Chapter 2
Chlorophyll Degradation in Green Tissues: Olives, Cabbage and Pickles

2.1 Chlorophyll Pigments

The chlorophylls are the main pigments in green plants, algae and other photosynthetic microorganisms. They belong to a class of pyrrole ring compounds known as porphins. Derivatives of porphins are called porphyrins. Phorbin, a porphyrin formed by the addition of another ring structure, serves as the basis for all chlorophyll molecules (Fig. 2.1). Different types of chlorophyll may arise depending on the chemical substituents on the R and R′ group (Fig. 2.2).

Chlorophylls are predominantly found in the chloroplasts of plant cells. They are of hydrophobic nature and are found alongside carotenoids, lipids and lipoproteins. The carotenoids serve an important physiological function. Carotenoids prevent

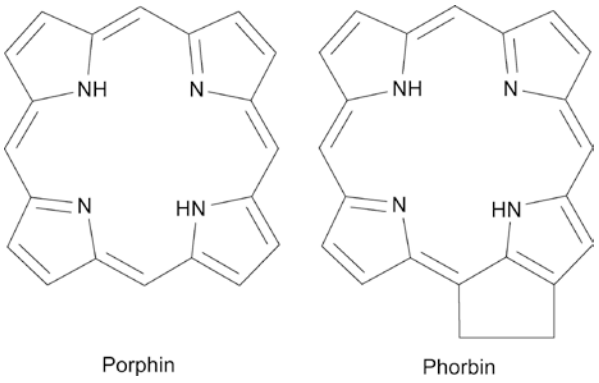

Fig. 2.1 Porphin is a compound made of 4 pyrole rings linked via methylene bridges. Phorbin is a porphin derivative with an attached 5-carbon ring structure.

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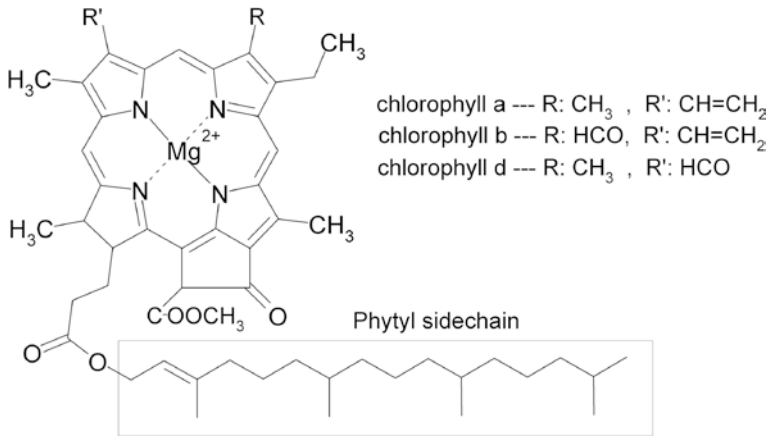

Fig. 2.2 The base molecule for chlorophyll compounds. Possible derivatives that can be obtained via the substitution of the R- and R'- groups are shown.

chlorophyll from acting as a photosensitizer in light-induced oxidation. The extraction of chlorophyll is often achieved through the use of generally non-polar solvents with some polar groups an example of which is acetone.

2.2 Chlorophyll Degradation

Chlorophyll absorbs visible light in in the wavelength range 400–500nm (blue) and 600–700nm (red). The remaining unabsorbed radiation is reflected, giving the chlorophylls their green color. When the chlorophyll molecule is altered or degraded, the absorption spectra may shift, leading to a change in color. This alteration may be natural or a result of food processing.

To the food scientist, chlorophyll loss represents a quality problem in the manufacture of processed plant products. The degradation of chlorophyll causes a shift in colour from brilliant green to olive-brown in processed foods. This results in an undesirable swamp-green appearance in foods. Chlorophyll loss, however, may serve as a useful measure of a plant's ripeness and freshness. In live plants, chlorophyll loss accompanies the natural process of senescence.

The degradation of chlorophyll results in the formation of five groups of intermediate compounds. The reason for their classification into groups rather than individual compounds is that the side chains of the chlorophyll molecule are often substituted with different groups, resulting in structurally similar, yet different compounds.

Several mechanisms contribute to the degradation of chlorophyll. The phytyl chain on chlorophyll can be cleaved off, or hydrolyzed, by the chlorophyllase enzyme to yield chlorophyllide. Chlorophyllase is dormant in live tissues but is

activated by heat during processing. The enzyme exhibits a temperature optimum between 60 and 80 °C (blanching temperatures) but loses activity if it is further heated. The phytyl chain may also be removed via non-enzymatic means. The dephytylation of the chlorophyll molecule does not cause any change in the color of the pigment.

