An overview on microalgae carotenoids and chlorophylls: focus in the bioaccessibility

Uma visão geral dos carotenoides e clorofilas microalgais: foco na bioacessibilidade

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RESUMO
Atualmente, a tendência mundial por hábitos alimentares cada vez mais saudáveis impulsiona consideravelmente a busca por alternativas naturais capazes de modular positivamente a saúde humana. Consequentemente, esse comportamento tem destacado produtos e ingredientes à base de microalgas. As microalgas são um grupo diversificado de microrganismos considerados uma fonte atrativa de várias moléculas biologicamente ativas. Em evidência estão os carotenoides e clorofilas, visto que potenciais funções biológicas promotoras da saúde têm sido constantemente associadas a essas biomoléculas. No entanto, para que esses compostos exerçam tais atividades, eles precisam ser bioacessíveis e absorvidos pelo corpo humano. Diante disso, essa breve revisão visa elucidar os principais aspectos relacionados aos carotenoides e clorofilas das microalgas, bem como abordar a bioacessibilidade e biodisponibilidade desses compostos.

Palavras-Chave: Microalgas, Biomassa, Biocompostos, Pigmentos Naturais, Carotenoides, Clorofilas, Bioacessibilidade, Biodisponibilidade.

ABSTRACT
Currently, the global trend towards healthier eating habits considerably boosts the search for natural alternatives capable of positively modulating human health. Consequently, this behavior has highlighted microalgae-based products and ingredients. Microalgae are a diverse group of microorganisms considered an attractive source of various biologically active molecules. In evidence are the carotenoids and chlorophylls, as potential biological health-promoting functions have been constantly associated with these biomolecules. However, for these compounds to exert such activities, they need to be bioaccessible and absorbed by the human body. Given this, this brief review aims to elucidate the main aspects related to carotenoids and chlorophylls from microalgae, as well as to approach the bioaccessibility and bioavailability of these compounds.

Keywords: Microalgae, Biomass, Biocompounds, Natural Pigments, Carotenoids, Chlorophylls, Bioaccessibility, Bioavailability.

1 INTRODUCTION

In recent decades there has been a global trend towards a lifestyle that contemplates a feed that goes beyond basic nutrition (Fanzo et al., 2020). This behavior strongly drives research and development in science and technology for new foods or food ingredients increasingly nutritious, functional, and sustainable (Tzachor et al., 2021). Consequently, it highlights microalgae, microorganisms considered potential sources for obtaining future food (Torres-Tiji et al., 2020).

This targeting is partly a consequence of metabolic versatility and microalgae biodiversity, making them an almost untapped resource of biologically active molecules (Tang et al., 2020). They are capable of biosynthesizing a wide range of phytochemicals such as carbohydrates, proteins, lipids, sterols, minerals, vitamins, and pigments (Jacob-Lopes et al., 2019).

In particular, fine chemical compounds found in microalgae are being successfully allocated to industrial sectors with increasing demands. This is the case of natural pigments such as carotenoids and chlorophylls that currently need emerging sources to consolidate the market need due to safety concerns with synthetic sources (Nwoba et al., 2020). The growing interest in this group of biomolecules stems from its effectiveness in promoting human health, which is why its use has potentially been directed to various applications in the food, pharmaceutical and cosmetic industries (Silva et al., 2020).

Microalgae are potential sources of a variety of carotenoids (Novoveska et al., 2019). They synthesize complex mixtures of these compounds, from structures found in higher plants, such as lutein, β-carotene and zeaxanthin, to microalgae-specific

carotenoids with enhanced bioactive abilities, such as echinenone, astaxanthin and canthaxanthin (Rodrigues et al., 2015; Patias et al., 2017; Nascimento et al., 2021; Nörnberg et al., 2021). Carotenoids are a vast group of terpenoid pigments in which many of these structures play a notable role as antioxidants, vitamin A precursors, and prevention and maintaining eye health (Rodriguez-Concepcion et al., 2018). In addition, scientific evidence suggests its role in reducing cardiovascular disease, cancer, obesity, immune function, diabetes, protecting neurons, among other important activities (Eggersdorfer and Wyss, 2018).

