Chlorella as a Source of Functional Food Ingredients: Short review

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Abstract. Chlorella is a well-known unicellular green algae species. It is considered as an important functional food due to the composition of bioactive compounds such as polyunsaturated fatty acids (PUFAs), polysaccharides, photosynthetic pigments, and phenolic compounds. These compounds have various possible therapeutic effects in the human body, such as anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, anti-cancer, anti-coagulant, anti-viral, and anti-enzymatic. This review highlights the importance and function of bioactive compounds from Chlorella and its potency as a source of functional food ingredients.

1. Introduction

Chlorella sp. belongs to the class Chlorophyceae from the division Chlorophyta [1]. Chlorella sp. cells are spherical with diameters ranging from 2 – 10 μm. The cells have a cup-shaped chloroplast equipped with a double enveloping membrane composed of phospholipids [2]. Chlorella sp. has a thick cell wall (100 - 200 nm) composed of chitin and cellulose that provides chemical and mechanical protection. In optimal condition, Chlorella sp. rapidly grows by a simple life cycle with metabolic pathways similar to higher plants [3].

Chlorella sp. dominates the microalgal market because of its economic values, such as GRAS, rapid cultivation, and high biomass [4]. Chlorella sp. has been utilized in various industries in cosmetics, food, pharmaceutical, and biofuel. This algal species comprises 51-58% of protein, 14-22% of lipid, 12-17% of carbohydrates, 4-5% of nucleic acids, and 0.4% of fiber. 60% of Chlorella protein content consists of amino acids; therefore, it can be directly absorbed by the human body [2]. Besides, Chlorella sp. accumulates biologically active compounds obtaining from secondary metabolism such as pigments and phenolic compounds. Several studies reported that Chlorella bioactive compounds have potential therapeutic activities, such as antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and anti-hyperglycemia. These have allowed them to be used as a source of nutritional ingredients [5,6].

In the last decade, people have become far more aware that foods contribute directly to their health. Consequently, research about natural origin with high biological value components is increasing. The high nutritional value of Chlorella has drawn significant attention to be developed as an innovative source for new food. Moreover, Chlorella can be used as functional foods defined as products that provide specific health benefits beyond basic nutrient requirements [7-8]. Therefore, Chlorella consumption potentially improves human well-being and life quality by decreasing the risk...
of diseases [9]. This paper discusses bioactive compounds with health benefits produced by *Chlorella* and their potential as functional food ingredients.

2. Bioactive compounds in *Chlorella* and its health potency

Bioactive compounds can be defined as compounds with a biological activity promoting good health [10]. The effects of the bioactive compound on an organism depend on their physicochemical and biological properties, such as bioavailability and chemical structure. The organisms involved and the dosage of use also play an essential role in determining bioactive compounds [10]. Foods rich in bioactive compounds that are regularly consumed positively affect human health by performing the antioxidant activity, thus decreasing the risk of numerous diseases including heart disease, diabetes, cancer, cataracts, and stroke [11,12].

*Chlorella* has been widely used as a dietary supplement for centuries. *Chlorella* has a GRAS certificate issued by the FDA, meaning safe for human consumption when grown and produced with proper hygiene and good manufacturing process [13,14]. The study found that *Chlorella* can produce various bioactive compounds such as polyunsaturated fatty acids (PUFAs) [14-17], polysaccharides [18-21], pigments [5,14,22], and phenolic compounds [23,24]. The experimental studies showed that *Chlorella* provides therapeutic properties such as antitumor, anticoagulant, antibacterial, antioxidant, antihyperglycemic, and immunomodulatory effects [5,17,25-27].

2.1 Polyunsaturated Fatty Acids (PUFA)

*Chlorella* synthesizes the long-chain polyunsaturated fatty acids (PUFAs). The production and composition of PUFAs depending on *Chlorella* strain and cultivation conditions (i.e., media, glucose concentration, and salt concentration) [15-17]. Ferreira et al. [15] reported that the oleic fatty acid (C18:1n9) dominated the *Chlorella* sp. PUFAs with the concentration of 35.1% (w/w). While, linoleic acid (C18:2n6), gamma-linoleic acid (GLA; C18:3n6), and alpha-linoleic (C18:3n3) were detected in *Chlorella homosphaera* in the concentration of 33.9% (w/w), 8.2% (w/w), and 2.3% (w/w). The accumulation of EPA (C20:5n3) and DHA (C22:4n6) was detected in *C. homosphaera* cultivated in BG-11 and increased in the addition of glucose. Besides, the production of omega-3 fatty acids (ω-3) was higher under salt stress by the increment of ALA and EPA in *Chlorella vulgaris* [16].

