Kinetics of Protein Quality Change in Egg Noodles Stored Under Constant and Fluctuating Temperatures[1]

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ABSTRACT

Loss of protein quality, measured by available lysine and extent of nonenzymatic browning, was studied in pasta. Pasta was stored for 48 weeks under constant conditions of 25, 35, 45, and 55°C and water activity (a_w) levels of 0.44, 0.52, 0.65, and 0.75. In addition, pasta at each a_w level was stored under a square wave temperature profile of 3½-day periods at 35 and 55° C. Data from the constant conditions were used to test the applicability of equations for predicting losses under the fluctuating condition. As expected, browning rate and loss of protein quality increased with increased temperature and a_w. The Q_{10} for browning and lysine loss ranged between 2 and 4. At 25° C, about 25% of the lysine was lost in 40–50 weeks, whereas at 45° C, the same loss occurred in 6–12 weeks. The amount of loss for the fluctuating condition was greater than that occurring at the mean temperature of 45° C. The Arrhenius relationship equations gave variable results in predicting the extent of change using steady-state data, although the absolute error was generally less than four weeks.

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Studies that analyze loss of protein quality of foods with respect to nonenzymatic browning are usually based on loss of the essential amino acid, lysine. Goldblith and Tannenbaum (1966), in a review of the literature before 1965, pointed out that lysine loss in foods is primarily caused by the Maillard reaction and can usually be estimated from the extent of browning. Lysine is usually lost more rapidly than other essential amino acids in the first stages of the Maillard reaction because of the free ε-amino group. However, Warren and Labuza (1977), Warmbier et al (1976a, 1976b), Eichner and Karel (1972), and Labuza and Saltmarch (1981b) demonstrated a substantial loss in available lysine before the visual development of brown pigments.

To understand the specific effects of water activity (a_w) and temperature on the rate of lysine loss, one can use a simplified mathematical model based on first order kinetics for up to about 50% loss. The loss of lysine can be described by:

$$\frac{dA}{d\theta} = -k_L(A) \qquad (1)$$

where θ = time, A = concentration of lysine at any θ, and k_L = rate constant (which is a function of a_w) in reciprocal time.

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If $A = A_o$ at time zero, this equation can be integrated into equation 2, which gives a straight line when $\ln (A/A_o)$ is plotted vs time:

$$\ln\left(\frac{A}{A_o}\right) = -k_L\theta \qquad (2)$$

where A_o = original concentration of lysine. The slope of this straight line is equal to k, the rate constant, and from studies at several temperatures, the Arrhenius activation energy (E_A) or the Q_{10} can be obtained, both of which give a measure of the temperature sensitivity of the reaction. E_A is invariable; therefore Q_{10} is a function of both temperature and the E_A as follows:

$$Q_{10} = \frac{\text{rate at temp } (T_m + 10)^\circ K}{\text{rate at temp } T_m^\circ K} = \frac{\text{shelf life at } T_m^\circ K}{\text{shelf life at } (T_m + 10)^\circ K} \qquad (3)$$

or

$$\log_{10}Q_{10} = \frac{2.2\,E_A}{(T_m)\,(T_m + 10)} \qquad (4)$$

where T_m = mean temperature and E_A is measured in calories per mole.

For the browning associated with protein quality loss, the data usually fit a zero order equation (Labuza and Saltmarch 1981b) of the form:

$$B = B_o + k_B\theta \quad (5)$$

where B = browning value at θ, B_o = initial value, and k_B = rate constant in browning value per unit of time. The E_as reported in the literature for lysine loss range from 10 to 38 kcal/mole, whereas Q_{10} values range from as low as 1.5 to a high of 4.7 (Labuza and Saltmarch 1981b).

With respect to a_w, most studies with dehydrated foods show a maximum rate of browning and of lysine loss in the 0.6–0.8 a_w range (Labuza and Saltmarch 1981b). For example, studies on dried milk (Holsinger et al 1970, Burvall et al 1977) indicated that as time, temperature, and moisture content are increased, the rate of protein quality loss increases. The maximum is due to the separate effects of mobility and dilution (Labuza 1980b).

With respect to pasta doughs, Fabriani and Frantoni (1972), using the 1-fluoro-2,4-dinitrobenzene (FDNB) procedure, found significant loss during high-temperature holding, as seen in Fig. 1. Unfortunately only an abstract was published and the type of pasta and the a_w were not presented. The data show only about 30% loss after storage for 18 months at 45°C, which seems to be minimal loss. The E_A calculated from this data is 13.3 kcal/mole. Other data by Cubadda et al (1968, 1970), using amino acid analysis and protein efficiency ratios, showed much more loss in less than 6–10 hr at 60–80°C, which suggests that the abstract of Fabriani et al (1972) may have been incorrect. These studies were done at an a_w of 0.08–0.11, which is quite low.

