Degradation Kinetics of Chlorophyll in Peas as a Function of pH

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ABSTRACT: The kinetics of chlorophyll degradation in pea puree were determined in a specially designed reactor with on-line pH control capability. Without pH control, the pH of the pea puree decreased continuously with heating due to acid formation; the pH was maintained within ±0.1 of the desired value with on-line pH control. Chlorophyll (both a and b) degradation followed the first-order reaction model. The temperature dependence of the rate constant was adequately modeled by the Arrhenius equation. The activation energy was independent of pH and was 17.5 kcal/mol and 17 kcal/mol for chlorophyll a and chlorophyll b, respectively. The degradation rate constant decreased log-linearly as the pH was increased. A mathematical model was developed to predict the chlorophyll concentrations as a function of time, temperature and pH.

KEYWORDS: kinetics, chlorophyll degradation, pH

Introduction

VEGETABLES ARE THERMALLY PROCESSED FOR MICROBIOLOGICAL safety; nevertheless, the addition of heat to vegetables causes losses of texture, flavor, color, and nutrients. One compound that is responsible for food color is chlorophyll. The stability of chlorophyll is affected by temperature and pH. Chlorophyll is more stable at higher pH (Gold and Weckel 1959; Sweeney and Martin 1961; Schwartz and Lorenzo 1990). Upon the loss of chlorophyll, olive-brown pigments are left, which are often unappealing to consumers (Clydesdale and Francis 1969; Clydesdale 1976, 1991; Baardseth and others 1988; Gnanasekharan and others 1992).

Knowledge of the degradation kinetics, including reaction order, rate constant and activation energy, is essential to predict the quality loss during thermal processing as well as storage. The kinetics of chlorophyll degradation have been conducted on a variety of vegetables, including spinach (Canjura and others 1991), snapbeans, okra and turnip greens (Jones and others 1963), asparagus, green beans and green peas (Hayakawa and Timbers 1977) and broccoli (Sweeney and Martin 1958). Chlorophyll degradation has been shown to follow a first-order model. The temperature-dependence of chlorophyll degradation has also been adequately described by the Arrhenius equation. The reported activation energies ranged from 10 to 30 kcal/mol (Lund 1977).

Since chlorophyll stability is known to be affected by pH, there have been different processes (Stevenson and Swartz 1942; Bendix and others 1952) and studies (Gupte and Francis 1964) intended to adjust the pH value of vegetables in retort processing with the hope of higher chlorophyll retention. It is necessary to determine the kinetics of chlorophyll degradation as a function of pH for estimating chlorophyll retention under the processing conditions of interest. Although there is a large body of literature dealing with the stability of chlorophyll in vegetables as affected by pH, most of the work has been qualitative rather than quantitative nature. Lajolo and Marquez (1982) studied the degradation of chlorophyll in a spinach system at low and intermediate water activities at four different pH conditions ranging from 5.9 to 6.8. They found that the degradation obeyed a first-order model regardless of the pH. The logarithm of the rate constant linearly related to pH.

In order to determine the chlorophyll degradation kinetics at a desired pH correctly, it is necessary to keep the pH constant during the entire course of a study. However, it is known that when vegetables are subjected to heat, pH continues to decrease. According to the study by Schwartz and von Elbe (1983), the pH of spinach changed from 7.06 to 6.0 during processing at 121 °C for the length of time required for a 90% reduction of chlorophyll b. The pH of peas was also found to decrease continuously upon heating (Ryan-Stoneham and others 1997). The reason for the pH change has been shown to be caused by the chemical reactions that lead to the formation of organic acids, primarily 2-pyrrolidone-5-carboxylic acid (Lin and others 1970, 1971; Clydesdale and others 1972). Some researchers have attempted to combat the pH changes of vegetables with buffer solutions; however, the buffering capability was normally overcome by the intrinsic pH changes

Ryan-Stoneham and others (2000) introduced a kinetic reactor with on-line pH control capabilities and demonstrated its uses and characteristics. The apparatus met the requirements of a kinetic reactor by accommodating a large, well mixed sample size of either very viscous liquids or semisolids while maintaining the temperature of the medium within ± 0.2 °C of the desired temperature and controlling the pH of the medium within ±0.1 pH units of the set-point pH.

There were two objectives. The first was to determine the kinetic parameters of chlorophyll degradation as a function of pH in the constant pH kinetic reactor at pH of 5.5, 6.2, 6.8 and 7.5. The second objective was to develop a mathematical model that related the rate constant of chlorophyll as a function of temperature and pH.

Materials and Methods

The constant pH kinetic reactor. The constant pH kinetic reactor has been described in detail by Ryan-Stoneham and others (2000). Briefly, the constant pH kinetic reactor consisted of a jacketed vessel that was heated by two circulating oil baths programmed at different temperatures, one for quick temperature come-up and another for temperature maintenance. An electrical heating tape encircling the reactor provided auxiliary heating, allowing for a tighter temperature control within ± 0.2 °C of the set-point temperature.

