Chlorophylls: Chemistry and Biological Functions

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14 Chlorophylls: Chemistry and Biological Functions

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14.1 Introduction

Chlorophylls are unique pigments with green color and are found in diverse plants, algae, and cyanobacteria (Inanc, 2011). The term chlorophyll is derived from the Greek chloros meaning “green” and phyllon meaning “leaf.” Isolation and naming of the chlorophyll was first carried out by Joseph Bienaimé Caventou (French pharmacist) and Pierre-Joseph Pelletier (French chemist) in 1817 (Gopi et al., 2014). Chlorophyll is made up of carbon and nitrogen atoms along with a magnesium ion in central position. Chlorophyll is found in almost every green part of plants, i.e. leaves and stem, within the chloroplast, the main organelle which contains the highest amount. Chloroplasts are found in the mesophyll layer, in the middle of plant leaves. Chloroplasts possess thylakoid membranes which contain green chlorophyll pigment. Chloroplast can be referred to as the “food factory” of the plant cell because it produces energy and glucose for the whole plant in association with CO2, water, and sunlight.

The name “chlorophyll” was first given to the chloroplast of higher plants only, but later it was extended to all photosynthetic porphyrin pigments (Vernon and Seely, 1966). It comes under the special class of compounds called tetrapyrroles because it contains four pyrrole rings joined together with a covalent bond, as are vitamin B12 and the heme molecule (Willows, 2004).

14.1.1 Importance

The main source of life on earth is the solar energy that is captured by green plants, algae, and various photosynthetic bacteria. Although there are different photosynthetic pigments such as carotenoids and phycobilins which entrap solar radiation, chlorophyll is the most important of these molecules. It converts solar energy into chemical energy that is used to build essential carbohydrate molecules (glucose) which are used as food source for the whole plant (Hynninen and Leppakases, 2002). The process can be described by the equation shown in Figure 14.1.

Broadly, the conversion process of solar energy into chemical energy is called photosynthesis. Chlorophyll pigment absorbs blue light and red light of solar radiation at 430 nm and 660 nm, respectively, and it reflects the green spectrum (Inanc, 2011) (Figure 14.2).

14.1.2 Types and Distribution of Chlorophylls

The numbers of naturally occurring chlorophylls may not yet be fully known. Chlorophyll a and chlorophyll b are the main components of photosystems in photosynthetic organisms. The types of different chlorophyll pigments present in nature in decreasing order of importance are given in Table 14.1.

Initially, chlorophyll was classified into four – chlorophyll a, chlorophyll b, chlorophyll c, and chlorophyll d (Vernon and Seely, 1966) – but later a new type of chlorophyll was discovered within stromatolite (a hard rock structure made by cyanobacteria) in western Australia, which was named chlorophyll f. Thus eventually chlorophyll was divided into five classes, a, b, c, d, and f.

14.1.2.1 Chlorophyll a

This type of chlorophyll is found in almost all photosynthetic organisms, i.e. plants, algae, cyanobacteria, and
aquatic species (Jordan et al., 2001; Nakamura et al., 2003). Previously it had been called chlorophyll α. It is found in all light harvesting complexes (LHCs) and both reaction centers (RCs) in organisms, photosystem I (PS I) and photosystem II (PS II). It absorbs mainly red light from the solar spectrum; the absorption peak is captured at 420 nm and 660 nm in organic solvents and at 453 nm and 670–480 nm in photosynthetic cells (in vivo). It works as primary donor in the RCs of PS I and PS II (Scheer, 1991).

Subsequently, two types of chlorophyll $a$, Ca 670 and Ca 680, were identified which are responsible for absorption of different wavelengths from the light spectrum (French et al., 1972). This observation was confirmed by curve analysis of the absorption spectra of many plants and algae. Various experiments were conducted, and the results reported were that Ca 670 possessed greater half-width than Ca 680 in the fraction in PS I, and Ca 680 possessed greater half-width than Ca 670 in PS II (French et al., 1969a, 1969b; French 1970).

14.1.2.2 Chlorophyll $b$

Previously, this was called chlorophyll $\beta$. Chlorophyll $b$ has been confirmed to be found in green algae and also in higher plants. It assists chlorophyll $a$ in the photosynthesis process. This pigment has yellow color in its natural state but absorbs blue light from the whole solar spectrum. For chlorophyll $b$ the characteristic absorption peak was observed at 453 nm and 625 nm in vitro conditions and at 480 nm and 650 nm in vivo (Strain et al., 1963).

14.1.2.3 Chlorophyll $c$

Chlorophyll $c$ is a brownish-golden colored pigment that accompanies chlorophyll $a$ in the photosynthesis process as an accessory pigment. It has three subclasses, named chlorophyll $c_1$, $c_2$, and $c_3$, which have been found in various algae (Beale, 1999). This pigment is widely assimilated in different marine organisms like diatoms, brown algae, and other marine algae (Smith and Benitez, 1955; Strain et al., 1963). The absorption peak of chlorophyll $c$ for the photosynthetic spectrum was obtained at 445 nm and 625 nm in organic solvent and 645 nm in vivo.

14.1.2.4 Chlorophyll $d$

Chlorophyll $d$ is the minor chlorophyll that was identified in red algae (Rhodophyta) by Strain (1958). It captures the extreme red end of the spectrum of sunlight. The absorption spectra were obtained for chlorophyll $d$ at 450 nm and 690 nm in vitro conditions and up to 740 nm on red band in vivo. It can be prepared in the laboratory with the help of permanganate by the oxidation of chlorophyll $a$ (Holt and Morley, 1959; Holt, 1961).