The overall purpose of this study was to determine the extent of browning and lysine loss in pasta stored under normal storage conditions of constant temperatures (25, 35, 45, and 55°C) and constant humidities (44, 52, 65, and 75%). In addition, pasta was also stored under fluctuating temperature conditions (35 and 55°C) to determine whether the actual loss that occurred could be predicted from the steady-state data using the methods of Kamman et al (1981) and of Labuza and Saltmarch (1981a) as originally developed by Labuza (1979).

MATERIALS AND METHODS

Pasta

The pasta used in the present study was "Wide Egg Noodle" (¼-in. width) obtained from Delmonico Foods, Louisville, KY. A fortified egg noodle was used to make lysine measurement easier.

Five hundred grams of pasta were humidified over specific saturated salt solutions at room temperature for four weeks under vacuum and covered to exclude light, as was done by Kamman et al (1981). The a_ws for the storage study were 0.44, 0.52, 0.65, and 0.75. Replicate 5-g samples of pasta from each a_w were sealed in aluminum laminated pouches and stored at 25, 35, 45, and 55°C for the steady-state accelerated shelf life tests. When removed for analysis, each sample was ground in a Waring Blendor for 90 sec and then ground again as finely as possible in a porcelain mortar just before use. Triplicate samples were analyzed in duplicate at zero time for each condition, and single samples were analyzed in duplicate at least 13 times during storage over a period of 16–48 weeks. The pasta for the fluctuating storage conditions was humidified in the same way at each of the four a_ws and then stored for alternate 3½-day periods, first at 55°C and then at 35°C. Samples were taken at the end of each week for 14 weeks. Previous work by Kamman et al (1981) showed that the come-up/come-down times were negligible and should not affect the kinetics. At a_w 0.75 no mold growth occurred.

Sorption Isotherm

To obtain the sorption isotherm, portions of the pasta were equilibrated at a_ws of 0.11, 0.32, 0.44, 0.52, 0.65, and 0.75 for four weeks in air at 25°C. After humidification, triplicate 1-g samples of ground pasta were dried at 70°C for 18 hr under vacuum at 28 in. of Hg. The a_ws were also determined using the vapor pressure manometric method (Lewicki et al 1978) to ensure that the samples were properly humidified. The resulting isotherm is shown in Fig. 2.

Extent of Browning: Three-Enzyme Digestion Method

The three-enzyme digestion method, originated by Choi (1949) and modified by Saltmarch et al (1980) was employed. The assay procedure basically uses a digestion at 37°C with trypsin, μ-chymotrypsin, and peptidase, followed by measurement of the absorbance of the filtrate at 420 nm. The results are reported as absorbance per 100 g, dry basis.

Available Lysine

Available lysine (using FDNB) was determined according to a modification of Booth's method (1971), which was adapted by Labuza and Saltmarch (1981a).

Two grams of powdered pasta were weighed into a 250-ml boiling flask and 10 ml of $NaHCO_3$ solution was added to each flask. The flask was then shaken for 1 hr at 37°C and 200 rpm in the New Brunswick Environmental Shaker Incubator model G 24 to disperse the sample. Then 15 ml of FDNB solution, which contained 0.4 ml of FDNB in 15 ml of 95% ethyl alcohol, was added. The flasks were shaken again at room temperature and 200 rpm for 3 hr in the same incubator. Thereafter, the samples were treated as in the Booth's method (1971). The results were reported on a dry solids basis.

RESULTS AND DISCUSSION

Figure 3 shows an example of the results for extent of browning at 45°C as a function of time at each of the four a_ws. Considerable scatter occurred, which did not happen in the Kamman et al (1981)

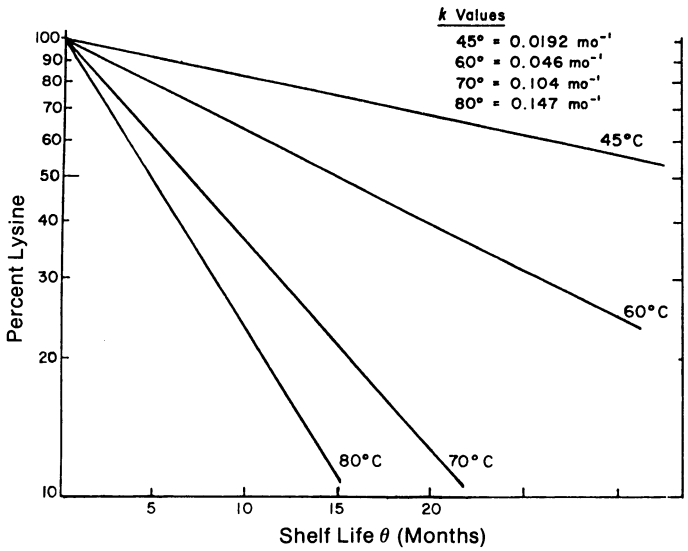

Fig. 1. Percent lysine retained as a function of storage temperature (from Fabriani and Frantoni, 1972). **K** = rate constant. θ = time.

