# Kinetic Model for Chlorophyll Degradation in Green Tissue

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A general mechanistic model was developed to describe chlorophyll degradation to pheophytin, chlorophyllide, and pheophorbide in coleslaw, pickles, and olives. Kinetic constants were determined for each commodity. The comparison of *in vitro* chlorophyllase catalytic constants from greened rye seedlings and *in vivo* rate constants determined from our whole tissue studies suggests that the conversions of chlorophyll to chlorophyllide and pheophytin to pheophorbide in coleslaw, cucumbers, and brined olives were the result of chlorophyllase activity. We developed a general model that allows for the quantitative comparison of chlorophyll degradation between commodities and thus enhances qualitative comparisons between commodities. In turn, the qualitative comparison could be useful in understanding and controlling the fate of chlorophyll in processed foods.

**Keywords:** *Chlorophyll; degradation; kinetics; model; coleslaw; pickles; olives*

## INTRODUCTION

The processing of fruits and vegetables induces structural and chemical changes to the tissue that often result in color changes. Many of these color changes are caused partly by enzymatic reactions and the release of organic acids from disrupted tissue. Chlorophyll, the principal pigment in all green plants, is highly susceptible to degradation during processing. The conversion of chlorophyll to pheophytin and pheophorbide results in a change from bright green to dull olive-green or olive-yellow (Gupte et al., 1963), which ultimately is perceived by the consumer as a loss of quality.

Chlorophyll degradation has been shown to follow different pathways depending on the commodity (Heaton et al., 1996a; White et al., 1963; Minguez-Mosquera et al., 1989). However, the mechanisms and kinetics of these reactions have only been partially characterized. Gupte et al. (1963) determined that the degradation of chlorophyll in spinach puree followed a first-order exponential decay, but did not determine the fate of this pigment. Schwartz and Elbe (1983) found similar results in their study of processed spinach, but developed the model one step further. They determined that chlorophyll was being degraded to pheophytin and subsequently to pyropheophytin. These authors were also able to determine the rate constants for the kinetic model they developed. No attempt, however, has yet been made to model the whole pathway of degradation of chlorophyll to chlorophyllide and pheophorbide. *In vitro* studies on chlorophyllase, the enzyme responsible for the conversion of chlorophyll to chlorophyllide and pheophytin to pheophorbide have, however, been performed (Tamai, 1979; Kuroka et al., 1981; Tanaka et al., 1982).

The purpose of this research was to develop a general mathematical model that describes chlorophyll degradation to chlorophyllide, pheophytin, and pheophorbide based on the results of a previous study by Heaton et al. (1996). The modeling procedure was also applied to previously published work on chlorophyll degradation in pickles (White et al., 1963) and olives (Minguez-Mosquera et al., 1989) to compare pathways and kinetic constants of chlorophyll degradation among the three different commodities.

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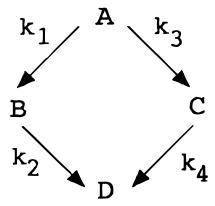
**Scheme 1**




## THEORY

Thus far, chlorophyll degradation in food systems has been observed to follow the two pathways shown in Scheme 1, where A, B, C, and D are chlorophyll, pheophytin, chlorophyllide, pheophorbide, respectively, and the $k$ terms are the rate constants for each degradation step. The degradation of chlorophyll outlined in Scheme 1 can be represented by the following set of differential equations:

$$dA/dt = -k_1A - k_3A \quad (1)$$

$$dB/dt = k_1A - k_2B \quad (2)$$

$$dC/dt = k_3A - k_4C \quad (3)$$

A mass balance on all species, and the concentration of D in time, is given by eq 4:

$$A_0 + B_0 + C_0 + D_0 = A + B + C + D \quad (4)$$

In eq 4, $A_0$ is the initial chlorophyll concentration, $B_0$ is the initial pheophytin concentration, $C_0$ is the initial chlorophyllide concentration, and $D_0$ is the initial pheophorbide concentration.

