D. Van Nostrand Company Inc., New York.
- Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Nat. Bur. Stand. (U.S.)* 81A: 89.
- Hannan, R.S. and Lea, C.H. 1952. Studies of the reaction between proteins and reducing sugars in the "dry" state. *Biochim. Biophys. Acta.* 9: 293.
- Hendel, C.E., Silveira, V.G., and Harrington, W.O. 1955. Rates of nonenzymatic browning of white potatoes during dehydration. *Food Technol.* 9: 433.
- Hodge, J.E. 1953. Dehydrated foods. Chemistry of browning reactions in model systems. *J. Agr. Food Chem.* 1: 928.
- Hsu, K.-H. and Fennema, O. 1989. Changes in the functionality of dry whey protein concentrate during storage. *J. Dairy Sci.* 72: 829.
- Hurrell, R.F. and Carpenter, K.J. 1981. The estimation of available lysine in food stuffs after Maillard reactions. In *Progress in Food and Nutrition Science. Maillard Reactions in Food*, C. Erickson (Ed.), p. 159. Pergamon Press Ltd., Great Britain.
- Jencks, W.P. 1969. Practical kinetics. Ch. 11. In *Chemistry and Enzymology*, W.P. Jencks (Ed.), p. 555. McGraw-Hill Book Co., New York.
- Jokinen, J.E., Reineccius, G.A., and Thompson, D.R. 1976. Losses in available lysine during thermal processing of soy protein model systems. *J. Food Sci.* 41: 816.
- Kaanane, A. and Labuza, T.P. 1985. Changes in available lysine loss reaction rate in fish flour due to an a_w change induced by a temperature shift. *J. Food Sci.* 50: 582.
- Kato, Y., Matsuda, T., Kato, N., and Nakamura, R. 1988. Browning and protein polymerization induced by amino-carbonyl reaction of ovalbumin with glucose and lactose. *J. Agric. Food Chem.* 36: 806.
- Kato, Y., Matsuda, T., Kato, N., and Watanabe, K. and Nakamura, R. 1986. Browning and insolubilization of ovalbumin by the Maillard reaction with some aldohexoses. *J. Agric. Food Chem.* 34: 351.
- Kim, M. N., Saltmarch, M., and Labuza, T.P. 1981. Non-enzymatic browning of hygroscopic whey powders in open versus sealed pouches. *J. Food Proc. Pres.* 6: 49.
- Kopelman, I.J., Meydud, S., and Weinberg, S. 1977. Storage studies of freeze dried lemon crystals. *J. Food Technol.* 12: 403.
- Labuza, T.P. 1982. Scientific Evaluation of Shelf-life. Ch. 3. In *Shelf-life Dating of Foods*, T.P. Labuza (Ed.), p. 41. Food and Nutrition Press, Trumbull CT.
- Labuza, T.P., Bohnsack, K., and Kim, M.N. 1982. Kinetics of protein quality change in egg noodles stored under constant and fluctuating temperatures. *Cereal Chem.* 59: 142.
- Labuza, T.P., Kaanane, A., and Chen, J.Y. 1985. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *J. Food Sci.* 50: 385.
- Labuza, T.P. and Massaro, S.A. 1990. Browning and amino acid loss in model total parenteral nutrition solutions. *J. Food Sci.* 55: 821.
- Labuza, T.P. and Saltmarch, M. 1981. The nonenzymatic browning reaction as affected by water in foods. In *Water Activity/Influence of on Food Quality*, L.B. Rockland and G.F. Stewart (Ed.), p. 605. Academic Press, New York.
- Lea, C.H. and Hannan, R.S. 1949. Studies of the reaction between proteins and reducing sugars in the "dry" state. 1. The effect of activity, of water of pH and of temperature of the primary reaction between casein and glucose. *Biochim. Biophys. Acta.* 3: 313.
- Lee, C.M., Sherr, B., and Koh, Y.-N. 1984. Evaluation of Kinetic parameters for a glucose-lysine Maillard reaction. *J. Agric. Food Chem.* 32: 379.
- Lenz, M.K. and Lund, D.B., 1980. Experimental procedures for determining destruction kinetics of food components. *Food Technol.* 34: 51.
- Loncin, M., Bimbenet, J.J., and Lengès, J. 1968. Influence of the activity of water on the spoilage of foodstuffs. *J. Food Technol.* 3: 131.
