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Review Paper / **Derleme Makale**

Chlorophyll: Structural Properties, Health Benefits and Its Occurrence in Virgin Olive Oils

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ABSTRACT

In recent years, virgin olive oil has acquired a distinguished position on the shelves of markets worldwide. Its increased demand is mostly related with the human health benefits of its consumption. Virgin olive oils contain a variety of health beneficial compounds like chlorophyll, which is a potential cancer preventative agent that has drawn significant attention recently. Biological activities attributed to chlorophyll derivatives consistent with cancer prevention include antioxidant and antimutagenic activity. It is also used as a food-coloring agent and known as natural green 3, and it has the E number E141. Chemical and physical properties of chlorophyll need to be determined in order to reduce its loss during food processing and storage. In this review, health benefits and structural properties of chlorophyll were presented, and its occurrence in virgin olive oil was reviewed.

Key Words: Chlorophyll, Olive oil, Health

Klorofil: Yapısal Özellikleri, Sağlık Açısından Faydaları ve Sızma Zeytinyağlarında Bulunuşu

ÖZET

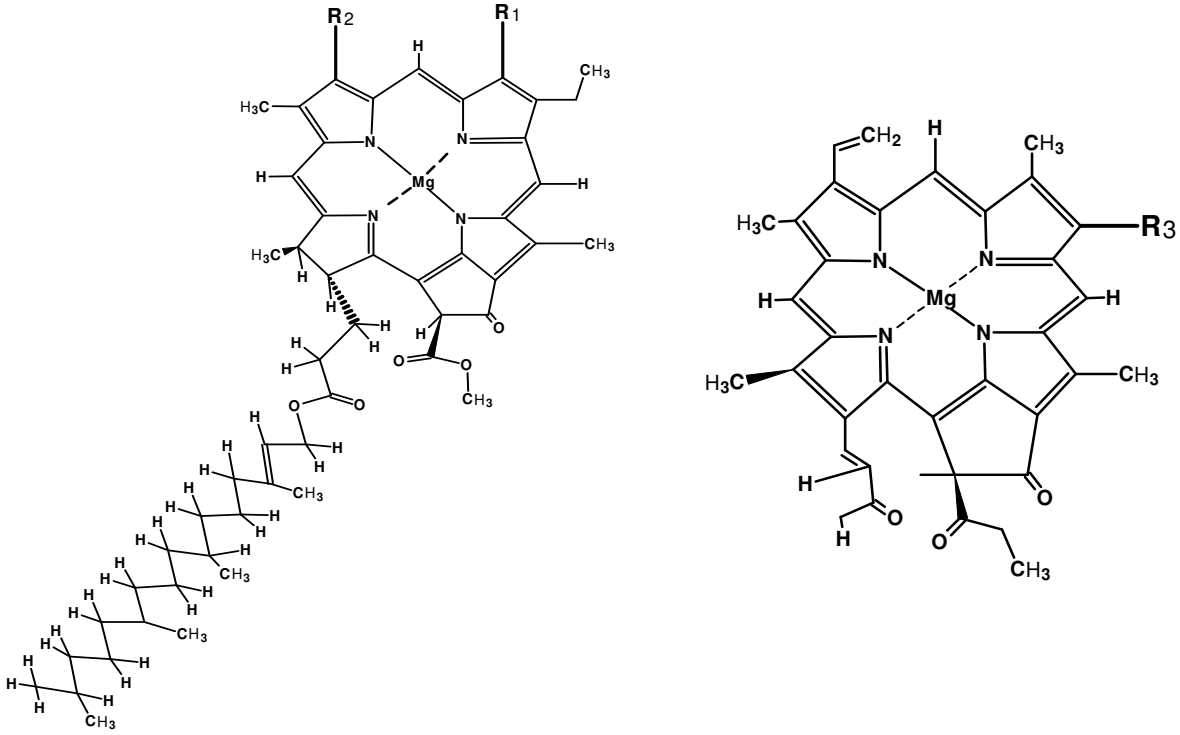
Son yıllarda, sızma zeytinyağı bütün dünyada marketlerin raflarında ayrıcalıklı bir konuma gelmiştir. Zeytinyağına talebin artmasının temel nedenleri arasında, tüketimi halinde insan sağlığına faydaları ile ilişkilidir. Sızma zeytinyağları son zamanlarda dikkatleri önemli ölçüde üzerine çeken, potansiyel kanser önleyici madde olarak bilinen klorofil gibi değerli bazı kimyasallar içerir. Kanseri önleyebilen klorofil türevlerinin biyolojik aktiviteleri antioksidan ve antimutajenik aktivitelerini içermektedir. Klorofil aynı zamanda, doğal yeşil 3 olarak bilinen gıda boyar madde olarak kullanılır ve E numarası E141'dir. Gıda işleme ve depolama sırasında oluşan klorofil kayıplarını azaltmak için klorofilin kimyasal ve fiziksel özelliklerinin bilinmesi gereklidir. Bu derleme çalışmasında, klorofilin sağlık faydaları ile yapısal özellikleri ele alınmış ve sızma zeytinyağında varlığı derlenmiştir.