Among ω-3 PUFAs, several studies reported the anti-inflammatory effect of DHA and EPA. *Chlorella* is rich in ALA, the precursor of DHA and EPA, which mainly converts to EPA but is limited to DHA [17,28]. Both EPA and DHA have an immunomodulatory effect that inhibits pro-inflammatory protein production [28]. The supplementation of EPA and DHA from Chlorophyceae to healthy and diabetic mice showed the reduction of cytokines secretion such as IFN-γ, TNF-α, and IL-4. The IFN-γ and TNF-α are the pro-inflammatory cytokines associated with diabetes. While, IL-4 is one of the pro-inflammatory modulators [29].

Several studies proposed that microalgae PUFAs have therapeutic activity by inhibiting the biosynthesis of the pro-inflammatory mediator. Sibi [30] reported that *Chlorella* lipid extract had an inhibition activity to lipase, ROS accumulation, and cytokinin production. Lipid extract of *Chlorella ellipsosidea* has the highest lipid inhibitory activity. ROS production was suppressed by lipid extract of *Chlorella protothecoides* and *C. ellipsosidea*. Moreover, a lipid extract from *C. vulgaris* succeeded to inhibit the TNF-α pro-inflammatory cytokine. An in-vivo study in rats showed that supplementation of *Chlorella pyrenoidosa* extract that is highly composed of PUFAs improved the glucose tolerance and altered the gut microbiota. Therefore, *C. pyrenoidosa* extract may be used as functional food ingredients to prevent diabetes [31].

2.2 Polysaccharides

*Chlorella* sp. is a rich source of polysaccharides with yield ranging from 13 to 19% [18]. The water-soluble polysaccharides of *Chlorella* sp. consist of neutral sugars, such as glucose, galactose, mannose, arabinose, xylose, ribose, fucose, and rhamnose [18-20,32]. Several studies reported the
therapeutic potency of Chlorella polysaccharides, such as anti-inflammatory, immunomodulatory, antioxidant, and anti-tumor [33-36].

The anti-inflammatory activity of Chlorella stigmatophora polysaccharides was found to be stronger than indomethacin, a commonly used anti-inflammatory drug [33]. Water-soluble polysaccharides from C. pyrenoidosa are beneficial to the immune system due to their immunomodulatory activities that activate porIL-1 protein expression, IL-1 mRNA expression, and IL-1 secretion in a macrophage model [34]. Polysaccharides from C. pyrenoidosa also have great potential as anti-tumor agents with activity against A549 in vitro and present antiviral, anti-hyperlipidemic, and anti-coagulant activities [35-36]. Other previous studies reported the immunostimulant effect of phosphorylated polysaccharides from C. pyrenoidosa, associated with a high proportion of arabinogalactan [37-38]. Soluble polysaccharides of C. vulgarishowed an antioxidant potential by a quantitative assay of ferric reduction. The sulfate fraction of polysaccharides was proposed to contribute to antioxidant activity [19]. Moreover, a study to diabetic mice reported that the Chlorella supplementation showed an anti-hyperglycemic effect suggested due to β-1,3-glucan [39].

2.3 Pigment

Chlorella produces important pigments divided into two major groups: chlorophyll and carotenoid [40]. Chlorella sp. contains about 5.5% chlorophyll and 0.5% carotenoid in normal conditions. Chlorophyll is a natural green pigment used by algae and plants for photosynthesis [41]. Carotenoid is a pigment produced by algae, plants, fungi, and bacteria, with color ranging from yellow to red. The carotenoid composition is affected by Chlorella species and culture conditions. The carotenoid types present in C. pyrenoidosa are β-carotene, α-carotene, lutein, zeaxanthin, astaxanthin, and neoxanthin. C. ellipsoidea contains violaxanthin, antheraxanthin, and zeaxanthin. C. vulgaris contains almost entirely of lutein [42-44].

Chlorophyll is one of the potential Chlorella antioxidant agents. Some studies showed that chlorophyll and its derivatives have various therapeutic uses, including wound healing and anti-inflammatory agent. Moreover, dietary chlorophyll derivatives provide antioxidant and radioprotective effects by inhibiting protein oxidation, membrane damage, DNA damage, and lipid peroxidation [45-46].

Generally, the health-promoting properties of carotenoids are related to their antioxidant activity. Carotenoid is an efficient antioxidant scavenging singlet molecular oxygen and radical [47]. Violaixin from C. ellipsoidea succeeded in inhibiting the cellular accumulation of nitric oxide in lipopolysaccharide (LPS)-stimulated RAW 264.7 mouse macrophage cells. Besides, violaxanthin suppressed the expression of the prostaglandin E2 (PGE2), the pro-inflammatory factor [48]. Cha et al. [44] evaluated the antiproliferative effect of carotenoid extract from C. ellipsoidea and C. vulgaris. Both Chlorella carotenoid extracts showed an apoptosis-inducing effect with higher inhibitory activity resulting from the extract of C. ellipsoidea. Moreover, the lutein and zeaxanthin of C. ellipsoidea have an anti-diabetic potency demonstrated by the α-glucosidase inhibitory activity by in-vitro analysis [49].