The pH of the system was monitored by a pH controller with automatic temperature compensation and maintained with am-

monia gas. Ammonia gas was chosen because it immediately reacts with water to act as a weak base upon contact with water; and it does not add additional volume to the reactor. When the pH deviated from the desired pH value, the controller activated a solenoid valve, allowing the flow of ammonia gas into the reactor. The flow rate of the ammonia was monitored by a flowmeter, and tightly controlled by a needle valve (R.S. Crum & Co., Mountainside, N.J., U.S.A.). The pH of the sample was controlled within ±0.1 pH units of the desired pH throughout the experiment. The reactor was designed to adequately mix viscous liquids as well as semisolids, as evidenced by uniform pH and temperature in the medium throughout the reactor during heating.

Sample preparation. Chlorophyll degradation was investigated in pea puree made by reconstituting 20% pea powder with 80% distilled water (w/w). Frozen green peas were purchased from a local supermarket and were freeze-dried in a Stokes commercial freeze-dryer (F.J. Stokes Machine Co., Philadelphia, Pa., U.S.A.). The freeze-dried peas were then ground by a Fitzmill comminuting machine (model D; W.J. Fitzpatrick Co., Chicago, Ill., U.S.A.). The pea powder was stored at -20 °C until used. The freshly made pea puree was allowed to hydrate for at least one hour before the start of an experiment.

The pH of the pea puree was adjusted to one of four desired pH values—5.5, 6.2, 6.8 and 7.5. For samples of pH 5.5 and 6.2, concentrated HCl was added to the distilled water and mixed well prior to the addition of the pea powder. For pea puree of pH 7.5, 1 N NaOH was added to the distilled water prior to the addition of the pea powder. The freshly prepared pea puree had a pH of 6.8.

Experiments. The chlorophyll degradation kinetics were determined at each pH with and without on-line pH control at 80, 90 and 100 °C.

The pea puree was heated in the reactor to the desired temperature. After time zero, samples were taken at predetermined time intervals. The samples were immediately cooled in an ice bath with agitation. Five grams of pea puree was mixed with 25 mL acetone and homogenized with a Tekmar tissuemizer (model SDT 1810; Tekmar Co., Cincinnati, Ohio, U.S.A.) for three minutes in order to release the chlorophyll into the acetone. The tissuemized pea puree was permitted to settle for approximately 20 s. The top layer of the acetone and chlorophyll mixture was siphoned off and filtered through a 0.22-mm filter paper (Fisher Scientific, Springfield, NJ). The samples were kept in a -4 °C freezer until quantification by high-pressure liquid chromatography (HPLC).

Chlorophyll quantification. The HPLC system consisted of the following components, all manufactured by Waters Associates (Milford, Ma., U.S.A.): a model 600 pump, a U6K injector, a Lambda Max model 481 detector and a 730 data module. The HPLC procedure used was that of Schwartz and von Elbe (1983). The isocratic mobile phase consisted of an ethyl acetate:methanol:water mixture (50:37.5:12.5 v/v/v) degassed with helium. Aliquots of 10 mL were injected into the HPLC. The concentration of chlorophyll, both a and b, were determined by the areas under the chromatogram peaks through the use of preestablished calibration curves. Experiments were duplicated.

Results and Discussion

FIGURES 1A AND 1B SHOW THE TIME-PH HISTORIES OF THE PEA puree with an initial pH of 7.5 heated for 18 h and an initial pH of 5.5 heated for 2 h, respectively, at 80 °C with and without on-line pH control. The duration of the heating period for each experiment was chosen for the concentration of chlorophyll b to reduce by 90%. Without on-line pH control, the pH of the sample dropped approximately 1.0 during the experiment; however, with the application of on-line pH control the pH remained within 0.1 of the desired value, namely for pH 7.5. In contrast, the pH change during heating at pH 5.5 was insignificant. Without on-line pH control, the pH dropped approximately 0.15 units, which was only slightly greater than the oscillation dictated by the type of pH controller used for on-line pH control. Although not shown here, the pH reduction at pH 6.2 was approximately 0.3 units and the pH shift at pH 6.8 was 0.6 units at the end of the experiments without on-line pH control. Since protons are products resulting from the heating of vegetables, the acid formation rate is slower due to the higher initial proton concentration (lower pH). At the same time, the chlorophyll degradation rate is faster at the lower pH. The combination of a shorter heating time and the slower acid production rate contributes to the insignificant pH changes at the two lowest pH values.