Ahahtar Kelimeler: Klorofil, Zeytinyağı, Sağlık

INTRODUCTION

Chlorophyll is a green pigment found in most plants, and its name is derived from the Greek *chloros* (green) and *phyllon* (leaf) [1, 2]. There are a few different forms of chlorophyll. C*hlorophyll a*, greenish-yellow in solution, is the primary photosynthetic pigment in green plants for the transfer of light energy to a chemical acceptor. Light that is absorbed provides the energy for photosynthesis. A green leaf absorbs blue light (mostly at 430nm) and red light (mostly at 660nm). It reflects the green wavelengths, appearing green to human eye. Chlorophyll a, alone, is found in blue-green and some red algae. Accessory pigments in photosynthesis transfer light energy to Chlorophyll a. One of these is *Chloropyll b*, blue-green in solution, found in higher plants and green algae with Chlorophyll a. *Chlorophyll c*

is also an accessory pigment found with Chlorophyll a in brown algae and diatoms. *Chlorophyll d*, together with Chlorophyll a, is in some red algae. All forms of chlorophyll are oil-soluble [3]. The chemical structures of chlorophyll forms are summarized in Figure 1 [4, 5]. Chlorophyll forms, except c, have a 'head' and a long 'tail'. The head consists of a porphyrin ring or tetrapyrrole nucleus, from which extends a tail made up of a 20-carbon grouping called the phytol. The tail of form c is short and links to porphyrin ring from C_{17}. In chlorophyll, the porphyrin is very similar in structure to the heme group found in hemoglobin, except that in heme the central atom is iron, whereas in chlorophyll it is magnesium.



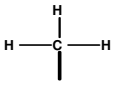
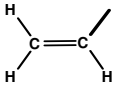
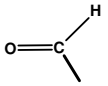
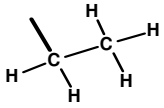
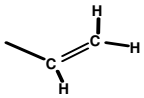
Chlorophyll	R_1	R_2	R_3	Empirical Formula
a				$C_{55}H_{72}O_5N_4Mg$
b				$C_{55}H_{70}O_6N_4Mg$
c_1				$C_{35}H_{30}O_5N_4Mg$
c_2				$C_{35}H_{28}O_5N_4Mg$
d				$C_{54}H_{70}O_6N_4Mg$

Figure 1. Structures of chlorophyll forms

REACTIONS OF CHLOROPHYLL

Chlorophyll is decomposed by heat and an olive-green color is produced. The time of heating and the temperature affect on the extent of decomposition, for example, the temperature is high in a pressure cooker and acidity is not decreased because the volatile acids are retained, thus the change is rapid. The usage of alkaline compounds such as alkaline water reduces acidity of medium [6]. However, if it is used at excess amounts, chlorophyll reacts with base. Figure 2 and 3 show simple reactions of chlorophyll with acid and base. Reaction of Chlorophyll *a* with acid removes the magnesium ion replacing it with two hydrogen atoms giving an olive-brown solid, phaeophytin-*a*. Hydrolysis of this (reverse of esterification) splits off phytol and gives phaeophorbide-*a*. Similar compounds are obtained if Chlorophyll b is used.