14.1.2.5 Chlorophyll $f$

Chlorophyll $f$ was the last main chlorophyll to be discovered. It was revealed in cyanobacteria from the deep stromatolites in the western region of Australia by Min

Table 14.1 Types of different chlorophylls in nature, in order of abundance from most (1) to least (7).

<table>
<thead>
<tr>
<th>Abundance</th>
<th>Chlorophyll types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorophyll $a$</td>
</tr>
<tr>
<td>2</td>
<td>Chlorophyll $b$</td>
</tr>
<tr>
<td>3</td>
<td>Chlorophyll $c$</td>
</tr>
<tr>
<td>4</td>
<td>Chlorophyll $d$</td>
</tr>
<tr>
<td>5</td>
<td>Chlorophyll $f$</td>
</tr>
<tr>
<td>6</td>
<td>Protochlorophyll</td>
</tr>
<tr>
<td>7</td>
<td>Bacteriochlorophyll</td>
</tr>
<tr>
<td>8</td>
<td>Chlorobium chlorophyll</td>
</tr>
</tbody>
</table>

Figure 14.2 Light absorption of different photosynthetic pigments.
Chen and his colleagues. Among all known types of chlorophyll, it is chlorophyll that can utilize the lowest solar light from the extreme end of the infrared spectrum for photosynthesis. The in vitro absorption and fluorescence of chlorophyll was obtained at 706 nm and 722 nm, respectively (Chen et al., 2010). Min Chen highlighted the need for a revised view of the dynamic role of chlorophyll, as “discovering this new chlorophyll has completely overturned the traditional notion that photosynthesis needs high energy light.” However, all pigments are often considered accessory pigments except chlorophyll.

In addition to the chlorophyll types described above, some other chlorophylls are found in nature, as discussed in the following sections.

### 14.1.2.6 Chlorophyll e
Chlorophyll e is a rare type, which was reported in golden-yellow algae named *Vaucheria hamata* and *Tribonema bombycinum* (Eugene and Govindjee, 1969). Its proper working mechanism has not been revealed clearly.

### 14.1.2.7 Protochlorophyll
Protochlorophyll occurs in very small amounts along with pheoporphyrin and protochlorophyllide. It is found in pumpkin seeds at the inner coat and in the dark-grown yellow leaves of seedlings (Madsen, 1963).

### 14.1.2.8 Bacteriochlorophyll
Bacteriochlorophyll is the main chlorophyll of various photosynthetic bacteria (French, 1969b; Sistrom and Clayton, 1964; Jensen et al., 1964). Several forms of bacteriochlorophylls have been reported, namely a, b, c, d, e, and g (Eimhjellen et al., 1963; Scheer, 1991).

### 14.1.2.9 Chlorobium Chlorophylls
These are abundantly found in Chlorobacteriaceae (green-sulfur bacteria). Chlorobium chlorophylls sometimes work in association with bacteriochlorophylls (Kaplan and Silberman, 1959; Moshentseva and Kondratieva, 1962; Mathewson et al., 1963).

### 14.1.3 Distribution of Chlorophylls among Photosynthetic Organisms
The distribution of chlorophylls in their natural state is summarized in Table 14.2 (Scheer, 1991; Chen et al., 2010).

<table>
<thead>
<tr>
<th>Types of chlorophylls</th>
<th>Presence and occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>All photosynthetic plants (excluding bacteria)</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>Higher plants and various green algae</td>
</tr>
<tr>
<td>Chlorophyll c</td>
<td>Brown algae and diatoms</td>
</tr>
<tr>
<td>Chlorophyll d</td>
<td>Various red algae, reported in Rhodophyta</td>
</tr>
<tr>
<td>Chlorophyll e</td>
<td>Golden-yellow algae (<em>Vaucheria hamata</em>)</td>
</tr>
<tr>
<td>Chlorophyll f</td>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Protochlorophyll</td>
<td>Found in yellow leaves of seedlings (grown in dark) and seed coat of pumpkin</td>
</tr>
<tr>
<td>Bacteriochlorophyll a</td>
<td>Purple and green bacteria</td>
</tr>
<tr>
<td>Bacteriochlorophyll b</td>
<td>Purple bacterium (<em>Rhodopseudomonas</em>)</td>
</tr>
<tr>
<td>Bacteriochlorophyll c</td>
<td>Reported in Chloroflexaceae, Chlorobiaceae</td>
</tr>
<tr>
<td>Bacteriochlorophyll d</td>
<td>Reported in Chloroflexaceae, Chlorobiaceae</td>
</tr>
<tr>
<td>Bacteriochlorophyll e</td>
<td>Reported in Chloroflexaceae, Chlorobiaceae</td>
</tr>
<tr>
<td>Bacteriochlorophyll g</td>
<td>Discovered in <em>Heliobacterium chlorum</em></td>
</tr>
<tr>
<td>Chlorobium chlorophylls</td>
<td>Green bacteria</td>
</tr>
</tbody>
</table>

### 14.2 Chemistry of Chlorophylls
The porphyrin unit has a very crucial role in nature because it participates in the fundamental skeleton of chlorophyll (Eugene and Govindjee, 1969). Many researchers have performed various experiments, and both analyzed the chemical properties and elucidated the structure of chlorophyll molecules (Aronoff, 1966; Seely, 1966). Research has revealed that the chlorophylls are tetrapyrroles, a cyclic form of porphyrin and chlorin (the parent molecule of all chlorophylls). This cyclic form creates an isocyclic ring with the help of —CH bridges. Chemically, chlorophylls possess a magnesium ion in the central position which is found connected with the tetrapyrole ring (Scheer, 1991). Moreover, chlorophylls are hydrophobic molecules because they contain phytol, an esterified isoprenoid C20 alcohol. The phytol (C20H30OH) possesses a double bond in the trans configuration (Gross, 1991).