The second major chlorophyll degradation mechanism is the abstraction of the Mg^{2+} ion bound to the center of the chlorophyll molecule to yield pheophytin. Under acidic conditions, the Mg^{2+} is replaced by H^{+} ions. The reaction may also be catalyzed by the magnesium dechelatase enzyme. The removal of the Mg^{2+} results in a shift of the absorption spectrum, leading to the appearance of an olive-brown, swamp-green color.

The degradation of chlorophyll to pheophorbide is a two-step process. These two steps can come in any order resulting in two distinct parallel pathways of chlorophyll degradation. In the first pathway, the phytyl chain from the chlorophyll molecule is cleaved to yield chlorophyllide. The Mg^{2+} ion bound to the center of the porphyrin ring is then replaced with H^{+} ions to yield pheophorbide.

The second pathway is chemically identical to the first pathway except that the order in which the steps occur are reversed. The Mg^{2+} ion in chlorophyll is first removed by the action of either acid or magnesium dechelatase to yield pheophytin. The phytyl chain in pheophytin is then removed to yield pheophorbide. The entire reaction and the parallel nature of the steps are outlined in Fig. 2.3.

2.3 A Kinetic Model of Chlorophyll Degradation

The following rate equations can be written for the degradation of chlorophyll as given in Fig. 2.4 where A stands for chlorophyll, B stands for pheophytin, C stands for chlorophyllide and D stands for pheophorbide.

$$\frac{d[A]}{dt} = -k_1[A] - k_3[A] \tag{2.1}$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] \tag{2.2}$$

$$\frac{d[C]}{dt} = k_3[A] - k_4[C] \tag{2.3}$$

$$\frac{d[D]}{dt} = k_2[B] + k_4[C] \tag{2.4}$$

Assuming no chlorophyll molecules are synthesized or introduced into the system, the four compounds are governed by the following mass balance:

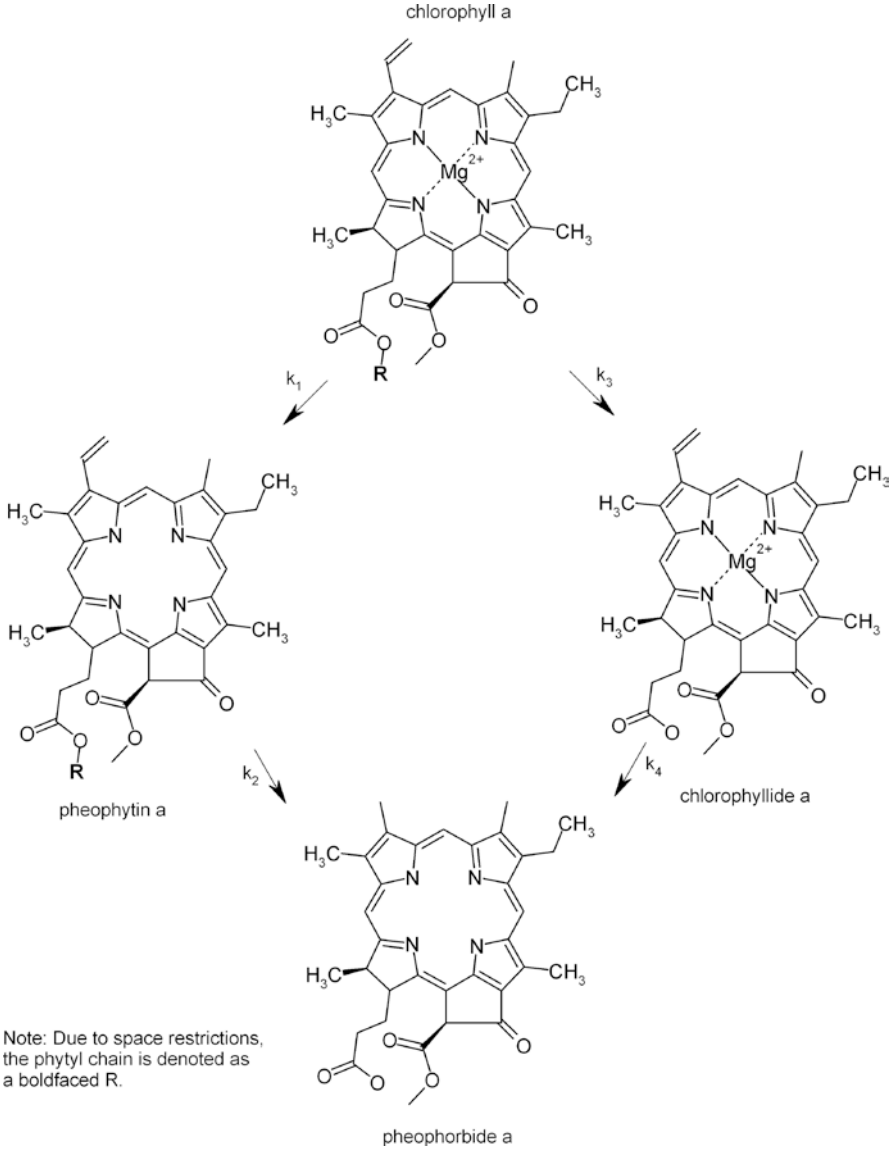

Fig. 2.3 The parallel reaction pathway for the conversion of chlorophyll *a* to pheophorbide a.

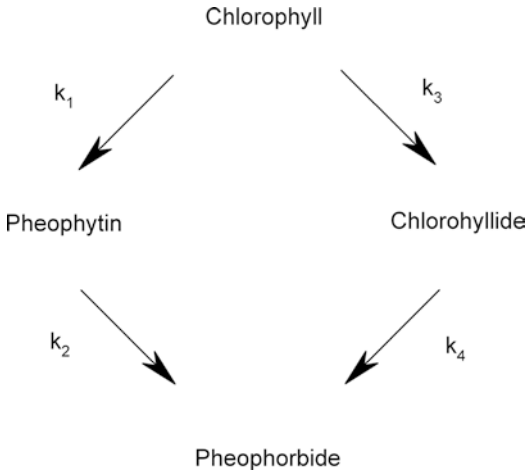

Fig. 2.4 A proposed kinetic model for the degradation of cholorophyll to pheophorbide.

$$[A_o]+[B_o]+[C_o]+[D_o]=[A]+[B]+[C]+[D] \tag{2.5}$$

where $[A_o]$ is the initial chlorophyll concentration, $[B_o]$ is the initial pheophytin concentration, $[C_o]$ is the initial chlorophyllide concentration and $[D_o]$ is the initial pheophorbide concentration. The following differential equations were solved to yield the following analytical solutions.

$$[A]=[A_o]e^{-(k_1+k_3)t} \tag{2.6}$$

$$[B]=\frac{k_1[A_o]}{k_2-k_1-k_3}\left[e^{-(k_1+k_3)t}-e^{-k_2t}\right]+[B_o]e^{-k_2t} \tag{2.7}$$

$$[C]=\frac{k_3[A_o]}{k_4-k_1-k_3}\left[e^{-(k_1+k_3)t}-e^{-k_4t}\right]+[C_o]e^{-k_4t} \tag{2.8}$$

$$[D]=[D_o]+\left([A_o]-[A]\right)+\left([B_o]-[B]\right)+\left([C_o]-[C]\right) \tag{2.9}$$

Instead of writing a separate differential equation describing the formation of pheophorbide, the quantity of pheophorbide was calculated by the re-arrangement of the mass balance equation. This assumes that pheophorbide is the ultimate product of chlorophyll degradation. This is a reasonable assumption since a study investigating this degradation pathway revealed that the pheophorbide concentration did not decrease even after 200 days.

The given solutions to the differential equations can be fitted to their corresponding data sets via nonlinear regression. Oftentimes, however, analytical solutions are impossible to obtain for differential equations describing more complex phenomena. A simple solution to this is to fit the data set to a numerical approximation of

the differential equation. The availability of more powerful computers and sophisticated data analysis software have allowed for the fitting of data directly to differential equations instead of the analytical solutions to these same differential equations. Moreover, all equations can be fitted to all data sets simulataneously, thus creating one unique set of parameter estimates, which are more accurate and robust. This is called multiple nonlinear regression. Multiple nonlinear regression is not discussed in this book.

2.3.1 Coleslaw by Heaton et al. [2]

In a previous study establishing the theoretical framework for the kinetic modelling of chlorophyll degradation in coleslaw, Heaton and others determined that chlorophyll degradation in coleslaw followed only one particular pathway out of the two pathways available. This pathway is the *Chlorophyll* → *Pheophytin* → *Pheophorbide* pathway. The data was fitted to the model on the assumption that chlorophyll does not degrade by the other pathway available, namely *Chlorophyll* → *Chlorophyllide* → *Pheophorbide*. This was evident from the experimental data.