These equations were simultaneously solved by integration, rearrangement, and substitution to obtain the following set of solutions:

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$$A = A_0\, e^{(-k_1 - k_3)t} \quad (5)$$

$$B = \frac{k_1 A_0}{k_2 - k_1 - k_3}[e^{(-k_1 - k_3)t} - e^{-k_2 t}] + B_0\, e^{-k_2 t} \quad (6)$$

$$C = \frac{k_3 A_0}{k_4 - k_1 - k_3}[e^{(-k_1 - k_3)t} - e^{-k_4 t}] + C_0\, e^{-k_4 t} \quad (7)$$

$$D = D_0 + A_0 + B_0 + C_0 - A - B - C \quad (8)$$

## MATERIALS AND METHODS

Data for modeling were obtained from White et al. (1963), Minguez-Mosquera et al. (1989), and Heaton et al. (1996), and were converted to units of $\mu$mol/100 g before being converted to mole percent, for example:

$$\%\ \text{pheophorbides} = \frac{P_{oa}P_{ob}}{C_aC_b + C_{da}C_{db} + P_{ya}P_{yb} + P_{oa}P_{ob}} \times 100 \quad (9)$$

In eq 9, $C_aC_b$ is the concentration ($\mu$mol/100 g) of chlorophyll *a* and *b*, $C_{da}C_{db}$ is the concentration ($\mu$mol/100 g) of chlorophyllide *a* and *b*, $P_{ya}P_{yb}$ is the concentration ($\mu$mol/100g) pheophytin *a* and *b*, and $P_{oa}P_{ob}$ is the concentration ($\mu$mol/100 g) of pheophorbide *a* and *b*. To ensure that the rate constants (data of Heaton et al., 1996) were not affected by this conversion, modeling was also performed with the data expressed as $\mu$mol/100 g. There were no differences (data not shown) between fits using data in units of mol % and $\mu$mol/100 g, thus confirming that our conversion procedure was acceptable. Conversion of the data of White et al. (1963) and Minguez-Mosquera et al. (1989) also allowed us to compare the *k* values of three different commodities. Data were fitted to the models by nonlinear least-squares methods with the software package Grafit (Leatherbarrow, 1992). Initial estimates for $k_1$, $k_2$, $k_3$, and $k_4$ were obtained by graphically estimating the slopes of their respective curves. The weighting used for determining the calculated curves was simple. The model was fitted to the experimental data until the lowest standard error was obtained for each calculation of kinetic constants.

Values of $A_0$, $B_0$, $C_0$, and $D_0$ were obtained from the data sets, fixed as constants, and not included in the error minimization procedures. Whenever a pathway was not operational, the rate constant for that particular step was set to zero and fixed as a constant. It was our experience that the smaller the number of parameters to be estimated ("floated"), the more stable and reliable the fitting procedure became. That is, solutions were less sensitive to initial conditions, and the precision of the solutions obtained was greater than if all parameters were floated. In such a complex kinetic scheme, the choice of boundary conditions is extremely important to avoid artifacts such as negative rate constants or negative initial pigment concentrations.

## RESULTS AND DISCUSSION

In the study of coleslaw browning by Heaton et al. (1996), the authors determined the pathway for chlorophyll degradation to be principally from chlorophyll to pheophytin to pheophorbide. In the model this translates to $k_3 = 0$, and no formation of C (chlorophyllide).

The experimental data were fitted using the following boundary conditions (parameters): $k_3 = 0$, $k_4 = 0$, $A_0 =$ 58.4 mol %, $B_0 = 27.9$ mol %, $C_0 = 0.1$ mol %, and $D_0 =$ 13.6 mol %. The calculated curves for this model and the experimental points are presented in Figure 1, demonstrating that the model describes the experimental results quite accurately. To ensure that the proposed model was not just coleslaw specific but could also be used to describe chlorophyll degradation in other foodstuffs, we modeled data from two other sources. White et al. (1963) described chlorophyll degradation in brined cucumbers. They postulated that the pathway for chlorophyll degradation was from chlorophyll to chlorophyllide to pheophorbide, and from chlorophyll to pheophytin, thus indicating that both pathways were operational. No conversion of pheophytin to pheophorbide was observed ($k_2 = 0$). The general model fits the observed data quite well, as shown in Figure 2, thus indicating that the model also accurately describes chlorophyll degradation in brined cucumbers. Boundary conditions (parameters) for this fitting procedure were as follows: $k_2 = 0$, $A_0 = 100$ mol %, $B_0 = 0$, $C_0 = 0$, $D_0 = 0$.