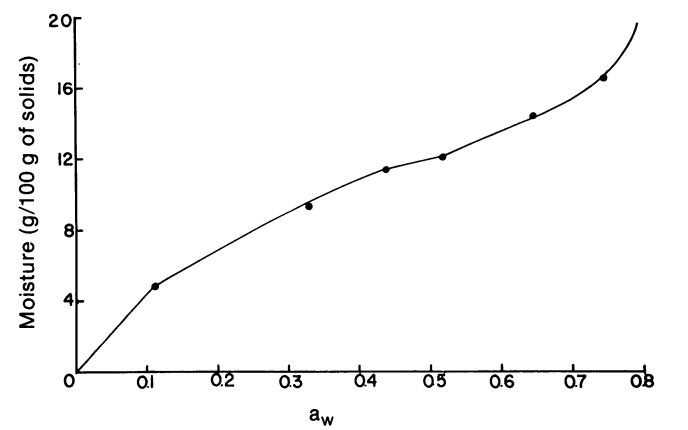

Fig. 2. Sorption isotherm (25°C) for pasta (moisture, dry basis, vs water activity [a_w] level).

studies of thiamin loss in pasta, although that may be because more data points were taken in the present study. Best straight lines based on zero order kinetics using least squares regression were calculated for the data at all temperatures and a_ws. Each set of data was analyzed statistically; the resulting kinetic data are shown in Table I. For each condition, the r^2 value and the rate constant ± the 95% confidence limits are shown. In addition, the time to reach a B of 50/100 g of dry solids (which was found to be when it was just noticeably brown) was calculated, using the upper and lower 95% confidence limits of the B_o and the upper and lower 95% confidence limits of the rate constant. This gives the most conservative estimate from the data, ie, the shortest time (using the highest initial browning value and highest rate constant in equation 5) and the longest time (using the lowest initial value and lowest rate constant). Table I shows that the rate of browning increases as temperature increases from 25 to 55° C. The poorest correlations for zero order are at the lowest temperature, at which the least change occurred.

TABLE I
Zero Order Browning Kinetics for Egg Noodles as a Function of Storage Conditions

Temperature (° C)		Water Activity 0.44	0.52	0.65	0.75
25	r^2	0.863	0.864	0.921	0.875
	k[a]	0.601 ± 0.196	1.005 ± 0.326	1.365 ± 0.326	0.783 ± 0.242
	θ_s[b]	46.2/97.8	26.7/59.0	21.5/40.0	35.1/73.9
35	r^2	0.861	0.961	0.981	0.850
	k	0.646 ± 0.196	1.052 ± 0.161	1.158 ± 0.426	0.874 ± 0.28
	θ_s	42.7/86.3	30.3/43.9	29.3/37.9	29.7/64.9
45	r^2	0.934	0.980	0.942	0.952
	k	1.602 ± 0.283	1.869 ± 1.190	2.168 ± 0.358	2.642 ± 0.395
	θ_s	19.0/30.6	21.3/26.11	13.7/22.2	11.0/17.7
55	r^2	0.906	0.969	0.889	0.924
	k	3.357 ± 0.574	3.971 ± 0.355	5.955 ± 1.115	6.162 ± 0.936
	θ_s	7.31/14.4	7.9/11.5	2.6/8.3	2.7/7.2
35/55	r^2	0.817	0.808	0.890	0.908
	k	2.693 ± 0.700	2.687 ± 0.721	4.001 ± 0.775	5.856 ± 1.026
	θ_s	9.8/22.1	9.3/21.7	6.1/12.8	4.0/8.9

[a] Rate constant in absorbance per 100 g of solid per week ±95% confidence limits.
[b] 95% confidence limits for time to reach B = 50 (min/max) in weeks.