### 14.2.1 Structures of Different Chlorophylls
Chemically, the basic skeleton of chlorophyll is composed of a cyclic tetrapyrole ring, which is a large planar structure of a symmetric arrangement in which the
four pyrrole rings are joined together by methine (−CH=) bridges, and four nitrogen atoms are coordinated with a central metal atom. In addition, they have a phytol group that confers a hydrophobic characteristic; the metal bound to the chlorophylls is magnesium. Therefore, this structure contains a chromophore of several conjugated double bonds responsible for absorbing light in the visible region, i.e. red (peak at 670–680 nm) and blue (peak at 435–455 nm). The reflection and/or transmission of the non-absorbed green light (intermediate wavelength) give the characteristic green color to plants and chlorophyll solutions. Higher plants, ferns, mosses, green algae, and the prokaryotic organism prochloron only have two chlorophylls (a and b); the remaining chlorophylls are present in algae and bacteria. The structures of different chlorophylls are described below:

### 14.2.1 Chlorophyll a

The molecular formula of chlorophyll a is C_{55}H_{72}MgN_{4}O_{5}. It contains a chlorin ring in which a magnesium ion is surrounded centrally by four nitrogen atoms (Taiz et al., 2006) (the structures of the different chlorophylls are shown in Figure 14.3). The side chains of various chlorophyll molecules determine the characters of other chlorophyll types and create changes in the absorption spectrum of solar light (Niedzwiedzki and Blankenship, 2010). Chlorophyll a possesses a long

![Figure 14.3 Structures of different chlorophylls.](image_url)
hydrophobic tail that anchors the molecule to alternative hydrophobic proteins within the thylakoid membrane of the plastid (Raven et al., 2005).

14.2.1.2 Chlorophyll b
The chemical formula of chlorophyll b is C\textsubscript{55}H\textsubscript{70}MgN\textsubscript{4}O\textsubscript{6}. There is only a minor difference between the structures of chlorophyll a and chlorophyll b; in the latter, a −CHO group is found in place of −CH\textsubscript{3} at the C-7 position (Figure 14.3).

14.2.1.3 Chlorophyll c
Chlorophyll c was confirmed as a mixture of magnesium tetradehydro- and hexadehydropheoporphyrin a\textsubscript{3}, mono- methyl ester (Ia, Ib) (Dougherty et al., 1970). It has three sub classes, c\textsubscript{1}, c\textsubscript{2}, and c\textsubscript{3}. Their respective formulas are C\textsubscript{55}H\textsubscript{58}MgN\textsubscript{4}O\textsubscript{5}, C\textsubscript{53}H\textsubscript{58}MgN\textsubscript{4}O\textsubscript{5}, and C\textsubscript{56}H\textsubscript{58}MgN\textsubscript{4}O\textsubscript{7}. The c\textsubscript{1} is considered the most common form of chlorophyll c; its structure differs from c\textsubscript{2} in that it contains an ethyl group in place of a vinyl group at the C-8 position (Figure 14.3).

14.2.1.4 Chlorophyll d
The molecular formula of chlorophyll d is C\textsubscript{54}H\textsubscript{70}MgN\textsubscript{4}O\textsubscript{6}. The structure of chlorophyll d was elucidated by Manning and Strain (1943). They revealed that it differs from chlorophyll a in that it has a formyl group in place of divinyl group in ring A of porphyrin (Larkum and Kuhl, 2005) (Figure 14.3).

14.2.1.5 Chlorophyll f
The molecular structure of chlorophyll f is similar to that of chlorophyll b, namely C\textsubscript{55}H\textsubscript{70}MgN\textsubscript{4}O\textsubscript{6}. However, after analysis using nuclear magnetic resonance (NMR), mass spectroscopy (MS), and high-performance liquid chromatography (HPLC), a major difference was revealed: that is, the existence of −CHO at the C-2 position of the porphyrin ring (Figure 14.3) (Willows et al., 2013).

14.2.2 Synthesis of Chlorophyll
Chlorophyll synthesis is a very crucial mechanism for the existence of photosynthetic organisms as well as of other creatures that are dependent on them. The chlorophyll biosynthesis process is highly complicated because of the complex combination of different enzymes and the many resulting compounds.

The C-5 pathway, a complex biosynthetic pathway, is responsible for naturally synthesizing the chlorophyll and other tetrapyrrole pigments because this pathway produces key precursors and the intermediate C-5 compound δ-aminolevulinic acid (ALA) (Wettstein et al., 1995; Tanaka and Tanaka, 2007). Chlorophyll biosynthesis is depicted in Figure 14.4.

The whole biosynthetic pathway of chlorophyll can be elaborated in the steps described in the following sections.

14.2.2.1 Forming of 5-aminolevulinic acid (ALA)
The first step in the C-5 pathway is glutamate activation by t-RNA\textsubscript{Glu}. A similar step is found in protein synthesis for glutamate incorporation (Hori and Kumar, 1996; Kumar et al., 1996). The second step is the formation of glutamyl-1-semialdehyde by the reduction of glutamyl t-RNA\textsubscript{Glu}, catalyzed by glutamyl t-RNA\textsubscript{Glu} reductase. In this step, t-RNA\textsubscript{Glu} is released. It has been revealed that illumination increases the activity of glutamyl t-RNA\textsubscript{Glu} reductase in seedling cotyledons of green cucumber (Masuda et al., 1996). The final step is the conversion of glutamate-1-semialdehyde into ALA by the transamination process. The C-5 pathway occurs in the plastids and is the only process that has been reported in plants. Apart from plants, ALA synthesis also takes place in animals and fungi, but the pathway is different and is known as the C-4 pathway. This pathway is found in mitochondria (Drechsler-Thielmann et al., 1993; Weinstein et al., 1993).