The experimental data were fitted using the following constraints: $k_3 = 0$, $k_4 = 0$, $A_{lim} = 6$ mol%, $B_{lim} = 20.5$ mol%, $C_{lim} = 0.1$ mol%, $A_o = 58.4$ mol%, $B_o = 27.9$ mol%, $C_o = 0.1$ mol%, $D_o = 13.6$ mol%. The assumption that chlorophyll only follows the *Chlorophyll* → *Pheophytin* → *Pheophorbide* pathway is embodied in the model by setting k_3 and k_4 equal to 0. A_{lim}, B_{lim} and C_{lim} denote the limiting concentration of chlorophyll, pheophytin and chlorophyllide, respectively. The limiting concentration denotes the minimum amount of the substance that exists in the system. Presumably, the limiting concentration is the concentration of each particular intermediate that is unavailable for degradation but is nevertheless present in the system. The difference between the reactant's concentration (for example, $[A]$) and its limiting concentration A_{lim}, $[A] - [A_{lim}]$, gives the concentration of reactant available to undergo degradation.

The calculated curves for this model and the experimental points are presented in Fig. 2.5, demonstrating that the model described the experimental results quite accurately.

2.3.2 Pickles by White et al. [6]

To ensure that the given differential equations also applied to chlorophyll degradation in other food systems, the model was used to describe chlorophyll degradation in brined cucumbers (pickles). White et al. noted that the pheophytin levels in the system studied remained constant after an initial increase early on in the experiment. This observation translates to $k_2 = 0$, that is, pheophytin is not

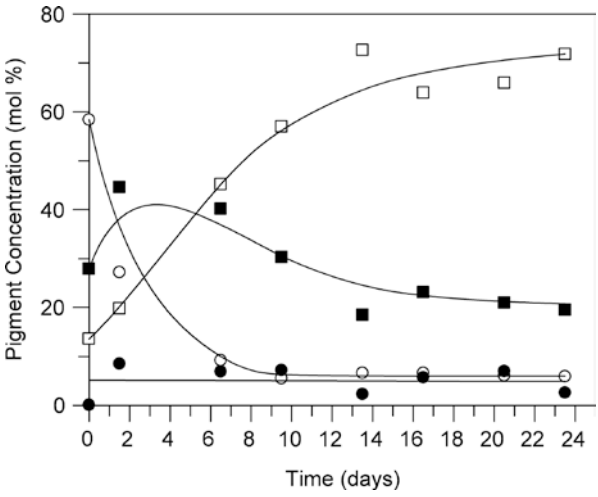

Fig. 2.5 Chlorophyll degradation data for processed coleslaw. (○) chlorophyll; (●) chlorophyllide; (■) pheophytin; (□) pheophorbide. The solid lines represent the curves obtained by regressing the analytic solutions to the data (Data by Heatonet al. [2]).

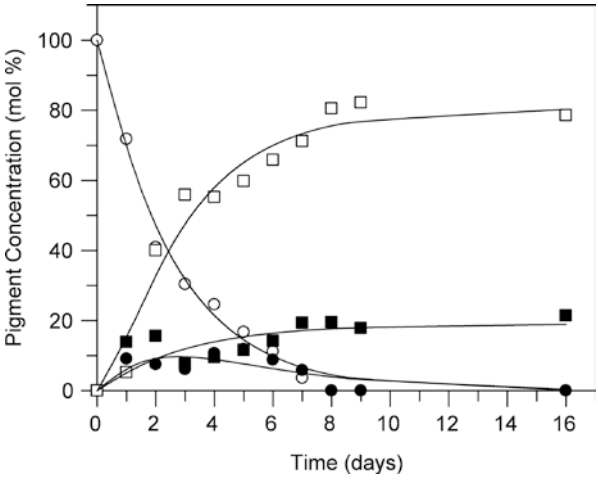

Fig. 2.6 Chlorophyll degradation data for brined cucumbers. (○) chlorophyll; (●) chlorophyllide; (■) pheophytin; (□) pheophorbide. The solid lines represent the curves obtained by regressing the analytic solutions to the data (Data by White et al. [6]).

further degraded to pheophorbide and that pheophytin accumulates in the food system, reaching a constant concentration towards the end of the observation period.

White's experimental data was fitted with the following boundary conditions: $k_2 = 0$, $A_o = 100$ mol%, $B_o = 0$, $C_o = 0$, $D_o = 0$, $A_{lim} = 0$, $B_{lim} = 21.4$ mol%, $C_{lim} = 0$, $D_{lim} = 78.6$ mol%. The experimental data and the resulting model curve is given in Fig. 2.6. The good fit observed implies that the model is also applicable to describe chlorophyll degradation in brined cucumbers.