The rate of browning should increase with increasing a_w up to a maximum and then fall, based on the effects of water in terms of solution and dilution (Labuza 1980b). At 25 and 35° C, the browning does show the expected maximum rate at an a_w of 0.65, but the rate increases continually with a_w for 45 and 55° C. This latter effect with increased a_w is similar to that found by Miller (1956) and Tsao et al (1978) for browning during extrusion and drying of soy and milk powder. Possibly, at higher temperatures, solubility of the reacting species is not limiting, and therefore, the dilution effect of increasing a_w (almost a 50% increase in water content from a_w 0.52 to a_w 0.75) is not controlling. Based on the 95% confidence interval at 25 and 35° C, a_w has no effect on the rate of browning, whereas at 45 and 55° C and in the fluctuating condition, the rate is significantly different (at the 0.05 level by the *t* test) between a_w 0.75 and either 0.44 or 0.52.

Because of the scatter of the data, the time to reach a browning value of 50 shows a wide range (almost twice the lower value) for most conditions. This indicates that even with many data points and absolute control of the external environment, exact prediction of shelf life cannot be done. This information should be noted by those especially interested in shelf life testing of any foods, including cereals.

Figure 4 shows the browning development for the product stored

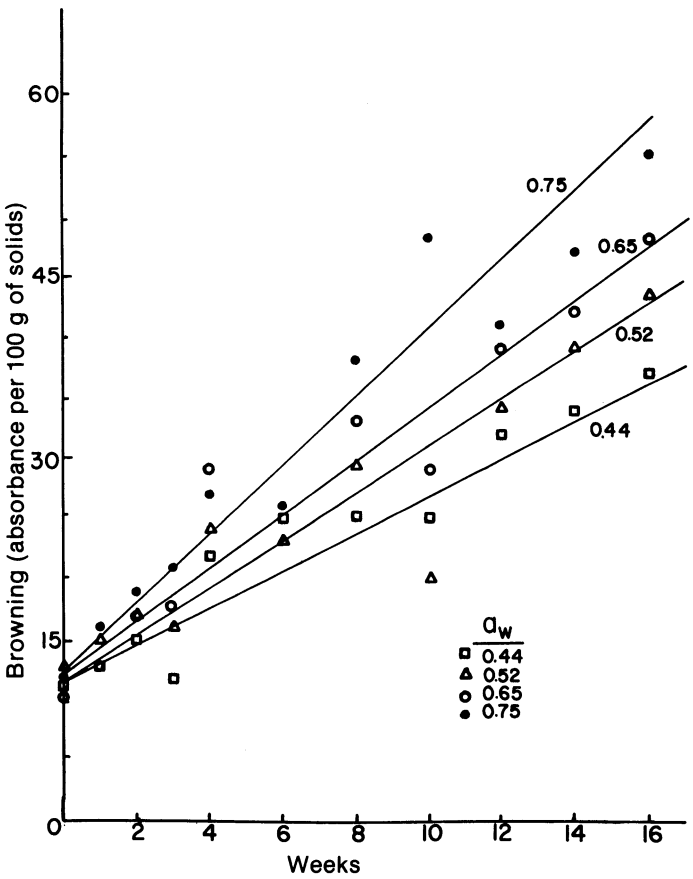

Fig. 3. Extent of nonenzymatic browning in pasta at 45° C and four water activity (a_w) levels.

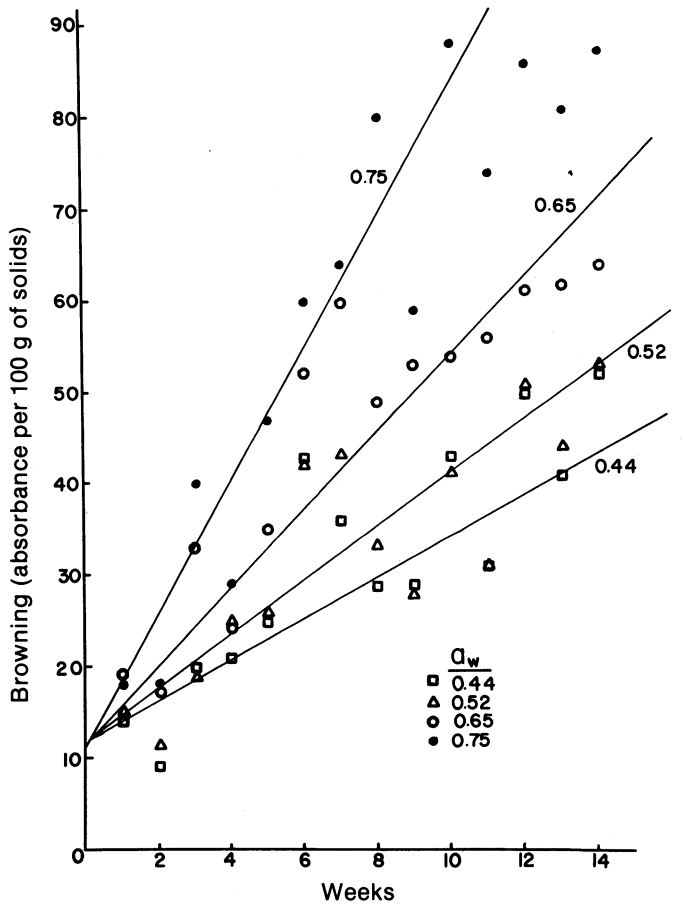

Fig. 4. Extent of nonenzymatic browning in pasta for 35/55° C square wave storage (3½-day periods) held at four water activity (a_w) levels.