14.2.2.2 Formation of Protoporphyrin from ALA
In this process, two ALA molecules create the monopyrrole porphobilinogen via the enzyme ALA dehydratase. The next step consists of the formation of hydroxymethylbilane, a tetrapyrrole from porphobilinogen. Four molecules of porphobilinogen are converted by the enzyme porphobilinogen deaminase (hydroxymethylbilane synthase). The condensation reaction takes place in this conversion (Jordan, 1991). The structure of the hydroxymethylbilane synthase enzyme was elucidated first among all the enzymes of tetrapyrrole biosynthesis (Louie et al., 1992).

In the next step, uroporphyrinogen III is formed from hydroxymethylbilane; the reaction is catalyzed by uroporphyrinogen III synthase. Uroporphyrinogen III is the first cyclic compound of the chlorophyll biosynthesis pathway (Leeper, 1994). The subsequent steps involve the decarboxylation of acetate chains of uroporphyrinogen III which gives coproporphyrinogen III, catalyzed by uroporphyrinogen decarboxylase. Proporphyrinogen IX formation takes place from coproporphyrinogen III by an oxidative decarboxylation reaction. Coproporphyrinogen oxidase is the catalytic enzyme for this decarboxylation reaction.

The last step, proporphyrinogen formation, is catalyzed by protoporphyrinogen oxidase, which has two features. First, this is the only enzyme of plant porphyrin
biosynthesis that is found in two organelles, mitochondria and plastid (Jacobs et al., 1982; Jacobs and Jacobs, 1984). Second, protoporphyrinogen is a colorless compound, as are previously formed intermediate compounds; protoporphyrinogen oxidase produces the first pigment, protochlorophyllide, of the chlorophyll biosynthesis process.

### 14.2.2.3 Addition of Magnesium Ion
The identification and analysis of photosynthetic gene cluster, a 46 kbp gene sequence, was the real discovery in the field of chlorophyll and bacteriophyll biosynthetic pathways. This gene cluster was identified in *Rhodobacter capsulatus* which possessed *inter alia* the genes responsible for the production of all the enzymes related to Mg branch formation in the biosynthetic pathway.

The insertion of the magnesium ion is catalyzed by the Mg chelatase enzyme which needs ATP for activation. Mg chelatase is a significant bottleneck enzyme that specifically binds to Mg ions only (Castelfranco et al., 1979).

### 14.2.2.4 Conversion of Magnesium Protoporphyrin into Protophloeyllide
This step involves esterification at position 6 of the propionic acid side chain to produce Mg protoporphyrin...
IX monoethyl ester; the reaction is catalyzed by S-adenosyl-L-methionine:Mg-protoporphyrin-\(O\)-methyltransferase. The catalytic mechanism of this enzyme was confirmed as a pingpong mechanism in wheat (Richards et al., 1987) and as an ordered mechanism in \textit{Rhodobacter sphaeroides} (Bollivar et al., 1994). Various investigations have demonstrated that Mg protoporphyrin IX works as a substrate for the methylation step. After that, an isocyclic ring is formed that constitutes divinyl protochlorophyllide. The divinyl protochlorophyllide (Mg-2,4-divinyl-porphorpyrin \textit{a5}) (MgDVP) is created by \(\beta\)-oxidation and cyclization at position 6 in ring C. This reaction is catalyzed by Mg protoporphyrin IX oxidative cyclase or MPE oxidative cyclase.

14.2.2.5 Reduction of Protochlorophyllide into Chlorophyllide

The photoreduction process occurs at this step, which converts protochlorophyllide into chlorophyllide \textit{a}, and the macrocycle (the dihydroporphyrin or chlorin) is developed. NADPH protochlorophyllide oxidoreductase is the enzyme responsible for photoreduction. A chromophore is found in the macrocycle that provides the green color to chlorophyll \textit{a}. Prothylakoids of etioplasts contain this enzyme.

14.2.2.6 Esterification of Chlorophyllide \textit{a}

This is the last step in the process of chlorophyll \textit{a} biosynthesis. Here, phytol, a C-20 isoprenoid alcohol, reacts with 7-propionic acid residue by an esterification process. Chlorophyll dihydrogeranylgeranyl ester and tetrahydrogeranylgeranyl ester are formed as intermediates when the geranyl geraniol esterified by chlorophyll \textit{a}, which is transformed into phytol by three gradual steps.

14.2.2.7 Biosynthesis of Chlorophyll \textit{b} via Chlorophyll \textit{a}

As discussed previously, the only difference between chlorophyll \textit{a} and chlorophyll \textit{b} is the presence of an aldehyde (formyl) group at ring B in place of a 3-methyl group. In the conversion process of chlorophyll \textit{b}, transformation occurs (Espineda et al., 1999). Earlier experiments suggested that chlorophyll \textit{a} was the precursor of chlorophyll \textit{b} (Rudiger, 1997).

14.2.3 Degradation or Catabolism of Chlorophyll

The global degradation of chlorophyll is found to be always equal to the global synthesis of chlorophyll (Hendry et al., 1987). Senescence of plant leaves involves a chlorophyll degradation process, and there are various biological phenomena that degrade chlorophylls such as flowering, fruit ripening, germination, bleaching, etc. These mechanisms are considered to be part of the aging processes. However, the chlorophyll degradation process is not fixed or the same in all cases (Gossauer and Engel, 1996). Table 14.3 demonstrates some natural and biological mechanisms in which chlorophyll breakdown occurs (Hendry et al., 1987).

