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## Introductory Chapter: Chlorophyll Molecules and Their Technological Relevance

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Chlorophylls were the major tool used, long before science was able to recognize the existence of plants and animals; our predecessors identified this distinction and slowly provided technological evidence that chlorophyll was responsible for the green color of the plants.

The name chlorophyll was proposed by Pierre Joseph Pelletier and Joseph Bienaimé Caventou in 1818 to designate the Green substance that could be extracted from the leaves with the aid of alcohol [1–3]. However, only about a century later, in 1911, the study of this interesting molecule gained more importance by Richard Willstätter, who obtained for the first-time pure chlorophyll and established its correct molecular formula  $C_{55}H_{70}MgN_4O_6$  [2]. A year later, Willstätter showed that chlorophyll obtained from a wide variety of sources was a mixture of two compounds, chlorophyll-a and chlorophyll-b, to which he assigned the correct molecular formula  $C_{55}H_{70}MgN_4O_5$  and  $C_{55}H_{70}MgN_4O_6$ , respectively. Nevertheless, the detailed understanding of the structures of these molecules was the result of the studies of Fischer and collaborators, who were the first to delineate the structure of porphyrin ring [2–5].

These compounds are complex organic molecules formed by derivatives of porphyrin, a macrocyclic structure, asymmetric, totally unsaturated. Basically, chlorophyll molecules are conjugated tetrapyrroles to which a cyclopentanone ring jointly with the third ring linked together by bridges methylene, with the central atom, magnesium (**Figure 1**). This structure also contains at the C-17, a propionic acid chain esterified with the phytol, diterpene alcohol [3, 6–8].

Chlorophyll-b differs from chlorophyll-a by the presence of aldehyde residue instead of the methyl group at position 7. The synthesis of this compound is given by the oxidation of the methyl group to aldehyde [9, 10] through the enzyme oxygenase, which catalyzes this conversion [11].

Structurally, the chlorophyll molecules differ from each other, due to the degree of saturation of the pyrrolic rings. For example, chlorophyll-c contains fully unsaturated phytylporphyrin (double alloys C17–C18), whereas the other chlorophylls are C17–C18, phytylchlorins (Figure 1). These differences in chlorophyll macrocycle saturation have profound consequences on the absorbance spectrum. For example, chlorophyll-a, chlorophyll-d, and chlorophyll-f phytylchlorins have approximately equal intensities of absorption in blue, red, and green. On the other hand, the phytylporphyrins of chlorophyll-c absorb themselves weakly in red and more intensely around 450 nm [6, 12, 13].

The spectral differences of the chlorophyll molecules are reflected in the key in which bodies are present that the chlorophyll-a appears more bluish green, chlorophyll-b bright green, chlorophyll-c yellowish green, chlorophyll d bright forest green, and chlorophyll-f emerald green [6]. This diversity of shades of green makes these organisms potential source of natural colorants [14].