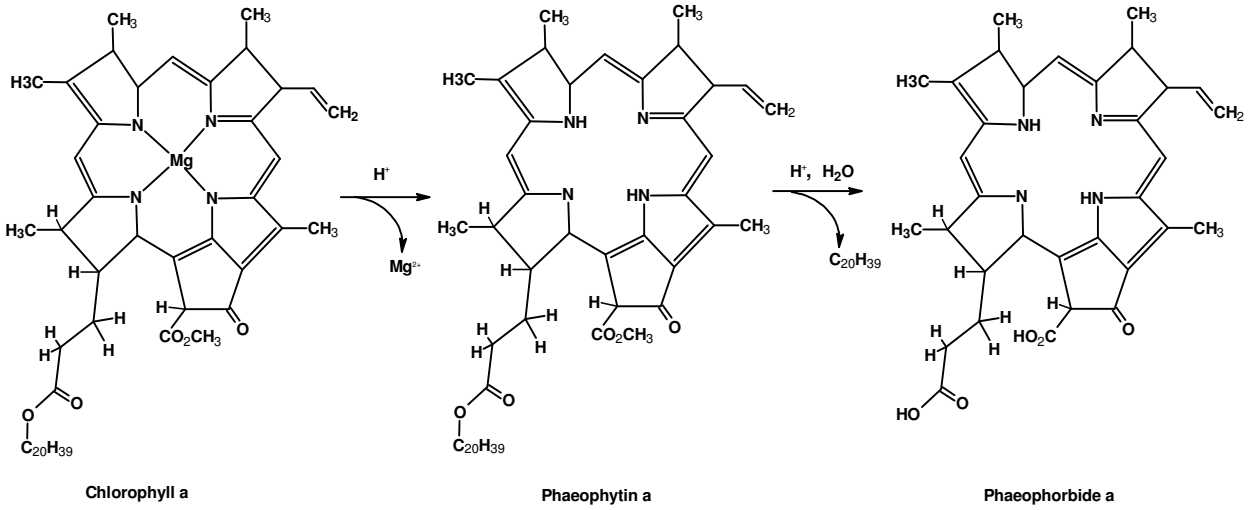


Figure 2. Reaction of Chlorophyll *a* with acid

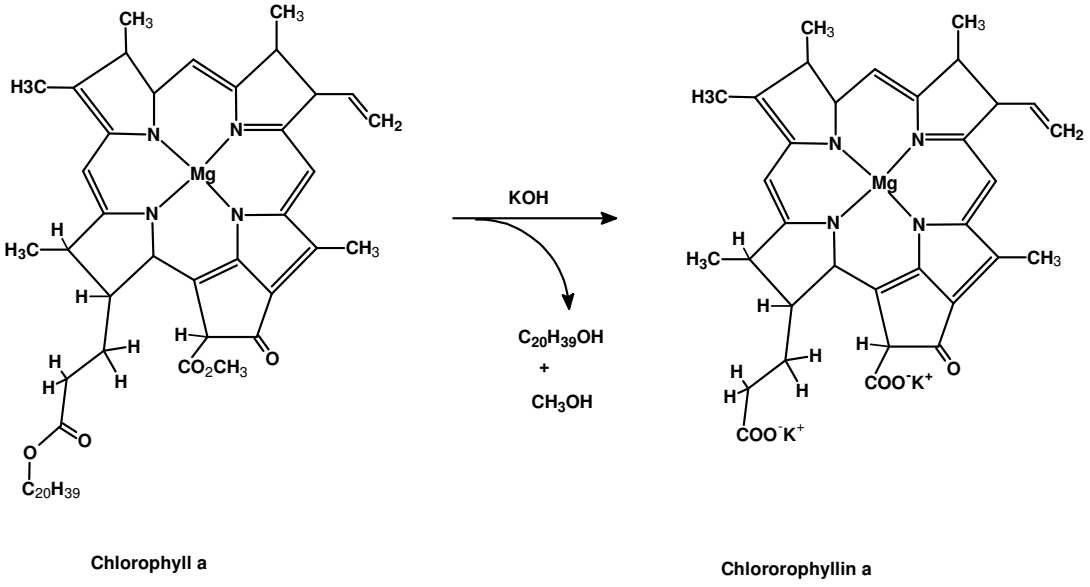
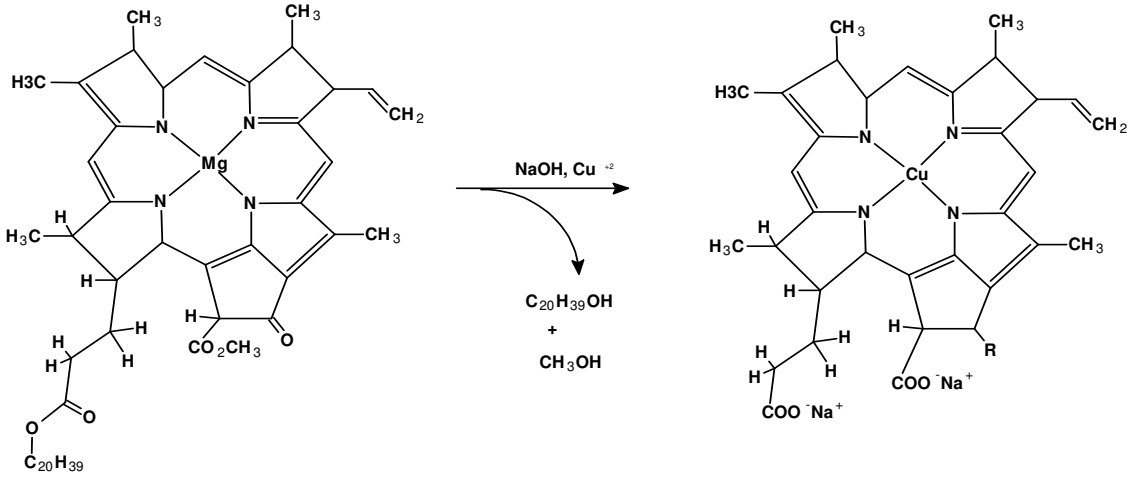


Figure 3. Reaction of Chlorophyll *a* with base

If chlorophyll is reacted with a base, it forms a series of phyllins, magnesium porphyrin compounds. Treatment of phyllins with acid gives porphyrins. In addition, the other one of reaction product is chlorophyllin being a semi-synthetic mixture of sodium copper salts [7]. Chlorophyllin is a water-soluble salt obtained by alkaline hydrolysis of chlorophyll with replacement of the magnesium by copper and methyl and phytyl ester groups by sodium. Trisodium copper chlorin e_6 and disodium copper e_4 are two compounds commonly found in commercial chlorophyllin mixtures (Figure 4) [8].



R	The derivative
=0	Disodium Copper Chlorin e_4
COO^-Na^+	Trisodium Copper Chlorin e_6

Figure 4. Synthesis of chlorophyllin

HEALTH BENEFITS AND USAGE

Chlorophyllin, chlorophyll derivative, is used as a food additive and alternative medicine. As a food-coloring agent, chlorophyllin is known as natural green 3 and has the E number E141. The major food groups contributing to dietary intake of copper complexes of chlorophylls and chlorophyllins are sugar confectionery, desserts, sauces and condiments, cheese and soft drinks. The ADI for copper complexes of chlorophylls and chlorophyllins is 15 mg/kg body*day [9]. As alternative medicine, Chlorophyll has positive effects on inflammation, oxidation, and wound