<table>
<thead>
<tr>
<th>Condition and factors</th>
<th>Part affected (chlorophyll breakdown)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the point of life cycle initiation</td>
<td></td>
</tr>
<tr>
<td>1 Germination of seed</td>
<td>Cotyledons loss</td>
</tr>
<tr>
<td>2 Initiation of first leaves</td>
<td></td>
</tr>
<tr>
<td>3 Maturation of inflorescence</td>
<td>Bracts loss</td>
</tr>
<tr>
<td>4 Fruit ripening</td>
<td>Green color loss of fruit</td>
</tr>
<tr>
<td>During the life cycle</td>
<td></td>
</tr>
<tr>
<td>Turnover (continuous synthesis)</td>
<td>Degradation of chloroplast parts</td>
</tr>
<tr>
<td>Premature death</td>
<td></td>
</tr>
<tr>
<td>Biotic (disease, harvesting, grazing, etc.)</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Climatic (UV rays, temperature, darkness)</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Edaphic (deficiency of water and minerals)</td>
<td>Whole plant</td>
</tr>
</tbody>
</table>

Source: Hendry et al., 1987.

Catabolic products derived from chlorophyll a and chlorophyll b have been reported among algae. The responsible modification behind this is the esterification hydrolysis of the methyl ester group at ring E. In higher plants, catabolites are hydrogenated in a few metabolic steps, and after ring opening a reductase reaction is indicated for higher plants (Gossauer and Engel, 1996). Subsequently, modification includes hydroxylation reactions, found only at ethyl group of ring B in Brassica napus and at side chains of ring A and B in Hordeum vulgare. This change facilitates transport into storage and vacuole for further formation of malonic acid ester and β-glucoside in Brassica napus.

14.3 Presence and Distribution in Fruits and Vegetables

Chlorophyll is mostly composed of a lipophilic derivative which includes chlorophyll a and b and is found in fresh fruits and green vegetables (Kimura and Rodriguez-Amaya, 2002). It is frequently found in the range 1000 to 2000 ppm in some species (Khachik et al., 1986). Chlorophyll is a widely distributed plant pigment (Table 14.4) in all green fruits and vegetables, and it is the most abundant pigment on earth. Chlorophylls are registered as food additives used to give color and other properties to a variety of foods and green beverages.

Generally, chlorophyll comprises 0.6% to 1.6% of plants on a dry weight basis; however, wide variation has been noted. In nature, six classes of chlorophyll exist in plants and photosynthetic organisms: a, b, c, d, e, and f. However, only a and b are predominant in angiospermic plants, while c, d, e, and f are found in different photosynthetic algal and diatomic species. Chlorophyll a is predominant over chlorophyll b by a 3:1 margin. Lichtenhaler (1968) surveyed the molar ratio of chlorophyll a to b in higher plants in 24 species, and the range was from 2.56 to 3.45. The a:b ratio was approximately 3 depending upon species. But the ratio can vary due to different environmental factors and growth conditions, and particularly with high exposure to sunlight.

The concentration of chlorophyll correlates with the photosynthetic capacity of plants, which gives some indication of the physiological status of the plant system (Gammon and Surfus, 1999).

14.4 Biological Functions of Chlorophyll

Chlorophyll is an extremely important biomolecule found in green plants. Plants utilize chlorophyll and sunlight to make food materials. Chlorophyll is important not only as a color pigment and for its physiological role in plants, but also for its health benefits (Liu et al., 2007).

Chlorophylls have been the subject of extensive research efforts because of their eminent role in plant physiology and their role as derivatives in the food sector. They have a major role in interrupting diverse diseases such as cancer, cardiovascular, and other chronic diseases (Sangeetha and Baskaran, 2010). Chlorophyll is used for bad breath, and it reduces colostomy odor. Chlorophylls have been used for a long time as a traditional medicine for therapeutic functions.

Humans have no potential to synthesize chlorophyll pigment, but they are able to deposit dietary chlorophyll as absorbed or with minor alteration to its structure (Larsen and Christensen, 2005). Healthcare providers use chlorophyll intravenously for the pain and swelling (inflammation) with pancreas problems (pancreatitis).

Common sources of chlorophyll used for medicine are alfalfa (Medicago sativa) and silkworm droppings. Early research suggests that chlorophyll not only is responsible for green color but also can prevent lung and skin cancer by intravenous injection along with the drug talaporfin, followed by laser therapy, in early stages of cancer.

Both natural and artificial commercial grade derivatives such as copper chlorophyll have been extensively used for their beneficial biological activities, which include wound healing, control of calcium oxalate crystal (Tawashi et al., 1980), and anti-inflammatory properties (Bowers, 1947; Larato and Pfau, 1970). Some reports do provide sufficient evidence for the beneficial effects of chlorophyll. However, many researchers have made claims regarding the healing properties of chlorophyll, but most have been disproved by the companies that are marketing them. Quackwatch (www.quackwatch.org) claims that no deodorant effect can possibly occur from chlorophyll added to products such as gum, foot powder, and cough drops (Kephart, 1955).

More evidence is essential to rate the effectiveness of chlorophyll for different biological uses.

Table 14.4 Distribution of chlorophylls in living organisms.