Figures 2a and 2b show the retention of chlorophyll a and b at all four pH values at 80 °C with and without on-line pH control. When a reaction is known to follow a first order kinetic model, the concentration of a reacting species is related to time at a constant temperature as follows:

$$\ln\frac{C}{C_o} = -kt \qquad (1)$$

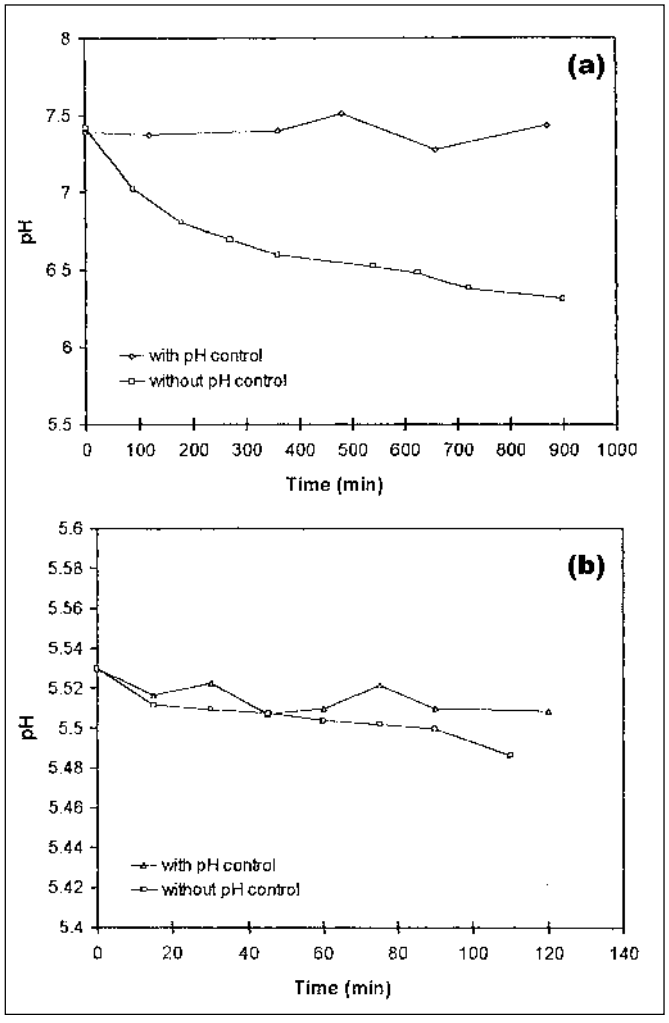

Figure 1—pH changes of pea puree heated in a constant pH kinetic reactor at 80 °C with and without pH control at (a) pH 7.5 and (b) pH 5.5

where C is the concentration at any time t, Co is the concentration at time zero, k is the rate constant at the reaction temperature, and t is time. The plot of the logarithm of the normalized concentration (C/Co) against time would yield a straight line with the rate constant equal to the negative of the slope.

The degradation followed a first-order kinetic model regardless of the initial pH which is consistent with that of Lajolo and Marquez (1982). It is interesting to observe that chlorophyll degradation followed a first-order model whether the pH remained constant or changed during the experiment. However, the rate constants were different, as indicated by the differences in the slopes, with and without on-line pH control when the pH was 6.8 and 7.5, and no significant difference was seen in the rate constant at the two lower pH values. Such behavior was expected because the pH histories of the pea puree were different with or without pH control when the initial pH values were 6.8 and 7.5. The pH histories with pH control were consistently higher than those without pH control. Since the chlorophyll degradation rate is quicker at lower pH, the chlorophyll degradation rate was faster without the application of on-line pH control. At the lower initial pH values of 6.2 and 5.5, the pH change was not significant enough to affect the rate constants; therefore, the rate constants were the same with and without pH control.

Figures 3, 4, 5 and 6 show the normalized concentrations of chlorophyll a and b in pea puree as a function of heating time at 80, 90 and 100 °C at pH 5.5, 6.2, 6.8 and 7.5, respectively, with on-line pH control. Regardless of temperature and pH, chlorophyll degradation followed a first-order reaction model as indicated by the linearity of the plots of the logarithm of the normalized concentration against time. From the slopes of the lines, the rate constant k was determined for each temperature and pH, for both chlorophyll a and b. It is apparent from the graphs that chlorophyll a degraded approximately 2.5 times faster than chlorophyll b independent of pH and temperature which is consistent with the findings of other researchers (Joslyn and MacKinney 1938; Tan and Francis 1962; Schwartz and Lorenzo 1991; Canjura and others 1991; Steet and Tong 1996). The heating times were chosen in order to go through one log cycle reduction in chlorophyll b concentration necessary to confirm the order of the reaction taking into account the experimental noises (Lund 1977). The standard deviations for some data points, normally toward the end of the experiments, were larger than expected. The reason for the variation was unknown.