healing. Chlorophyll and chlorophyllin can form complexes structures with certain chemicals causing cancer such as aflatoxin-B_1 found in powders and extracts of many spices, herbs and higher plants or some heterocyclic amines found in cooked meat, or polycyclic aromatic hydrocarbons found in tobacco smoke [10-14]. The formations of these complex structures may interfere with gastrointestinal absorption of potential carcinogens, and the amounts of carcinogenic substances in susceptible tissues may be reduced [15] A number of the studies on cancer preventative effects of chlorophyll derivatives have been done [15, 16-26]. Chlorophyll is a good source of antioxidant nutrients. Antioxidant nutrients such as vitamins A, C and E help to neutralize harmful molecules (free radicals) in the body that can cause damage to healthy cells. Many studies support that chlorophylls and its derivatives have antioxidant properties [27-30] but some studies shown that chlorophyll was responsible for a pro-oxidant effect on the oxidation of oils [31-33]. The pro-oxidant and antioxidant properties of chlorophylls and its derivatives depend on the presence of light, when in dark medium chlorophylls and its derivatives act as antioxidant otherwise pro-oxidant. Calcium oxalate stones are better known as kidney stones. Chlorophyllin may inhibit the growth of calcium oxalate dihydrate; being considered to be a primary phase in calcium oxalate stone formation [34-38]. Therapeutic properties of chlorophyll can be summarized as followings [21];