<table>
<thead>
<tr>
<th>Chlorophyll type</th>
<th>Occurrence</th>
<th>Chlorophyll type</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>Universal</td>
<td>Chlorophyll d</td>
<td>Various algae</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>Mostly plants</td>
<td>Chlorophyll e</td>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Chlorophyll c</td>
<td>Various algae</td>
<td>Chlorophyll f</td>
<td>Cyanobacteria</td>
</tr>
</tbody>
</table>
14.5 Changes in Chlorophyll during Processing of Fruits and Vegetables

Chlorophyll degradation is a unique phenomenon that happens during the processing of fruits and vegetables. Color is one of the important sensory characteristics and plays a vital role in the acceptability of food items in the food industry. Chlorophyll \( a \) imparts blue-green color whereas chlorophyll \( b \) imparts yellow-green color. They also possess different thermal stabilities (Steet and Tong, 1996). Chlorophyll \( b \) was reported to be thermally more stable, whereas chlorophyll \( a \) was found to be thermally unstable (Schwartz and Elbe, 1983; Canjura et al., 1991; Schwartz and Lorenzo, 1991). In the temperature range 70–100 °C, green peas showed degradation of chlorophyll \( a \) and \( b \) following a first-order kinetics model. The degradation of chlorophyll \( a \) was reported to be 12–18 times faster than that of chlorophyll \( b \), and was found to be primarily dependent upon the temperature regime during the processing; at the same time, this indicated the higher susceptibility of chlorophyll \( a \) in a thermal environment (Erge et al., 2008). The higher thermal stability of chlorophyll \( b \) has been attributed to the electron-withdrawing effect of its C-3 formyl group (Belitz and Grosch, 1987).

Chlorophylls are highly susceptible to degradation during processing conditions, resulting in food color changes (Schwartz and Elbe, 1983). The simple reaction mechanism that occurs with the chlorophyll molecule during degradation is shown in Figure 14.5. The chlorophyll degradation in foods may occur via chemical and biochemical reactions. Chemical reactions involve the formation of pheophytin from the chlorophyll by the replacement of magnesium ions from the porphyrin ring. The magnesium replacement can occur by acidic substitution, by heat treatment, or after the action of Mg chelatase. Decarbomethoxylation may occur during strong heat treatment, leading to the conversion of pheophytin to pyropheophytin (Schwartz et al., 1981; Diop et al., 2011).

Krautler and Matile (1999) and Matile et al. (1999) described the role of chlorophyllase, Mg dechelatase, and pheophorbide \( a \) oxygenase in chlorophyll degradation. Among these enzymes, chlorophyllase catalyzes the first step in chlorophyll catabolism and removes the phytol chain from the porphyrin ring to form chlorophyllide. Lipoxygenase is widely distributed in vegetables and is involved in off-flavor development and color loss. The latter is due to hydroperoxide and radical formation by oxidation of lipids, which can destroy chlorophyll and carotenoids during frozen storage (Vamos-Vigyazo and Haard, 1981; Adams, 1991). Lopez-Ayerra et al. (1998) concluded that lipid oxidation was related to chlorophyll degradation in spinach. Peroxidases are assumed to play an important role in chlorophyll degradation, a process accompanying ripening and senescence in most fruits and vegetables. Peroxidase is responsible for off-flavor development during storage of canned products, especially of non-acidic vegetables which often exhibit high levels of activity. They impair not only the sensory properties and, hence, the marketability of the product, but also its nutritive value (Vamos-Vigyazo and Haard, 1981). Martinez et al. (2001) reported that chlorophyll degrading peroxidase activity in strawberry and canola seeds decreased with chlorophyll content in their ripening stage, while the reverse occurred in mustard.

Kato and Shimizu (1985) reported that a phenolic peroxidase \( \text{H}_2\text{O}_2 \) system was involved in \textit{in vitro} bleaching of chlorophyll. The phenolics active in the system were derivatives of monophenols having an electron-attracting group in the \textit{para} position. Yamauchi et al. (2004) concluded that in peroxidase-mediated chlorophyll degradation, peroxidase oxidizes the phenolic compounds with hydrogen peroxide and forms the phenoxy radical. The phenoxy radical then oxidizes chlorophyll to colorless low molecular weight compounds through the formation of C-13-hydroxychlorophyll-\( a \) and a bilirubin-like compound as an intermediate.

It is very important to consider the factors affecting chlorophyll degradation during the processing of fruits and vegetables. Diverse factors are responsible for degradation, including exposure to dilute acids, heat, and oxygen (Tonucci and Von Elbe, 1992). Under thermal processing conditions, chemical and structural variations...
take place in the chlorophyll which in turn lead to changes in color (Canjura et al., 1991; Heaton et al., 1996). In green cellular tissues subjected to various processing conditions, chlorophyll undergoes distinct types of degradation reactions. The changes in the chlorophyll molecule during heating have been discussed by Gauthier-Jaques et al. (2001). Demetalation and epimerization were observed during heat treatment, and prolonged heating leads to additional demethoxycarbonylation of the molecule. Demethoxycarbonylation also occurs during the canning of vegetables. Dephytlation of chlorophyll is achieved enzymatically during fermentation and storage and is often observed together with demetalation.

In food products, chlorophyll degradation studies revealed that chlorophyll degrades to pheophorbide via pheophytins or chlorophyllide. Schwartz and Lorenzo (1990) demonstrated that chlorophyll degradation terminates at pheophytide. However, studies by Matile et al. (1992) and Ginsburg et al. (1994) have shown that chlorophyll degradation continues beyond pheophorbide to colorless compounds. Heaton et al. (1996) observed that chlorophyll degradation of cabbage heads in cold storage did not lead to pheophorbide accumulation, leaving the degradation of pheophorbide to colorless byproducts as the only explanation.

Chlorophyll degradation has been shown to follow different pathways depending on the commodity (Heaton et al., 1996). However, the mechanisms and kinetics of those reactions have been only partially characterized. Heaton and Marangoni (1996) summarized the accepted mechanism of chlorophyll degradation in fruits and vegetables and extended it to include the degradation of pheophorbides into colorless compounds. Takamiya et al. (2000), in a study on the mechanism of chlorophyll degradation using gene cloning, determined that after successful removal of phytol and Mg$^{2+}$ from the chlorophyll molecule by the use of chlorophyllase and Mg dechelatase, pheophorbide is cleaved and reduced to yield a colorless, open tetrapyrrrole intermediate.