2.4 Phenolic Compound

Phenolic compounds contribute to the microalgae chemical protective mechanisms, including metal contamination, settlement of bacteria, and ultraviolet radiation [50]. Besides, phenolic compounds protect the important cell components, such as cell membranes, nucleic acids, structural proteins, and cellular enzymes against free radicals [51]. Phenolic compounds found in C. vulgaris are chloroglucinol, ferulic acid, apigenin, and p-Coumaric acid [23, 52]. The phenolic compounds extracted from Chlorella sorokiniana are caffeic acid, ferulic acid, p-Coumaric acid, and cinnamic acid [24].

Phenolic compounds have medicinal properties such as antioxidant, anti-tumour, and antibacterial. Previous studies reported that the Chlorella phenolic compounds were the major contributor of antioxidative properties showed by DPPH and FRAP analysis. Chlorella phenolic compounds could
prevent the tumorigenesis of the liver cells by inhibiting the lipid peroxidation on the cell membrane, neutralizing cellular free radicals, and preventing DNA damage. A study reported the antibacterial properties of methanol extract-containing phenolic compounds from *C. vulgaris*, demonstrating by the inhibition zone of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* [23, 24, 53].

3. Application of *Chlorella* in Functional Food

Functional food can be considered as foods that are not intended only to provide humans with necessary nutrients but also contain one or more functional ingredients that can promote health and well-being. The increasing demand for such foods has driven research correlated with natural origin components with high biological value. Microalgae, such as *Chlorella*, are sources for new products that have excellent potential as functional food ingredients. *Chlorella* has been widely used as a food supplement and healthy food [13]. The Chlorella daily intake dose of 3 - 10 grams per day provides health effects for the human body. Recent studies have shown that *Chlorella* has a well-balanced chemical composition. Therefore, *Chlorella*-supplemented food products can enhance nutritional value [54].

*Chlorella* has been widely used as a dietary supplement for centuries. The microalgal market is filled with Chlorella in powder and tablet due to its ability to bind heavy metals like mercury, which are considered toxins and remove them out of the body. Recently, some research has been done regarding the development of food products prepared from *Chlorella* biomass. Food products, including croissants, pasta, yogurt, and cookies [55-58], have been incorporated with *Chlorella* (Table 1). The addition of *Chlorella* biomass into those food products has resulted in attractive and innovative products with increasing functional nutrients, especially essential amino acids, antioxidants, polyunsaturated fatty acids, and vitamins. Moreover, *Chlorella* addition could also raise the viability of probiotics in fermented dairy products, like yogurt [59].

| Product  | Dosage                          | Nutritional Effect                                      | References |
|----------|---------------------------------|--------------------------------------------------------|------------|
| Croissants | Enrichment of *C. vulgaris* biomass at the concentration of 1%, 2%, and 3%. | Fat and ash content significantly increased.              | [55]       |
| Pasta     | Enrichment of *C. vulgaris* biomass at the concentration of 0.5%, 1%, and 2% | Fat and ash content significantly increased.              | [56]       |
| Yogurt    | Addition of *C. vulgaris* biomass at the concentration of 0.25%, 0.5%, and 1% | The viability of the probiotics in yogurt significantly increased | [57, 59]   |
| Cookies   | Fortification with 2, 4, and 6 g of *Chlorella* sp. powder. | Protein, fat, and ash content significantly increased    | [58]       |

The incorporation of *Chlorella* into food products was able to improve the nutritional value and the sensory qualities of food products. The addition of *C. vulgaris* enhanced the appearance, taste, and also texture of croissants [55]. These results are in agreement with Fradique et al. [56], who reported that the panelists preferred pasta prepared with *C. vulgaris*, particularly those with higher concentrations (2%) due to their innovative and attractive appearances. Cookies with the addition of *C. vulgaris* also has higher textural characteristics. This result can be related to the high protein content
of Chlorella. The protein molecules may have reinforced the dough system and affected the water absorption process, promoting the increase of cookies firmness [58].

The growing number of researches related to Chlorella as functional food ingredients showed that Chlorella has a great opportunity to be developed as food for the future. Consumers also have a high demand for food to prevent disease, boost mental health, and improve life quality. Hence, the global Chlorella ingredient market is expected to grow and meet human nutritional needs. The lack of knowledge about the benefits of Chlorella may also be a major challenge hampering the growth of the Chlorella market [60].

4. Conclusion
In recent years, Chlorella has attracted significant attention as sources of natural and biologically active compounds that positively affect human health. These natural compounds, such as polyunsaturated fatty acids (PUFA), polysaccharides, carotenoids, and phenolic compounds. It has been proved that Chlorella has therapeutic properties such as antioxidant, anti-inflammatory, immunomodulatory, and antidiabetic. Therefore, Chlorella is a potential source of functional food ingredient which can be developed as sustainable future food. However, more research must be conducted, especially for raising awareness among the general public about the benefits of Chlorella to withstand the challenges of the future market.

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