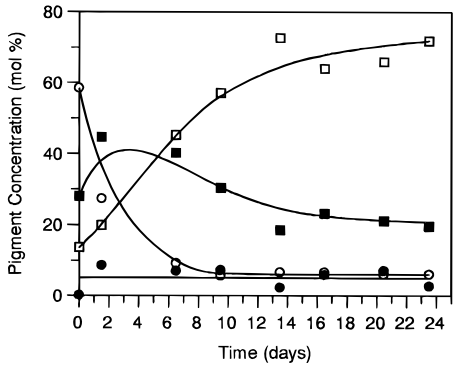


**Figure 1.** Chlorophyll degradation in processed coleslaw stored at 5 °C in polyethylene bags as a function of time: (○), chlorophyll *a* and *b*; (■) pheophytin *a* and *b*; (●) chlorophyllide *a* and *b*; (□) pheophorbide *a* and *b*. The points represent the experimental data, and the curves represent the behavior predicted by the kinetic model.

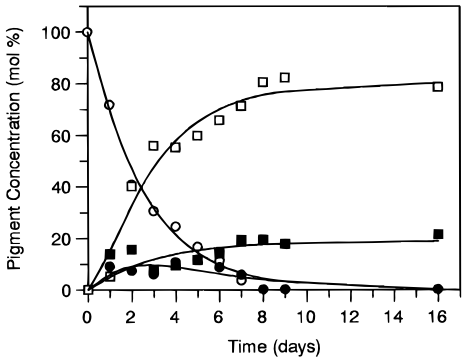


**Figure 2.** Chlorophyll degradation in whole brined cucumbers as a function of time: (○) chlorophyll *a* and *b*; (■) pheophytin *a* and *b*; (●) chlorophyllide *a* and *b*; (□) pheophorbide *a* and *b*. The points represent the experimental data, and the curves represent the behavior predicted by the kinetic model.

To further test the usefulness of the model, we modeled the data on chlorophyll degradation in brined olives of Minguez-Mosquera et al. (1989). Minguez-Mosquera et al. (1989) determined that the pathway for chlorophyll degradation in olives was the same as it was in brined cucumbers. No conversion of pheophytin to pheophorbide was observed in this case either ($k_2 = 0$). As illustrated in Figure 3, the model once again fits the data quite well. Boundary conditions (parameters) for this fitting procedure were as follows: $k_2 = 0$, $A_0 = 100$ mol %, $B_0 = 0$, $C_0 = 0$, $D_0 = 0$.

The ability of the model to adequately describe chlorophyll degradation in three different commodities under different storage and processing conditions suggests that the model may be useful in describing

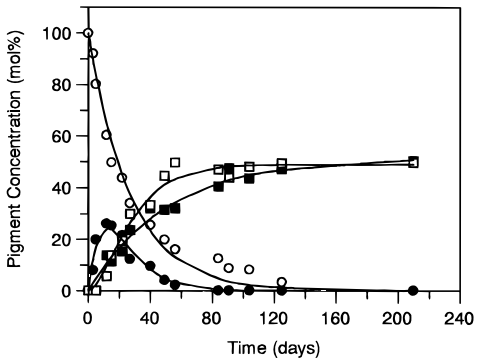


**Figure 3.** Chlorophyll degradation in whole brined olives as a function of time: (○) chlorophyll *a* and *b*; (■) pheophytin *a* and *b*; (●) chlorophyllide *a* and *b*; (□) pheophorbide *a* and *b*. The points represent the experimental data, and the curves represent the behavior predicted by the kinetic model.

chlorophyll degradation in a wide variety of processed foods. As can be observed in Table 1, $k_1$ and $k_2$ values derived from the data of Heaton et al. (1996) are quite consistent even though they have been derived from different data sets (i.e., from the modeling of chlorophyll degradation, pheophytin accumulation and degradation, and pheophorbide accumulation). Comparing the first-order rate constants for chlorophyll degradation in the various commodities, it can be noticed that the rate of chlorophyll degradation to pheophytin is greatest in coleslaw ($k_1 = 0.54$/day), followed by pickles ($k_1 = 0.084$/day), and smallest in olives ($k_1 = 0.023$/day). This reaction is enhanced under acidic conditions, so the probable reason for the observed differences is the pH of the products. Coleslaw reaches its minimum pH (4.8−4.6) during the first day of storage (Heaton et al., 1996), whereas the other commodities take 5−15 days to reach the equivalent pH. Therefore, one would expect the rate of conversion of chlorophyll to pheophytin to be greatest in coleslaw.