under the square wave fluctuating temperature (35/55° C) condition. Considerable scatter occurred, as in the constant temperature condition. What is noteworthy is that storage at 35/55° C shows a much faster average rate of reaction (significant at the $P = 0.05$ level) than does storage at the T_m of 45° C. This is as predicted by Labuza (1979) and as found by Kamman et al (1981) for the thiamin loss in pasta and by Labuza and Saltmarch (1981a) for browning in sweet whey powders.

To determine whether the steady-state data can be used to predict the extent and rate of browning for the 35/55° C fluctuating condition, the E_{AS} were determined using the Arrhenius relationship, which states that a plot of log k versus reciprocal absolute temperature should be a straight line (Labuza 1980a). Statistical methods by Freund (1967) were used, in which the 95% confidence limits of E_A are determined by linear regression of the ln of the upper and lower 95% confidence limits of k as a function of reciprocal absolute temperature. This is equivalent to using each data point as an individual experiment and calculating k value from the initial browning value and the extent of browning at each θ. Although the latter method increases the degrees of freedom significantly (for this study it would be about 13), one gets the same results as by using the 95% confidence limits of the k values. As can be seen in the Arrhenius plot of Fig. 5, the 25° C data do not fit the expected linear relationship with the higher temperatures. This was true at each a_w. This anomaly could be due either to a shift in mechanism or to the fact that at 25° C the rate is so slow that 48 weeks of storage were not enough to get a true estimate of the rate constant. Because the fluctuating study was done at 35/55° C, the E_{AS} and Q_{10S} were calculated without the 25° C data. This resulted in a larger 95% confidence limit (Table II) because two degrees of freedom were lost, but the r^2 values were still quite high. If only the average k values were used, as in most published studies, the r^2 values all exceed 0.999, which is quite good; however, the 95% confidence limits exceed the E_A value because only one degree of freedom is left (in fact, all values would be 4.6 times as large as are shown), which results in a useless interpretation.

Table II shows that the estimated average E_A based on the 95% confidence limits is not statistically different (at $P = 0.05$) as a function of a_w as has been reported in previous studies in which statistical tests were not used. Because of the large confidence limits, the Q_{10} value range as calculated from equation 4 is also large. For pasta, the E_A and Q_{10} for browning are less than usually reported in the literature (20–40 kcal/mole, eg, Labuza and Saltmarch 1981b), but if the 95% confidence limits are used, only the value at a_w 0.52 falls out of this range. This could throw into question most reported values and suggest that the proper experimental design should include more temperatures to increase the degrees of freedom and thereby reduce the confidence interval. Adding one more temperature will reduce the confidence interval by ±12% (based on $P = 0.05$ from standard t tables), and two temperatures will reduce it by ±17%, assuming that the limits of the new data are inside the prior data. This reduction is not worth the extra expense and work of using more temperatures, especially if the analytical error is ±10%, except as a means to test for lack of fit of the Arrhenius model.

Using these steady-state data, the effective temperature (T_{eff}) of the fluctuating study was calculated and compared to predicted values. The T_{eff} of a fluctuation is several degrees above the mean temperature and is predicted from both the Q_{10} of the reaction and