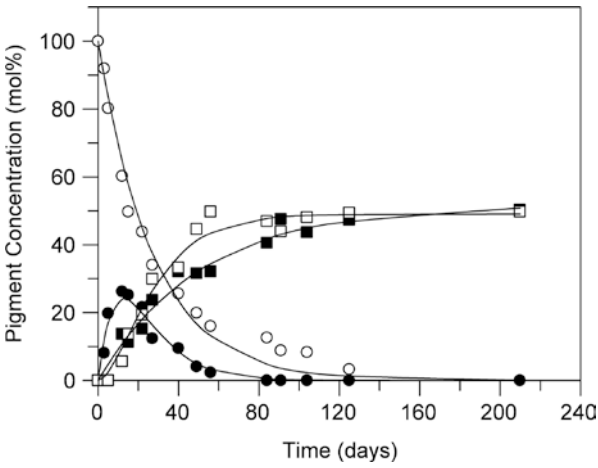

Fig. 2.7 Chlorophyll degradation data for whole brined olives. (○) chlorophyll; (●) chlorophyllide; (■) pheophytin; (□) pheophorbide. The solid lines represent the curves obtained by regressing the analytic solutions to the data (Data by Minguez-Mosquera et al. [4]).

2.3.3 *Olives by Minguez-Mosquera et al. [4]*

To further test the utility of the model, experimental data on the degradation of chlorophyll in processed olives was fitted to the given model. Minguez-Mosquera et al. proposed a similar pathway of chlorophyll degradation as that proposed by White. Fig. 2.7 shows the experimental data and the modelled curve. Once again, the closeness-of-fit indicates that the model is also valid for brined olives.

2.3.4 *Relating Kinetic Parameters to Degradation Mechanisms*

The kinetic parameters derived from fitting the analytical solutions of the model to the data is given in Table 2.1. Consider the degradation of *Chlorophyll* → *Pheophytin*. It is believed that this reaction occurs rapidly under acidic conditions. At low enough pH, H^+ ions can displace the Mg^{2+} ion chelated to the porphyrin ring. A glance at the k_1 constants of the different data sets reveal that the formation of pheophytin from chlorophyll was fastest in coleslaw ($k_1 = 0.54$ day^{-1}), followed by the pickles ($k_1 = 0.084$ day^{-1}) and then by the olives ($k_1 = 0.023$ day^{-1}). The vastly greater k_1 constant observed in the early portion of the coleslaw experiment is due to the almost instantaneous drop in the pH of coleslaw, presumably caused by the addition of salad dressing.

Similarly, no change was observed in the chlorophyllide concentration in coleslaw since the beginning of the experiment. This is the basis for the abovementioned assumption $k_3 = 0$ and $k_4 = 0$ in Heaton *et al*'s data. However, the degradation of chlorophyll to chlorophyllide was the primary initial pathway observed in brined olives ($k_3 = 0.033$ day^{-1}) and pickled cucumbers ($k_3 = 0.29$ day^{-1}). A likely explanation as to the high rate of conversion from *Chlorophyll* → *Chlorophyllide* (relative to

Table 2.1 Kinetic constants (mean ± standard deviation) derived from fitting the individual analytical solutions of the model to the data

Food system	k_1 (day^{-1})	k_2 (day^{-1})	k_3 (day^{-1})	k_4 (day^{-1})
Pickled cucumbers (White et. al)	0.084 ± 0.015	0	0.29 ± 0.01	1.12 ± 0.70
Brined olives (Minguez-Mosquera et. al)	0.023 ± 0.005	0	0.33 ± 0.013	0.056 ± 0.020
Coleslaw (Heaton et. al)	0.54 ± 0.06	0.22 ± 0.05	0	0

the corresponding rates of conversion to pheophytin) is the high activity of the chlorophyllase enzyme in the unprocessed olives. Prior to brining and fermentation, the pHs of both the cucumber and the olives are neutral. Chlorophyllase has high activity at neutral pH. Furthermore, the pH of the olives and the cucumbers remained at the neutral optimum for several days --- ample time to degrade chlorophyll to chlorophyllide. Subsequent fermentation results in a decrease in pH, completing the degradation pathway by converting chlorophyllide to pheophorbide. This decrease in pH also resulted in the conversion of chlorophyll to pheophytin as observed in Figs. 2.6 and 2.7.

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