Tables 1 and 2 compare the rate constants of chlorophyll a and b with and without on-line pH control at all temperatures and pH conditions studied. Similar to the reasons stated previously, little or no difference in the rate constants at pH 5.5 and 6.2 was expected and found. The differences increased as the

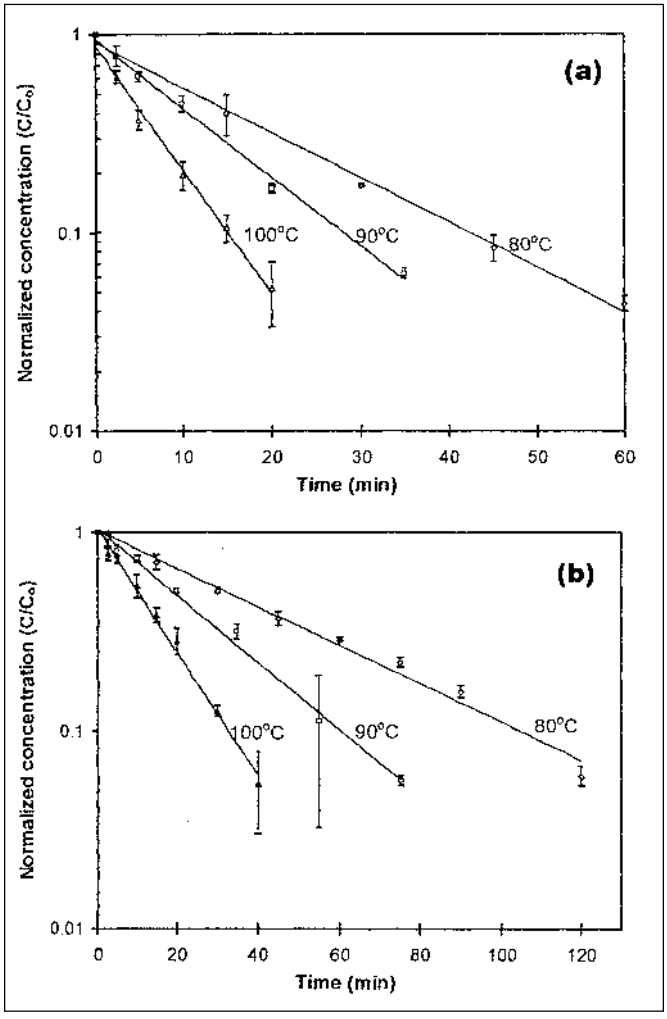

Figure 3—Chlorophyll degradation in pea puree at pH 5.5 heated at 80, 90 and 100 °C with on-line pH control (a) chlorophyll a and (b) chlorophyll b

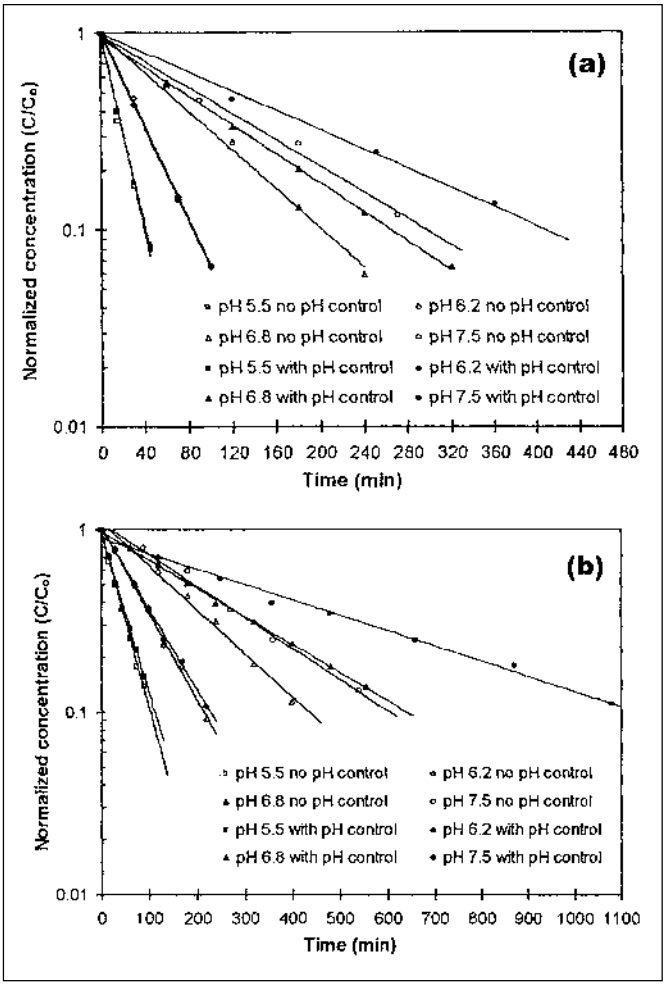

Figure 2—Plot of normalized concentration against heating time with or without pH control at pH 5.5, 6.2, 6.8 and 7.5 heated at 80 °C for (a) chlorophyll a and (b) chlorophyll b

Table 1—Rate constants with and without on-line pH control at 80, 90 and 100 °C for chlorophyll a.