Variation in pH is one of the important factors that influence the variations in the color of chlorophylls by the conversion of chlorophylls to pheophytins (Minguez-Mosquera et al., 1989). During the processing of peas for puree formation, three chlorophyll-linked degraded products were reported at high pH under high-temperature, short-time conditions after the storage of puree at room temperature (Buckle and Edwards, 1969). Schwartz and Lorenzo (1990), in a study on continuous aseptic processing and storage, concluded that oxidation during storage was not a dominant factor in chlorophyll degradation and color loss. A major problem in thermal processing of green vegetables is the continuous decrease in pH values due to acid formation. To avoid this problem, Ryan-Stoneham and Tong (2000) conducted degradation studies in a specially designed reactor with an online pH control capability. The authors concluded that the activation energy was independent of pH, with a magnitude varying between 17 and 17.5 kcal/mol for both chlorophyll $a$ and $b$. The degradation rate was found to decrease linearly as the pH increased.

It was demonstrated that chlorophylls have higher stability at alkaline pH conditions (Clydesda and Francis, 1968). Gupte and Francis (1964) reported that the use of magnesium carbonate in combination with high-temperature, short-time processing (at 150 °C) initially improved chlorophyll retention in pureed spinach. However, the effect was not stable during storage. Magnesium compounds resulted in the formation of hard white crystals of magnesium-ammonium-phosphate (Eheart and Odubi, 1973). Ahmed et al. (2002) studied the color degradation kinetics of coriander leaf puree; however, they worked on puree at unmodified pH. The effect of pH (3–8) and microbial growth on green color degradation of blanched broccoli during storage was investigated by Gunawan and Barringer (2000). Color degradation accelerated when broccoli was submerged in McIlvaine’s buffer at decreasing pH. Pheophytinization followed first-order reaction kinetics. The logarithmic values of reaction rate constants were linearly correlated with the ambient pH up to pH 7. Acids containing a benzene ring were found to cause a faster color change than acids with simple chains due to their hydrophobicity. There is synthesis of olive-brown pheophytins under the acidic conditions, which interchanged the magnesium in the chlorophyll with the two hydrogen ions (Mangos and Berger, 1997; Van Boekel, 1999, 2000).

When the process temperatures are high and the pH of the tissue is low, pheophytins are formed in the processed vegetables (La and Von Elbe, 1990). The excessive heating leads to the formation of pyropheophytins as a result of removal of the carbomethoxy group from the pheophytins (Schwartz and Elbe, 1983; Canjura et al., 1991; Mangos and Berger, 1997). The treatment of broccoli juice at temperatures more than 60 °C leads to the degradation of chlorophyll $a$ and $b$ to pheophytin $a$ and $b$ (Weemaes et al., 1999a, 1999b). Pheophorbides are formed by the loss of magnesium from the chlorophyllides under the acidic environment (Heaton et al., 1996). The hydrolysis of the phytol chain in pheophytins produced olive-brown colored pheophorbides (Heaton et al., 1996). Higher temperature sensitivity was found in mustard leaves than in spinach in relation to color (Ahmed et al., 2004).

The thermal degradation of chlorophyll pigment in thermally processed green vegetables is an aspect of vital importance for the food processing sector. The color degradation of green peas occurred when green peas were subjected to temperatures of 70, 80, 90, and 100 °C to analyze the effect of heat treatment (Erge et al., 2008). It was reported that chlorophyll $a$ and chlorophyll $b$
followed a kinetics model of the first order during degradation, which was recorded using a tristimulus colorimeter (Baardseth and Von Elbe, 1989). The blanching of green peas was studied under temperatures of 70, 80, 90, and 100°C in solutions of buffers with pH of 5.5, 6.5, and 7.5. The pH effect on the degradation of chlorophyll was noted, and the results showed the rise in degradation rate of chlorophyll $a$ and chlorophyll $b$ with the decrease in pH (Koca et al., 2007). The constant degradation rate of chlorophyll $a$ and chlorophyll $b$ were highly related to the changes in the green pea CIE $a^*$ color values, when the products were at a pH of 5.5 or 6.5. The $a^*$ parameter is related to the greenish color of the food; thus the degradation of chlorophylls was followed by a reduction in the CIE LAB parameter. Moyano et al. (2008) reported a correlation between the chlorophyll content of a broad range of virgin olive oils and their lightness.

The degradation of green color of broccoli (Brassica oleracea) by blanching at a storage temperature of 7°C was carried out; the broccoli was then submerged in McIlvaine's buffer at a pH of 3–8, and it was observed that chlorophyll degradation increased as the pH decreased (Gunawan and Barringer, 2000). The thermal degradation of chlorophyll and chlorophyllides in a puree of spinach at temperatures of 100 to 145°C (2–25 min) and 80 to 115°C (2.5–39 min), respectively, led to the formation of derivatives chlorophylls, pyrophorophylls, phaeophytins, and pyroprochlorophylls (Canjura et al., 1991). Ahmed et al. (2000) studied the effect of particle size on chlorophyll content and total color of green chili puree. Both chlorophyll content and green color varied significantly with processing temperature and mesh size. Chlorophyll content increased with increased mesh fineness, but decreased linearly with processing temperature. Up to a 60% loss in chlorophyll was noted after heating at 100°C for 15 minutes.

Chlorophyll degradation and yellowing of peel in lime (Citrus latifolia Tan.) is one of the dominant problems worldwide. Application of UV-B treatment at 8.8 kJ/m² led to effective delay in the reduction of chlorophyll content. The phaeophorbide content decreased, followed by an increase in the pheophytin $a$ content during later stages of storage. Therefore, the study concluded that there was mitigation of degradation of chlorophyll by the application of UV-B irradiation (Srilaong et al., 2011).