Heaton et al. (1996) determined that there was no significant change in the concentration of chlorophyllide over time in coleslaw, indicating that this pathway was not operational. In pickles and olives, however, the degradation of chlorophyll to chlorophyllide and then to pheophorbide was the predominant degradative pathway, and the accumulation of pheophorbide via pheophytin did not occur. The pH of both pickles and olives remained neutral for several days after the initiation of the experiment; thus, chlorophyllase activity would have also remained relatively high, thereby allowing for the enzymatic conversion of chlorophyll to chlorophyllide. As the pH of the system dropped, the chemical conversion of chlorophyllide to pheophorbide was enhanced. This enhancement is depicted in the large $k_3$ values for both pickles (0.29/day) and olives (0.033/day) relative to their $k_1$ values (0.084 and 0.023/day, respectively). The drop in pH also caused chlorophyll to degrade to pheophytin. This effect was more predominant in olives than in pickles.

As a final point, it would be interesting to compare the *k* value for the *in vitro* conversion of chlorophyll to chlorophyllide, or pheophytin to pheophorbide by the enzyme chlorophyllase to the *in vivo* rate constants obtained from the modeling work on chlorophyll degradation in whole tissue. From the data of Tanaka et al. (1982) we obtained a $k_3$ value of 6.96/day for chlorophyllase in green rye seedlings. This value was obtained by dividing the $V_{max}$ of chlorophyllase by its $K_m$ value, which yielded a first-order rate constant. This value is relatively high compared with our determined $k_2$ and $k_3$ values (Table 1), but when one takes into consideration the temperature and pH differences between the various experiments, the difference can be justified. According to Tanaka et al. (1982), a change in pH from 7.5 to 4.0 causes a decrease in activity by 30%, giving a modified $k_3$ value of 4.87/day. A decrease in temperature from 30 to 10 °C would further decrease activity by 80% (Tanaka et al., 1982), which would in turn reduce the $k_3$ value to 0.9744/day. The value would be further reduced if the temperature curve from the data of Tanaka et al. (1982) was extrapolated to 5 °C. This value is now in the same range as the $k_2$ and $k_3$ values reported in Table 1. This similarity further supports the hypothesis that the conversions of chlorophyll to chlorophyllide and pheophytin to pheophorbide were the result of chlorophyllase activity (Heaton et al., 1996).

In conclusion, we have developed a general mechanistic model that describes chlorophyll degradation in green plant tissue. The model can discriminate between the pathway(s) of degradation and thus quantitatively define which pathways are operational or predominate under various conditions. The ability of the model to quantify chlorophyll degradation provides a better means of comparing the relative rates of green pigment degradation in various commodities. By being able to determine the rate and pathway of chlorophyll degradation, the model will lead to a better understanding of chlorophyll degradation in foods. These results will aid

**Table 1. Kinetic Constants for Pigment Degradation in Coleslaw, Pickles, and Olives As Determined by Fitting Experimental Data to the Mechanistic Model Developed in This Study**

| data source | $k_1$ | $k_2$ | $k_3$ | $k_4$ |
|---|---|---|---|---|
| White et al. (1963) | | | | |
| eq 5 | 0.11 | 0 | 0.28 | − |
| eq 6 | 0.065 | 0 | 0.28 | − |
| eq 7 | 0.24 | 0 | 0.10 | 0.43 |
| eq 8 | 0.075 | 0 | 0.31 | 1.82 |
| av (SE) | 0.084 (0.015) | 0 | 0.29 (0.01) | 1.12 (0.70) |
| Minguez-Mosquera et al. (1989) | | | | |
| eq 5 | 0.018 | 0 | 0.018 | − |
| eq 6 | 0.010 | 0 | 0.010 | − |
| eq 7 | 0.027 | 0 | 0.056 | 0.083 |
| eq 8 | 0.035 | 0 | 0.034 | 0.068 |
| av (SE) | 0.023 (0.005) | 0 | 0.033 (0.013) | 0.056 (0.020) |
| Heaton et al. (1996) | | | | |
| eq 5 | 0.59 | − | 0 | − |
| eq 6 | 0.42 | 0.27 | 0 | − |
| eq 8 | 0.60 | 0.17 | 0 | − |
| av (SE) | 0.54 (0.06) | 0.22 (0.05) | 0 | − |

processors in determining the optimum shelf-life of their product. Understanding the mechanism responsible for the discoloration of tissue will undoubtedly lead to higher quality food.

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