	80 °C			90 °C			I00 °C		
pH	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*
5.5	0.046	0.054	14.8	0.08	—	—	0.16	—	—
6.2	0.022	0.025	12	0.046	0.042	9.5	0.082	0.08	2.5
6.8	0.0085	0.013	34.6	0.016	0.021	23.8	0.034	0.046	26.2
7.5	0.004	0.0099	59.6	0.008	0.016	50	0.017	0.031	45.2

*$(k_{without} - k_{with})/k_{with} \times 100\%$

initial pH increased. Extracting information from previous studies, taking into account the effects of pH on kinetics of chlorophyll degradation in vegetables, the researchers did not consider the possibilities of pH changes in the data analysis.

The rate constants as a function of pH and temperature obtained in Figures 3 to 6 are shown in Figures 7a and 7b for chlorophyll a and b, respectively. The Arrhenius equation of the following form has been widely used to relate the rate constant to temperature:

$$\ln k = \ln A_o - \frac{E_a}{R}\frac{1}{T} \quad (2)$$

where k is the rate constant, A_o is a pre-exponential constant, E_a is the activation energy, R is the gas constant (1.987 cal/mol K), and T is the temperature in Kelvin. The plot of the logarithm of the rate constant against 1/T would be a straight line with the negative of the slope equal to E_a/R. The temperature-dependence of both chlorophyll a and b followed the Arrhenius relationship because of the linearity of the plots. The activation energy was independent of pH as indicated by the parallel behavior of the data points. Although not shown here, when the logarithm of the rate constant was plotted against pH, a linear relationship was obtained. A similar observation was reported by Lajolo and Marquez (1982).

The following mathematical model was then proposed to re-

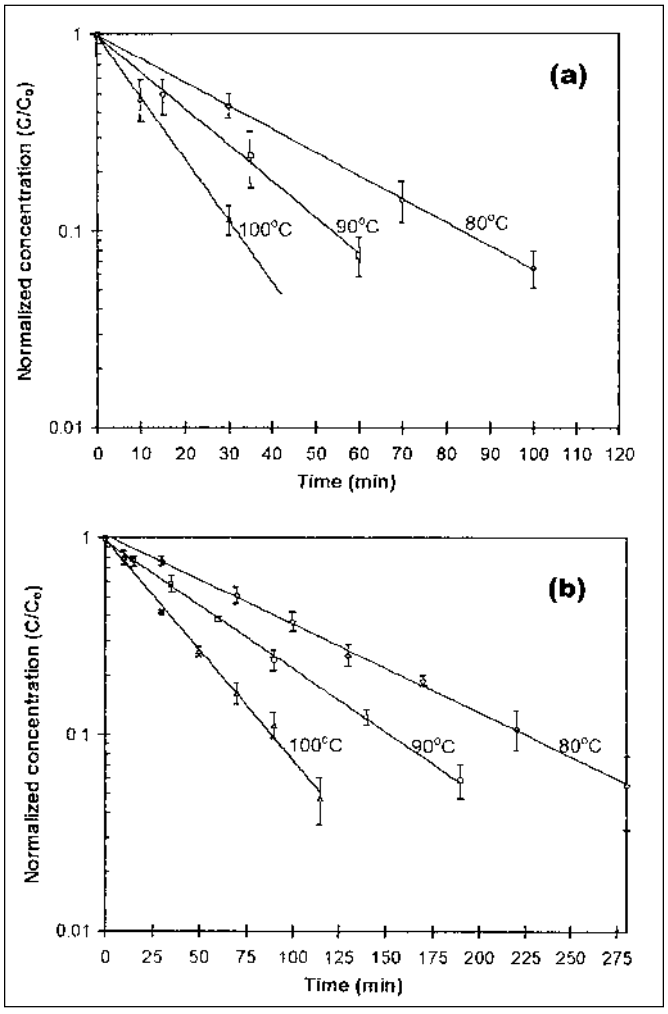

Figure 4—Chlorophyll degradation in pea puree at pH 6.2 heated at 80, 90 and 100 °C with on-line pH control (a) chlorophyll a and (b) chlorophyll b