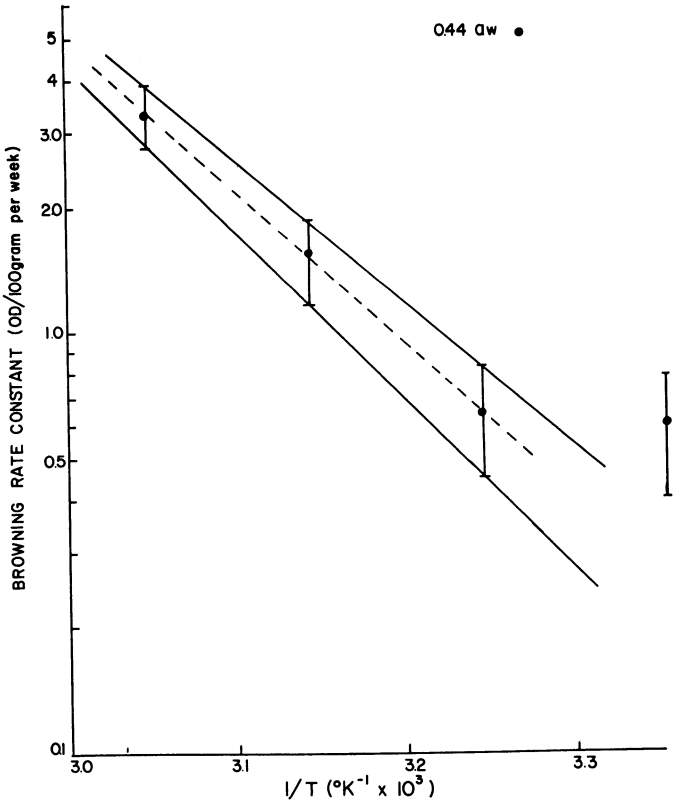

Fig. 5. Activation energy plot for nonenzymatic browning in pasta held at 0.44 water activity (a_w). Bars show temperatures (left to right) of 55, 45, 35, and 25° C. **T** = temperature.

TABLE II
Temperature Dependency for Nonenzymatic Browning in Pasta Stored Under Various Conditions

	Water Activity			
	0.44	**0.52**	**0.65**	**0.75**
E_A[a] (kcal/mole ± 95% confidence limits)	16.89 ± 7.95	12.49 ± 4.02	16.95 ± 9.57	20.06 ± 8.00
r^2 (ln k vs 1/T)	0.897	0.949	0.858	0.923
Q_{10}				
Average	2.27	1.83	2.28	2.65
Range	1.54–3.34	1.51–2.23	1.43–3.62	1.79–3.91
Actual T_{eff}[b] based on k with ± 95% confidence limits				
Average	52.1	50.9	51.1	54.1
Range	48.4–55.0	45.8–55.0	48.4–53.6	52.1–55.0
Actual T_{eff} based on average k	51.9	49.4	50.8	54.0
T_{eff} based on equation 6				
Average	48.6	47.7	48.6	49.2
Range	46.9–49.9	46.8–48.6	46.6–50.2	47.6–50.3

[a] Activation energy.
[b] Effective temperature.

the amplitude of the fluctuation. For any reaction, the ΔT_{eff} above the mean is:

$$\Delta T_{eff} = 10 \cdot \frac{\ln(\Gamma_s)}{\ln(Q_{10})} \quad (6)$$

where

$$\Gamma = \tfrac{1}{2}\left[Q_{10}^{\frac{a}{10}\left(\frac{T_m + 10}{T_m + a}\right)} + Q_{10}^{\frac{-a}{10}\left(\frac{T_m + 10}{T_m - a}\right)} \right], \quad (7)$$

a = amplitude in ° C (° K), and ΔT_{eff} = ° C above T_m equivalent to fluctuating condition. In addition, the effective rate constant for the fluctuating condition is:

$$k_{eff} = k_{T_m} \Gamma_s \quad (8)$$

where k_{T_m} is the rate constant at the mean temperature of the fluctuation.

The calculation was done in two ways, first by using the E_A value based on the 95% confidence limits, and second by using the E_A value calculated from the mean browning rate at each a_w. The 95% confidence limits are also shown based on the first method and using the 95% limits of Q_{10} in equation 6. In all cases comparing the average T_{eff} values, the actual value is greater than that predicted from equation 6, ie, the rate of browning is greater than that based on pure kinetics. This could suggest a history effect, ie, a faster rate than expected occurs at the lower temperature of the fluctuation because of intermediates produced at the higher temperature that change the mechanism at the lower temperature. One would expect this to be more prominent with increased water content or a_w. However, the average values of equation 6 fall with the 95% confidence limits except at a_w 0.75. Thus one could state that the predictions are quite good, and therefore, steady-state data are useful in predicting losses for any temperature distribution. This conclusion is needed if true shelf life for open dating of foods is to be possible (OTA 1978). One should note that the conditions imposed on calculating the effective temperature range used the extremes of all the confidence ranges.

The same mathematical analyses were performed on the data for loss of lysine as measured by the FDNB method. Figure 6 shows the data for the square wave condition as an example. Table III lists the r^2 and rate constant values (±95% confidence limits) for lysine loss in addition to the range of times to 25% loss, which would be of biological significance from the standpoint of nutritional labeling. In this case, the 25% loss was based on reducing the initial value of

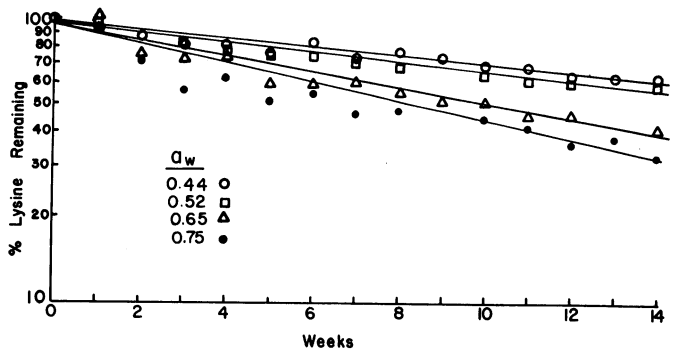

Fig. 6. Percent of lysine remaining in pasta stored at 35/55° C square wave conditions (3½-day periods) and held at four water activity (a_w) levels.