- Mauron, J. 1981. The Maillard reaction in food; A critical review from the nutritional standpoint. In *Progress in Food and Nutrition Science. Maillard Reactions in Food*, C. Erickson (Ed.), p. 4. Pergamon Press Ltd., Great Britain.
- Mizrahi, S., Labuza, T.P., and Karel, M. 1970a. Computer-aided predictions of extent of browning in dehydrated cabbage. *J. Food Sci.* 35: 799.
- Mizrahi, S., Labuza, T.P., and Karel, M. 1970b. Feasibility of accelerated tests for browning in dehydrated cabbage. *J. Food Sci.* 35: 804.
- Narayan, K.A. and Andreotti, R.E. 1989. Kinetics of lysine loss in compressed model system due to Maillard reaction. *J. Food Biochem.* 13: 105.
- Petriella, C., Chirife, J., Resnik, S.L., and Lozano, R.D. 1988. Solute effects at high water activity on nonenzymatic browning of glucose-lysine solutions. *J. Food Sci.* 53: 987.
- Ringe, M.L. and Love, M.H. 1988. Kinetics of protein quality change in an extruded cowpea-corn flour blend under varied steady-state storage conditions. *J. Food Sci.* 53: 584.
- Saguy, I. and Karel, M. 1980. Modeling of quality deterioration during food processing and storage. *Food Technol.* 34: 78.
- Saltmarch, M., Vagnini-Ferrari, M., and Labuza, T.P. 1981. Theoretical basis and application of kinetics to browning in spray-dried whey food systems. In *Progress in Food and Nutrition Science. Maillard Reactions in Food*, C. Erickson (Ed.), p. 333. Pergamon Press Ltd., Great Britain.
- Schnickels, R.A., Warmbier, H.C., and Labuza, T.P. 1976. Effect of protein substitution on nonenzymatic browning in an intermediate moisture food system. *J. Agric. Food Chem.* 24: 901.
- Stamp, J.A. and Labuza, T.P. 1983. Kinetics of the Maillard reaction between aspartame and glucose in solution at high temperatures. *J. Food Sci.* 48: 543.
- Taira, H., Taira, H., and Sukukai, Y. 1966. Japan J. Nutr. Food. 18: 359. [In Labuza, T.P. and Saltmarch, M. (1981)].
- Tsao, T.F., Frey, A.L., and Harper, J.M. 1978. Available lysine in heated fortified rice meal. *J. Food Sci.* 43: 1106.
- Warmbier, H.C., Schnickels, R.A., and Labuza, T.P. 1976. Nonenzymatic browning kinetics in an intermediate moisture model system: Effect of glucose to lysine ratio. *J. Food Sci.* 41: 981.
- Warren, R.M. and Labuza, T.P. 1977. Comparison of chemically measured available lysine with relative nutritive value measured by Tetrahymena bioassay during early stages of nonenzymatic browning. *J. Food Sci.* 42: 429.
- Wilkinson, R.W. 1961. A simple method for determining rate constants and orders of reaction. *Chem. Ind.* 1395.
- Wolf, J.C., Thompson, D.R., and Reineccius, G.A. 1977. Initial losses of available lysine in model systems. *J. Food Sci.* 42: 1540.
- Wolf, J.C., Warthesen, J.J., Thompson, D.R., and Reineccius, G.A. 1981. Mathematical model for predicting free lysine and methionine losses during thermal processing of fortified foods. In *Progress in Food and Nutrition Science. Maillard Reactions in Food*, C. Erickson (Ed.), p. 405. Pergamon Press Ltd., Great Britain.

Ms received 5/17/90; revised 3/28/91; accepted 7/9/91.

Presented in part at the 1989 IFT Annual meeting, Chicago, June 25-29. We thank J. Jarboe, W.L. Porter, C.P. Dunne, and M. Lightbody, US Army Natick Research, Development & Engineering Center (Natick) and K.W. Whitburn, Framingham State college for review of the manuscript. We acknowledge valuable discussions on curve fitting with Ron Segars and Linda Hoke, Natick. The invaluable help of J. Jarboe in the analysis of cellulose, of E. Powers and D. Munsey in the determination of water activity, of Capt. K. Narayan, Tinker Air Force Base, Oklahoma in the creation of expert graphics and data processing software, and of A.M. Huwe for her excellent secretarial assistance is gratefully acknowledged.