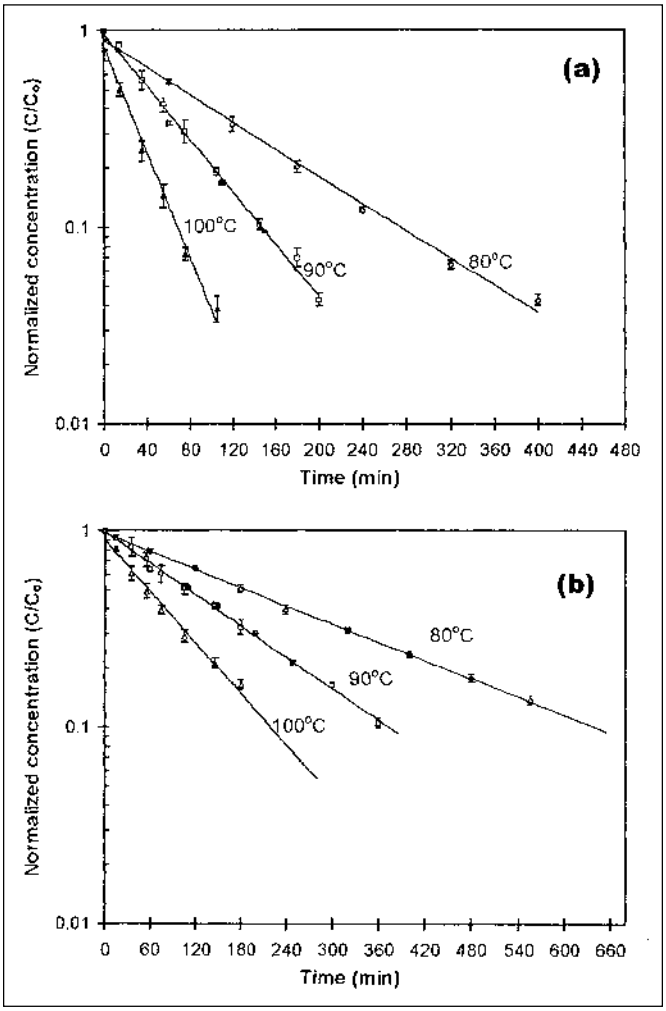

Figure 5—Chlorophyll degradation in pea puree at pH 6.8 heated at 80, 90 and 100 °C with on-line pH control (a) chlorophyll a and (b) chlorophyll b

Table 2. Rate constants with and without on-line pH control at 80, 90 and 100 °C for chlorophyll b.

	80 °C			90 °C			100 °C		
pH	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*	k with pH control (1/min)	k without pH control (1/min)	% Difference in k*
5.5	0.022	0.022	0	0.039	—	—	0.077	—	—
6.2	0.01	0.011	9	0.015	.016	6	c.031	0.033	6
6.8	0.0035	0.0055	36	0.006	0.01	40	0.013	0.019	31.6
7.5	0.002	0.0042	52.4	0.0031	0.007	55.7	0.008	0.015	46.7

$(k_{without} - k_{with}) / k_{with} \times 100\%$

late the rate constants to both temperature and pH:

$$\ln k = \ln A_o - \frac{E_a}{R}\frac{1}{T} + c_p * pH \quad (3)$$

where c_p is the pH coefficient describing the pH dependence.

The pre-exponential constant, the activation energy and the pH coefficients for chlorophyll a and b were determined through multiple linear regression on the experimental data. The following equations were found to best describe the rate constants as a function of temperature and pH for both chlorophyll a and b:

For chlorophyll a:

$$ln\ k = 28.38 - 8796.2(1/T) - 1.193pH$$

$$r^2 = 0.99$$

For chlorophyll b:

$$ln\ k = 25.53 - 8475.6(1/T) - 1.014pH$$

$$r^2 = 0.96$$

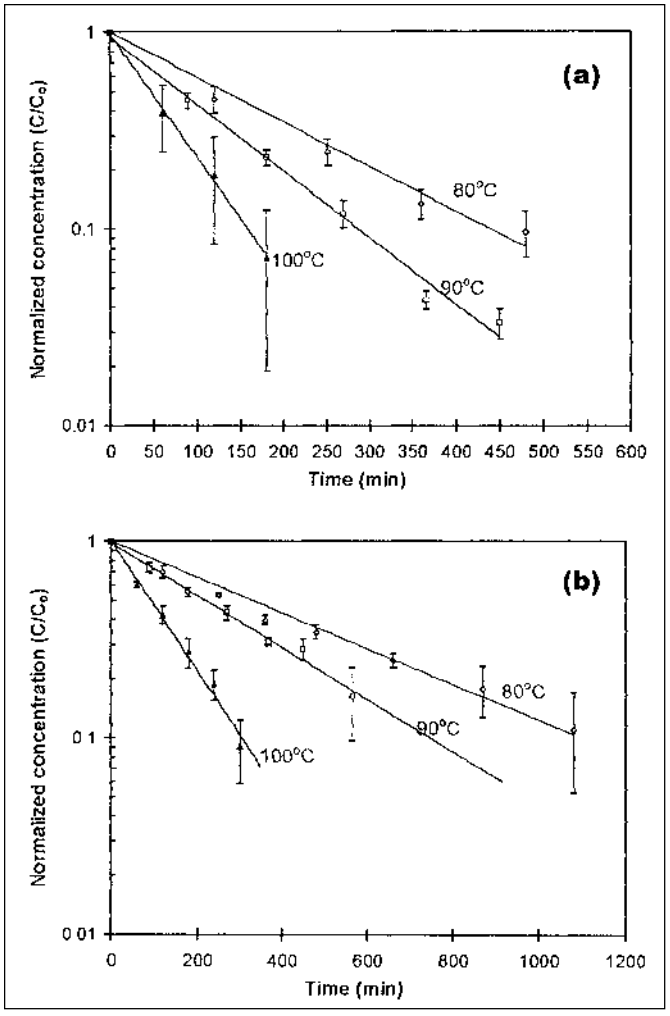

Figure 6—Chlorophyll degradation in pea puree at pH 7.5 heated at 80, 90 and 100 °C with on-line pH control (a) chlorophyll a and (b) chlorophyll b