Pistachio (Pistacia vera L.) kernels are green in color due to the presence of chlorophyll, which appeals to the food industry for its diverse uses. The degradation of the green color in pistachio is one of the unacceptable features. The heat treatments applied to pistachio during roasting lead to degradation of chlorophyll $a$ and $b$ to pheophytins and pyrophorophylls, which ultimately impacts the quality and market value of pistachio kernel (Pumilia et al., 2014).

In the case of Thai lime (Citrus aurantifolia Swingle ‘Paan’) the postharvest hot water treatment delays chlorophyll degradation and maintains quality during storage. It is reported that the enzyme activities degrading chlorophylls, i.e. chlorophyllase, chlorophyll-degrading peroxidase, pheophytinase, and Mg-dechelation activities, were mitigated by hot water treatment (Kaewsuksaeng et al., 2015). The processing of vegetables like broccoli florets, spinach leaves, and green peppers under high pressure does not degrade chlorophylls, whereas high-pressure, high-temperature processing leads to the degradation of both chlorophylls, and chlorophyll $a$ was found to be unstable at 70°C compared to chlorophyll $b$. At 117°C both chlorophylls were degraded (Sanchez et al., 2014). The blanching treatment of vegetables followed by freezing led to chlorophyll retention in frozen green asparagus, French bean, and peas (Lisiewska et al., 2010).

A previous study related the effect of microwave and conventional cooking on the chlorophyll pigment in peas, leek, squash, broccoli, spinach, and green beans. Except in peas, the reported results showed chlorophyll $a$ to be more resistant than chlorophyll $b$ in five of the six vegetables. There was an increase in the pheophytins of all the vegetables after cooking. Boiled leek showed loss in chlorophyll $a$ and chlorophyll $b$, whereas in the case of boiled and microwaved peas, there was retention of chlorophyll $a$ and chlorophyll $b$. The formation of pheophytin $a$ and $b$ was reported at higher levels in boiled squash and boiled green beans. The maximum amounts of pheophytins were reported to be formed in boiled vegetables, and the minimum levels in microwaved vegetables (Turkmen et al., 2006). HPLC (reverse phase gradient) was used to monitor the effect of freezing ($–22°C$) on chlorophyll $a$ and $b$ content in Padrón peppers and green beans. In the case of unblanched beans there was considerable reduction in the pigments within 1 month, whereas after 11 months the pigments remained stable; almost similar results were reported in blanched beans. Chlorophyll reductions in blanched beans were reported to be induced by blanching. The reduction of chlorophyll $a$ was also reported in Padrón peppers (Oruna-Concha et al., 1997).

The quantitative determination of chlorophyll $a$ and $b$ and pheophytins $a$ and $b$ under refrigerated storage conditions, followed by industrial processing of spinach, showed that pheophytins $a$ and $b$ were the prominent chlorophyll derivatives formed under refrigerated conditions at 8°C for 3 weeks (Lopez-Ayerra et al., 1998). The heating of spinach leaves showed degradation of chlorophylls $a$ and $b$, and the effect was found more under microwave cooking or blanching than under steaming or baking. Pheophytins $a$ and $b$ were formed under steamed conditions, whereas under the effect of microwave cooking pyrochlorophylls $a$ and $b$ were formed (Teng and Chen, 1999).

The effect of salt on the degradation of visual green color “-a” in spinach puree under a temperature range of
50–120 °C, open pan cooking, and pressure cooking, was studied. Salt showed a protective action against the degradation of the green color (Nisha et al., 2004). In canned slices of kiwi fruit, chlorophyll was found to degrade when heated at 100 °C for 5 minutes (Robertson, 1985).

Therefore, in conclusion, chlorophyll pigments in horticultural crops require very specific conditions to avoid degradation under various processing environments. The congenial temperature conditions during thermal and non-thermal processing should be optimized to mitigate the degradation of chlorophyll pigments in different vegetables and fruits. However, under other processing steps like blanching, the pH plays an important role in relation to the degradation of chlorophyll pigments. The susceptibility of degradation of chlorophyll during processing of different vegetables is largely dependent on the different processes employed with a particular horticultural crop. The processing methodology along with its intensity during processing of vegetables or fruits is also a crucial factor which affects the degradation of chlorophylls in horticultural crops.

### 14.6 Conclusions and Research Needs

Chlorophyll plays a vital role in the photosynthetic processes in plants universally. Its biological role for human health facilitates its importance for nurturing good health. It acts as potent antioxidant, enhances blood clotting activity, assists with hormonal balancing, has importance for detoxification, and improves digestion. The presence of chlorophyll in diverse food items emphasizes its importance; it is a natural colorant which appeals to customers in the items in which the presence of high chlorophyll content is acceptable. The majority of raw vegetables contain chlorophyll, and therefore chlorophylls act as a potent biomarker to check the freshness of vegetables. It is commonly observed in fruits that chlorophyll is degraded during the ripening process and converted to other pigments like lycopene, anthocyanin, etc. The emerging food processing sector worldwide should develop the engineering technology for the processing of chlorophyllous fruits and vegetables by stabilizing the chlorophyll pigment, and making it bioavailable. The processes in which the maximum amount of chlorophyll pigments could be retained can be optimized to the utmost levels for keeping the stability of chlorophyll pigments under consideration while processing fresh green vegetables. Novel methodologies should be developed for the processing of chlorophyll-rich vegetables and fruits. There is an ample need to apply biotechnological interventions to facilitate the long-term preservation of chlorophylls by enhancing shelf life.

### References


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