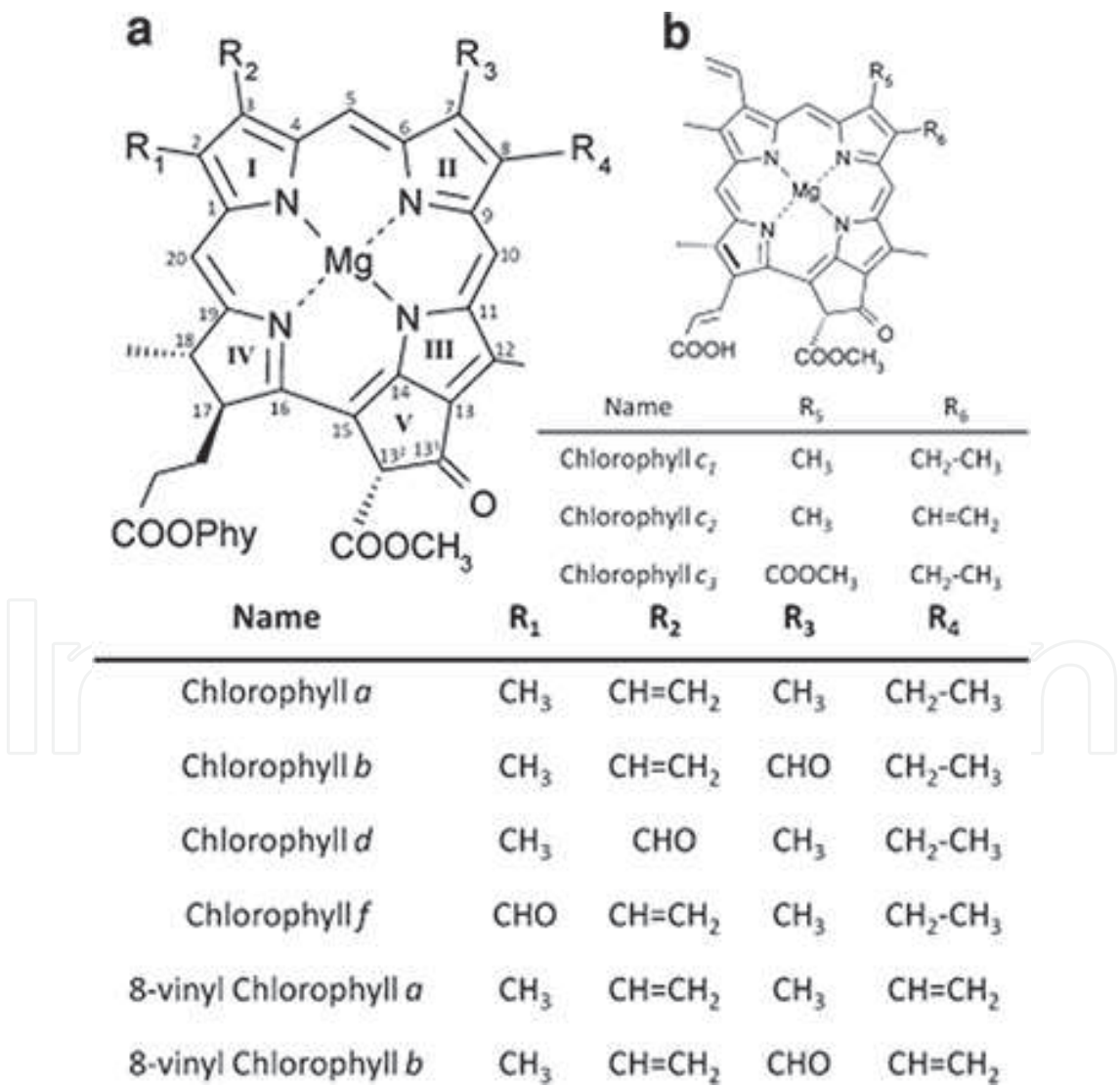


Figure 1. Structure of the side chain variables of chlorophyll molecules.

In addition, a chlorophyll molecule consists of two distinct parts: hydrophile, the macrocycle, and a hydrophobic part, phytol chain. The most hydrophilic segment of the macrocycle is the cyclopentanone ring and the propionic ester group (17 positions). The hydrophilic and hydrophobic character directly influences the choice of solvent for extraction, which, from a quantitative or qualitative point of view, is an extremely important aspect when it focuses on the technological relevance of chlorophyll molecules [15–17].

Given this scenario, analysis techniques such as liquid chromatography coupled to ultraviolet and mass spectrum allowed demonstrating that currently a total of five molecules, so-called chlorophyll, have been found in nature. All of them occur in the cyanobacterium, which is cosmopolitan microalgae, prokaryotic, Gram negative, with metabolism preferentially photoautotrophs, able to perform photosynthesis oxygen production, eukaryotic algae, and higher plants. These microorganisms demonstrate considerable biocatalytic potential in biotechnological processes due to their robustness and require nutritional simplicity [18]. Chlorophyll-a is present in all organisms that perform photosynthesis and chlorophyll-b oxygen (**Figure 1**), which are the most abundant pigment in green algae, is found in higher plants in nature, and other similar structures are used as accessory pigments in photosynthetic process, called chlorophyll-c, chlorophyll-d, chlorophyll-e, and chlorophyll-f [6–8, 13]. These specific pigments present in the cells of the photosynthetic organisms influence efficiency of dispersion of light, because each species has its own distinct characteristic pigment [9].

Chlorophyll-a is the most abundant and important structure of all chlorophylls, corresponding to approximately 75% of the green pigment found in nature. This molecule has bioactive properties with extensive use in pharmaceutical and food industry, is considered a high added value, and may reach high values on the market. The bioactive potential of the cells is associated with a complex structure of conjugated connections in pyrrolic rings, allowing an oxidant and antioxidant, acting without free radical sequestration [7]. In addition, it is related to nutraceutical properties, playing an important role in health, through its anti-inflammatory and vascular constrictor, which makes this molecule an important ingredient in food formulations, cosmetics, and drugs [16].

Another molecule that differs from chlorophyll-a, to present the first pyrrole ring, a substitution of the vinyl C3 group for the chlorophyll-a molecule for a formyl group, was called chlorophyll-d. This molecule was discovered 70 years ago, but only in 1996, an important property of this molecule was observed [6, 9, 13], performing a function equivalent to chlorophyll-a in oxygenic photosynthesis [7, 13]. The properties of chlorophyll-d, like its photosynthetic power associated with the difference in wavelength of absorption in relation to chlorophyll-a, make this a potential component molecule to be employed in a biorefinery. In the last years, it has been argued that there is a strong interest in the development of technologies for the production of natural molecules [16, 19, 20].

On the other hand, chlorophyll biomolecules are highly prone to change, because their chemical structure is rich in double bonds combined. In this sense, in order to increase stability, chlorophyll undergoes changes in its molecule, replacing the atom of  $Mg^{2+}$  for  $Cu^{2+}$ , resulting in the call copper chlorophyllin, which is stable and can be used in hydro- or lipo-soluble formulations [21].

In summary, the broad application of chlorophyll makes it a molecule of great importance for the global market. However, there is still doubt about its application due to its chemical instability. In this way, the extension of studies to elucidate the open gaps will be of extreme relevance to expand the scientific knowledge base and its relation to the industrial application.

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