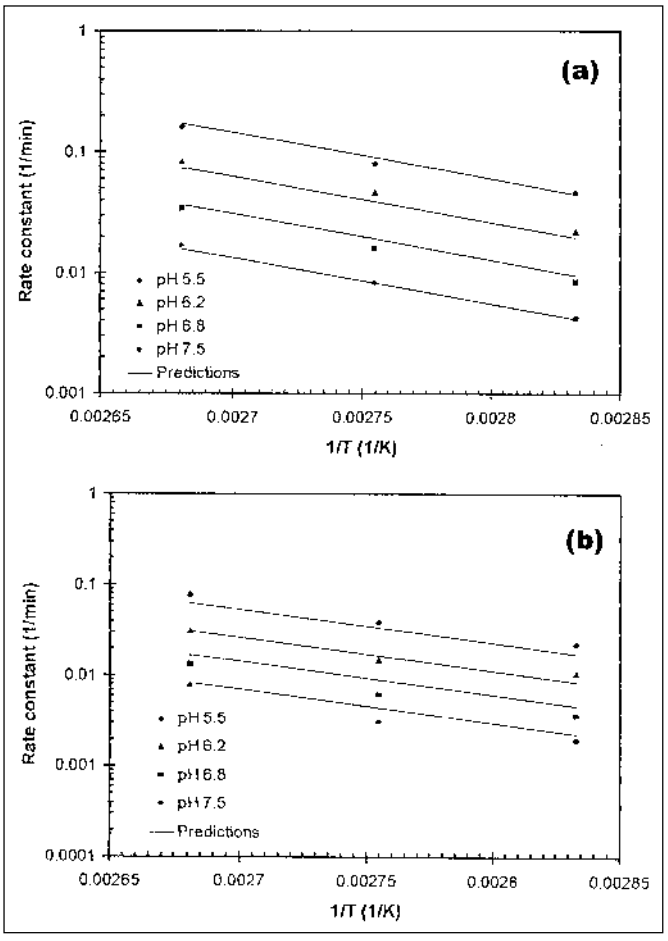

Figure 7—Arrhenius plots of chlorophyll degradation in pea puree as a function of temperature and pH (a) chlorophyll a and (b) chlorophyll b

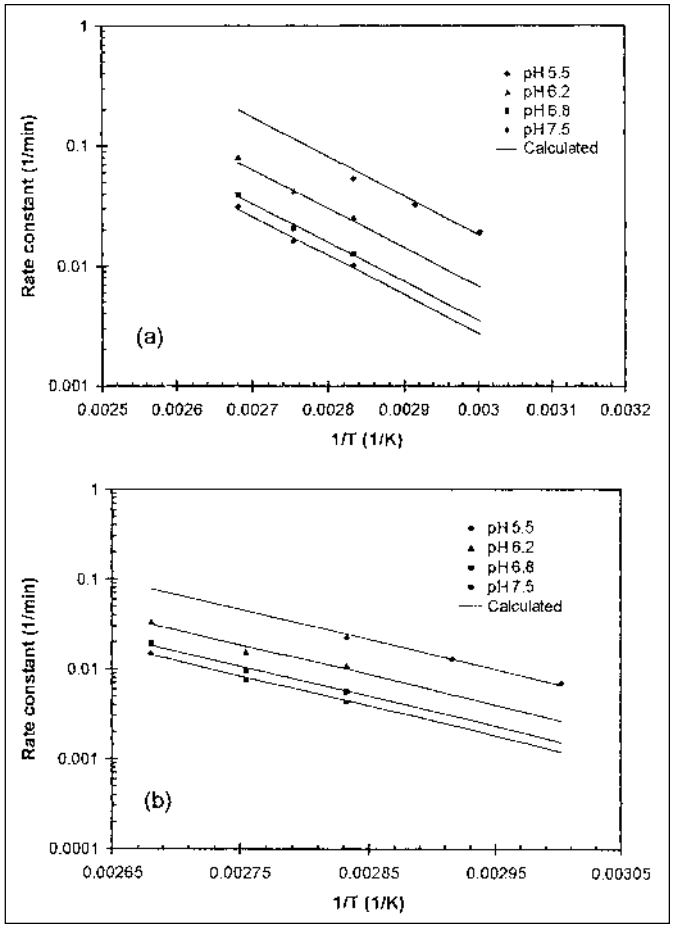

Figure 8—Arrhenius plots of chlorophyll degradation in pea puree without on-line pH control (a) chlorophyll a and (b) chlorophyll b