- Stimulating immune system
- Benefit against sinusitis, fluid buildup, and skin rashes
- Ability to help combat anemia
- Eliminating molds in the body
- Purifying the blood and the organism, cleaning it of toxins.
- Ability to help prevent cancer and is being used in cancer therapy.
- Cleaning the intestines
- Ability to help to rejuvenate and energize the body
- Detoxification of the liver
- Ability to normalize blood pressure
- Combating bad odors, bad breath as well as body odor; due to the magnesium salts that it contains.

OCCURRENCE OF CHLOROPHYLL IN VIRGIN OLIVE OIL

Glutamic acid is converted to 5-aminolevulinic acid (ALA) in the first phase of chlorophyll biosynthesis (Figure 5). Glutamic acid is attached to a transfer RNA molecule in this reaction. Porphobilinogen (PBG) then forms by condensing two molecules of ALA, which ultimately form the pyrrole rings in chlorophyll. A porphyrin structure occurs from four molecules of PBG in second phase. This phase consists of six distinct enzymatic steps, ending with the product protoporphyrin IX. The formation of the fifth ring is in third phase, which the cyclization of one of the propionic acid side chains to form protochlorophyllide. NADPH is used for the reduction of one of the double bonds in ring D in the pathway. The final step in the chlorophyll biosynthetic pathway is the attachment of the phytol tail, which is catalyzed by an enzyme called chlorophyll synthetase

[39]. Their presence in olive oil depends on olive fruits (*Olea europea, L.*) genetic factors (olive variety), the stage of fruits ripeness, environmental conditions, the extraction process and storage conditions. For examples, three (Cerasuola, Nocellara, Biancolilla) Sicilian monovarietal virgin olive oils obtained from selected mills from the main producing areas of Sicily (Italy) during the 2004–2005 season contains 26.1, 31.97 and 24.95 ppm of total chlorophylls respectively [40]. The amounts of Chlorophyll a in five commercial Cornicabra virgin olive oil samples, collected from industrial oil mills located in the area of Toledo and Ciudad Real (Castilla-La Mancha), were determined during the crop seasons 2000/2001. There were statistically significant differences between samples in the contents of Chlorophyll a, with concentrations ranging from 2.20 to 43.0 mg/kg [41]. For Portuguese olive oils from three different Protected Designation of Origin (PDO), one from the north ‘‘Azeite de Trás-os-Montes” PDO, one from the centre ‘‘Azeites da Beira Interior” PDO, and one from the south of Portugal ‘‘Azeite de Moura” PDO, the amount (the total) varied between 3.72 mg/kg, in the ‘‘Azeite de Trás-os- Montes PDO” oil, and 6.22 mg/kg, in the ‘‘Azeite de Moura PDO” samples [42]. The content of chlorophyll pigments in olive oil samples (n=40) collected from households throughout Greece (August–September 2003) varied within a range of 8.6 to 68.3 mg/kg [43]. Chlorophyll pigment levels of Greek virgin olive oils from various geographic areas and cultivars obtained during the 1995-1996 and 1996-1997 seasons were found between 2.6 and 64.1 mg/kg [44]. Criado et al. [45] determined the contents of total chlorophylls in thirty virgin olive oil samples from various olive oil mills, from all over the region of ‘‘Les Garrigues” (Catalonia, Spain), obtained in two successive crop seasons. Total chlorophylls: 2.28 mg/kg (mean), 3.92-0.858 mg/kg (range) in the crop seasons, corresponding to 2002/03 (12 samples, period November 1 to December 15) and respectively 4.73 mg/kg (mean), 9.22-0.870 mg/kg (range) in 2003/04 seasons (18 samples, period November 1 to January 15). Kahramanmaraş olive oil samples obtained from olive oil processing factory with the traditional and continuous centrifugation systems in 2009-2010 seasons. For classical and continuous systems, the amounts of total chlorophylls were determined between 33.11-76.11 and 29.19-45.23 mg/kg, respectively [46].