TABLE III
First Order Kinetics for Lysine Loss in Egg Noodles as a Function of Storage Conditions

Temperature (° C)		Water Activity 0.44	0.52	0.65	0.75
25	r^2	0.59	0.933	0.944	0.896
	k[a]	5.69 ± 3.82	8.78 ± 2.11	5.00 ± 0.97	4.13 ± 1.06
	$\theta_{25\%}$[b]	30.3/153.8	26.4/43.1	48.2/71.4	55.8/93.7
35	r^2	0.751	0.898	0.901	0.831
	k	6.81 ± 2.91	10.60 ± 2.37	7.52 ± 1.57	10.77 ± 3.4
	$\theta_{25\%}$	29.6/73.8	22.2/34.9	31.6/48.3	20.3/39.0
45	r^2	0.832	0.880	0.963	0.974
	k	17.05 ± 5.08	34.02 ± 3.33	42.60 ± 5.51	61.13 ± 7.41
	$\theta_{25\%}$	13.0/24.0	6.8/11.2	5.9/7.8	4.2/5.4
55	r^2	0.971	0.983	0.947	0.940
	k	41.99 ± 5.05	52.35 ± 4.37	78.64 ± 11.7	93.28 ± 14.14
	$\theta_{25\%}$	6.1/7.8	5.1/6.0	3.2/4.3	2.7/3.6
35/55	r^2	0.919	0.946	0.924	0.909
	k	32.99 ± 5.88	37.33 ± 5.36	63.47 ± 12.09	75.16 ± 13.63
	$\theta_{25\%}$	7.4/10.6	6.7/9.0	3.8/5.6	3.2/4.7

[a] k value × 10^3 ± 95% confidence limits, in weeks $^{-1}$.
[b] 95% confidence limits (min/max) for 25% loss of lysine in weeks, based on initial value of 392 mg/100 g.

TABLE IV
Lysine Content as a Function of Time for Egg Noodles Stored at 55° C and 75% rh

Time (weeks)	Lysine (mg/100 g of dry solids)	A/A_o[a]
0	392 ± 10	1
1	325	0.837
2	287	0.739
3	219	0.563
4	221	0.569
5	179	0.462
6	198	0.510
7	187	0.482
8	153	0.395
9	148	0.381
10	140	0.362
12	128	0.331
13	112	0.288
16	88	0.23

[a] Ratio of concentration of lysine to original concentration.

TABLE V
Temperature Dependency for Lysine Lossin Egg Noodles Stored Under Various Conditions

	Water Activity 0.44	0.52	0.65	0.75
E_A[a] (kcal/mole ± 95% confidence limits)	15.16 ± 9.14	12.77 ± 5.16	19.46 ± 5.89	21.56 ± 5.4
r^2 (ln k vs 1/T)	0.733	0.860	0.916	0.941
Q_{10}				
Average	2.07	1.86	2.57	2.85
Range	1.34–3.25	1.45–2.39	1.93–3.42	2.19–3.70
Actual T_{eff}[b] based on k ± 95% confidence				
Average	53.7	49.6	52.4	51.4
Range	50.9–56.0	47.1–51.8	50.1–54.3	49.5–53.1
T_{eff} based on equation 6				
Average	48.3	47.8	49.1	49.5
Range	46.2–49.8	46.6–48.8	47.9–50.0	48.5–50.2

[a] Activation energy.
[b] Effective temperature.

TABLE VI
Comparison of Prediction to Actual Extent of Protein Quality Change for a 35/55° C Square Wave

	Browning[a]				Lysine Loss[b]			
	0.44	0.52	0.65	0.75	0.44	0.52	0.65	0.75
Q_{10}	2.27	1.83	2.28	2.65	2.07	1.86	2.57	2.85
Γ_s	1.34	1.18	1.35	1.50	1.27	1.19	1.47	1.59
k_{T_m} (at 45° C)	1.602	1.869	2.168	2.642	0.01705	0.03402	0.04260	0.06113
Predicted time (weeks)	18.6	18.2	13.7	4.5	13.3	7.11	4.6	2.96
k_{eff} (actual)	2.693	2.687	4.000	5.856	0.03299	0.03733	0.06347	0.07516
Actual time (weeks)	14.9	14.9	10.0	6.8	8.7	7.7	4.5	3.8
Error, % (Δ/actual)	+25	+22	+37	−51	+53	−7.6	+2.2	−22

[a]To absorbance = 50/100 g of solids.
[b]To 25% loss, by 1-fluoro-2, 4-dinitrobenzene method.