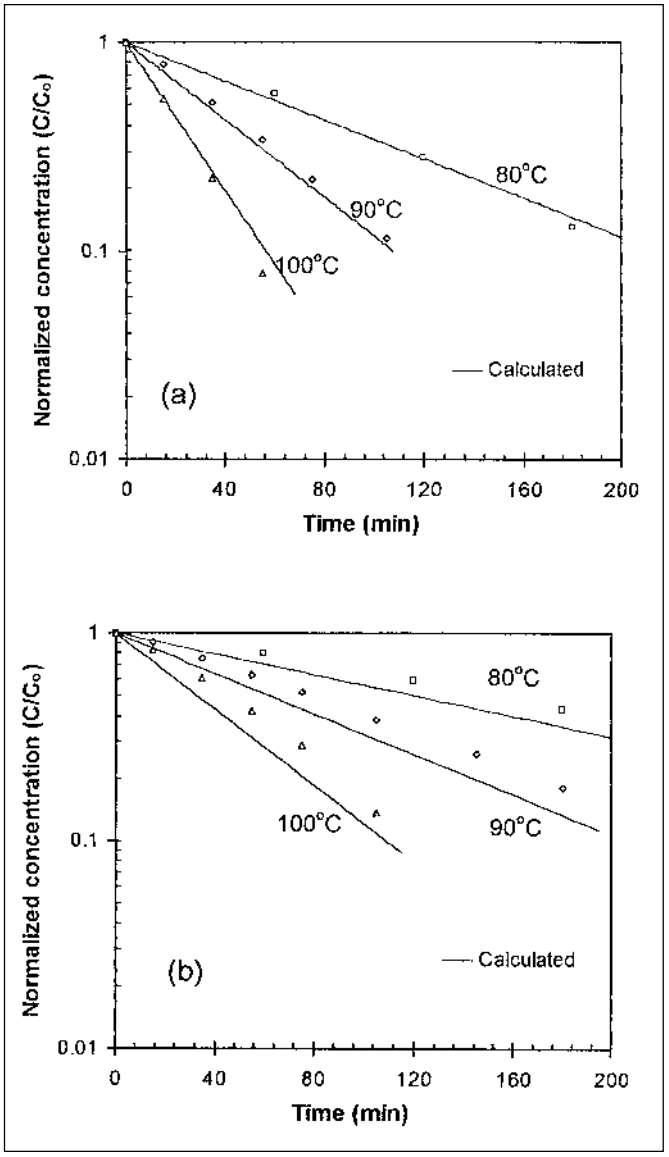

Figure 9—Predictions of chlorophyll retention as a function of heating time at 80, 90 and 100 °C without on-line pH control at pH 6.8 using a calculated rate constant based on the median pH (a) chlorophyll a and (b) chlorophyll b.

The predicted rate constants for chlorophyll a and b as a function of temperature and pH using equations 4 and 5 are also shown as the solid lines in Figures 7a and 7b, respectively. The predictions agreed well with the experimental data. The activation energies for chlorophyll a and b were 17.5 and 17.0 kcal/mol, respectively, which compare well with those published earlier by others (Hayakawa and Timbers 1977; Steet and Tong 1996). The similarity of the pH coefficients suggested that the pH-dependence of chlorophyll a and b were comparable.

Figures 8a and 8b are the Arrhenius plots describing the temperature-dependence of chlorophyll a and b degradation, respectively, at the four initial pH values of 5.5, 6.2, 6.8 and 7.5 without on-line pH control. It can be concluded that with or without on-line pH control, independent of the initial pH, the temperature-dependence always followed the Arrhenius relationship. An average Ea of 14.8 kcal/mol and 15.3 kcal/mol for chlorophyll a and b, respectively, were determined from the slopes of the lines.

It is clear from this study and other published investigations that the pH of vegetables during thermal processing is not constant. The magnitude of pH changes is dependent upon the type of vegetable, the initial pH and the time-temperature history of the vegetable of interest (whether high-temperature short-time or low-temperature long-time). The prediction of chlorophyll concentration would be more accurate by treating pH as a variable and by knowing the chlorophyll degradation kinetics as a function of pH. However, the prediction procedure would be significantly complicated. A simpler approach can be applied to improve the accuracy of the prediction assuming the pH of the vegetable remained constant at an average pH value (between the initial and final pH values) and the rate constant at that average pH is known for a specified processing condition and product. Figure 9a and 9b show the chlorophyll a and b retention as a function of heating time at 80, 90 and 100 °C without on-line pH control when the initial pH was 6.8. For each temperature, the predicted line was calculated based on a rate constant at the median pH values through the use of equations (1), (4) and (5). The median pH values at each temperature were chosen based on the initial and final pH when chlorophyll a and b degraded 90%. The reasonably good agreement between the experimental data and prediction suggests that the simpler approach is adequate.

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