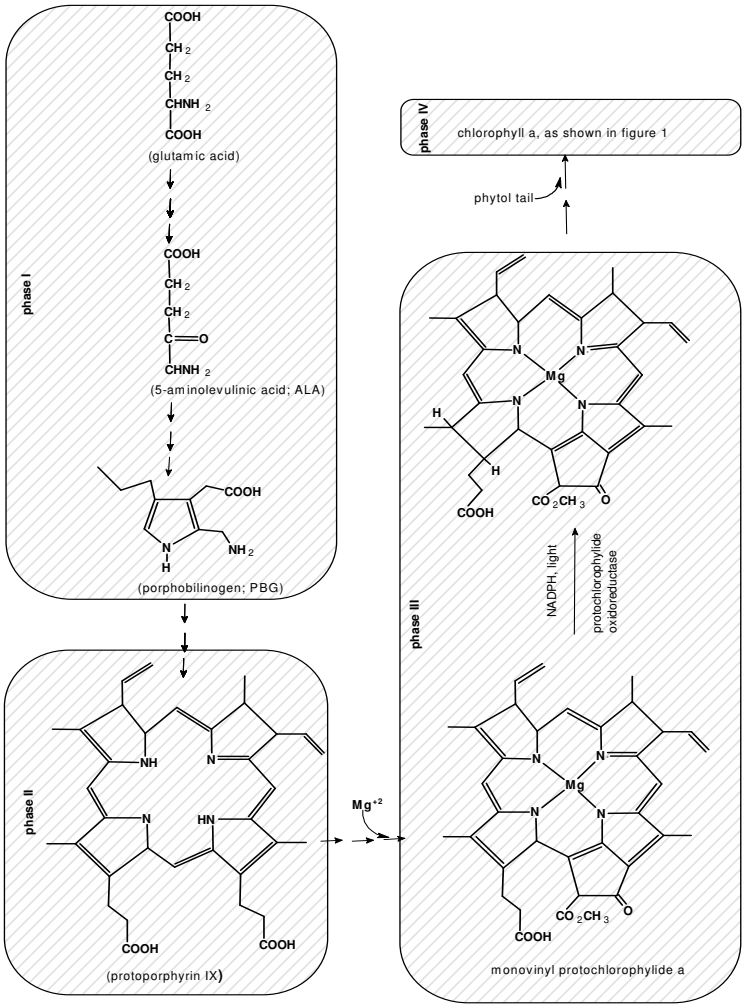


Figure 5. Biosynthesis of Chlorophyll *a*

CONCLUSION

Chlorophylls are pigments that give color to vegetables and several fruits, where they play key roles in photosynthesis. Chlorophylls cannot be synthesized by animal tissues, though animal cells can chemically modify them for assimilation. Thus, these molecules must be obtained from foods. Several reports have demonstrated that plant pigments play important roles in human health. In fact, the potential health benefit of a diet rich in chlorophylls have been indicated in recent studies reporting their role as agents preventing some diseases. The extraction process, being one of many factors on which their presence in olive oil depends, causes chlorophyll losses, owing to structural transformation of pigments caused by liberation of acids, namely the transformation of chlorophylls into pheophytins by the removal of the Mg^{2+} ion. Chlorophyll pigments are responsible for the greenish hues of virgin olive oil. Among chlorophylls, pheophytin a, is found in major. In future, clear understanding of human health benefits of chlorophyll, will be more important in order to reduce its loss during food processing.

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