392 ± 10 mg of lysine per 100 g of dry solids to a value of 294 mg/100 g of solids. Even though the lysine measurement has a variance of only 2.6%, the correlations for first order were poorer than those for browning. Unfortunately, not enough samples were available to carry out every experiment through two half lives, which is necessary to absolutely substantiate a first order reaction. In fact, at 25 and 35° C over the period of the experiment (40–48 weeks), only 15 to 25% loss occurred, respectively, which reduces the reliability of the rate constant (Labuza 1979) despite the high r^2.

Table III shows that significant lysine loss could occur during consumer storage at 25° C. The maximum rate of loss appears to be at 0.52 a_w, which is close to the range for normally produced pasta. As with browning, storage at higher temperature shifts the maximum rate to the highest a_w. With respect to pasta shelf life, storage at 35° C could result in over 50% loss of protein quality if the product were held for more than 80 weeks. Fortunately this would not be the usual condition in this country. The data do indicate, however, that short-term (3–4 weeks) temperature abuse at ≥45° C would be very detrimental.

Protein quality loss does not appear to cease at 50% in pasta as was found by Wolf et al (1977a, 1977b) and Thompson et al (1976) in soy systems. For example, Table IV shows the results of lysine loss at 55° C and 0.75 a_w. Although a slowdown appears between three and seven weeks, at further times the loss exceeds 50%.

The calculated E_{AS} (range of 13–22 kcal/mole) for lysine loss in Table V compared favorably to the pasta value of 13.3 reported by Fabriani et al (1972). Based on the 95% limits, a_w does not seem to affect temperature sensitivity (either E_A or Q_{10}) of the reaction, as was noted for the browning reaction. In fact, Lee and Labuza (1975) found no affect of a_w on the E_A for ascorbic acid loss in model systems, although other data show both an increase and a decrease. Most likely, the effect is small and the reported differences are caused by the lack of statistical testing of the data. The purpose, in fact, of "doubt," ie, questioning a scientific theory, is to test data. The extensive testing in this study of a real food system, rather than of a model system, indicates that one must be much more careful in developing theory. Lastly, Table V shows that the predicted average T_{eff} is less than the measured average T_{eff}. As with browning, one can also conclude that the prediction equation for a first order reaction is not valid, or one can conclude, as was cited for browning, that possibly a history effect occurs. The confidence limits for the measured T_{eff} and predicted T_{eff} overlap, and thus, the prediction may in fact be quite good. Unfortunately, the prediction has a large range because it is based on the errors associated with the steady-state data and because some of the results seem to show a period in which very little loss occurs.

One last comparison can be made between the actual results of the fluctuating condition and the predictions based on the steady-state data. In Table VI, the time to reach the optical browning value and 25% lysine loss value is compared to the predicted time. For this prediction, only the average Q_{10} and Γ_s were used for each condition to show the error involved if no statistical analysis was used. As seen in Table VI, the prediction shows anywhere from a 2 to a 53% error. No pattern exists in the error with respect to either analytical method or a_w. In almost every case except two (for browning at a_w = 0.65 and for lysine at a_w = 0.75), the predicted average falls within the 95% limits for the actual values (Tables I and III, respectively). Based on this, one can say that use of kinetics and the theoretical equations developed for fluctuating conditions; storage tests at constant temperature rather than at fluctuating temperatures are more practical as they can be used for prediction under any condition. Other studies need to be done to confirm this, especially with sine wave and random variable storage conditions. As a last point, this study points out the problem that would occur in using very few sampling times to generate data. Many storage studies in the literature report sampling only at a zero time and two subsequent times. From three times, any reliable estimate of the rate is obviously impossible to get since the $t_{\alpha/2}$ statistic for one, degree of freedom is 12.71 at $P = 0.05$.

One suggestion is to use at least eight independent zero-time values to weight the regression closer around the initial value (this is especially important for first order reactions, in which the lower value gets weighted more heavily) and then have at least an equal number of determinations spaced out over the storage period. This results in 14 degrees of freedom with a $t_{\alpha/2}$ of 2.15, making for a much better estimate of the error. If sensory panels are used to generate data, however, the cost of the test may be prohibitive.

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