Chlorophylls are ubiquitous molecules in microalgae, which have a large and diverse content of this class of pigments, including Mg-free chlorophyll (pheophytin), dephytylated chlorophyll (pheophorbide), and its oxidized and epimer derivatives, as well as types of chlorophyll a, b, c, d and f (Zepka et al., 2019; Fernandes et al., 2021). They are widely used as a natural food coloring agent and have antioxidant, wound healing, and antimutagenic properties (Sarkar et al., 2020). In addition, other activities such as the ability to reduce the risk of some types of cancer, obesity control, anti-inflammatory properties, antimicrobial activities, antiviral activity, immunostimulatory activity, and anti-parasite activity are constantly associated with the ingestion of these biomolecules (Pérez-Gálvez et al., 2017; Saide et al., 2020).

However, for these bioactive compounds to play a role in promoting health and biological functions, they need to be bioaccessible for intestinal uptake and subsequent systemic distribution in the human body (Kopec and Failla, 2018). These parameters are monitored through bioaccessibility and bioavailability assays and are strictly necessary for studies aimed at applying biocompounds as a functional or nutraceutical component (Dima et al., 2020).

In this sense, this brief review brings together a description of the main characteristics of carotenoids and microalgae chlorophylls, with the main focus on aspects related to the bioaccessibility and bioavailability of these compounds.

2 MICROALGAE

Microalgae are a heterogeneous group of microscopic organisms with a complex taxonomy, as they designate organisms that are very different from one another in terms of origin, chemical composition and morphology. Constitute a polyphyletic group of unicellular photosynthetic microorganisms, ubiquitous, mostly present in aquatic systems, with planktonic and benthic habits (Lourenço, 2006; Sathasivam et al., 2017).

According to Guiry and Guiry (2021), under the name of microalgae, organisms with two types of cell structure are included, phylogenetically classified as prokaryotic, with representatives in the groups Cyanophyta and Prochlorophyta; eukaryotic structure, with representatives in the groups Glaucophyta, Rhodophyta, Ochrophyta, Haptophyta, Cryptophytes, Dinophyta, Euglenophyta, Chlorarachniophyta, and Chlorophyta.

Because they have high metabolic versatility, these microorganisms can be cultivated under different modes for the production of biomass, such as photoautotrophic cultivation that involves the photosynthesis process, heterotrophic cultures, where it is necessary to insert organic carbon sources and mixotrophic cultivation, which comprises both autotrophic and heterotrophic conditions (Hu et al., 2018; Pang et al., 2019). Microalgae can also grow up to 50 times faster than terrestrial plants, thus achieving a higher rate of CO_2 fixation (Mountourakis et al., 2021). Thus, due to the substantial capacity of some microalgae species bioconverts organic material and nutrients present in wastewater and mitigate atmospheric CO_2 (Khan et al., 2018), these microorganisms offer an alternative to conventional forms of agro-industrial effluent treatments, with the simultaneous generation of co-products in the form of biomass (Rodrigues et al., 2014; Fernandes et al., 2017).

In turn, they are microorganisms capable of biosynthesizing, accumulating and secreting a wide variety of metabolites in response to growing conditions, many of which are high-value substances with industrial applications and health benefits (Vieira et al., 2020). However, the biodiversity of microalgae represents an almost untapped resource, since of the possible 20,000 to 800,000 existing species, relatively about 40-50,000 species have been studied in detail from a biochemical and physiological point of view (Suganya et al., 2016).

In terms of exploration and biotechnological use, the following groups stand out: cyanobacteria (Cyanophyta), chlorophytes (Chlorophyta) and diatoms (Ochrophyta). In contrast, the most abundant classes are (Bacillariophyceae), green algae (Crysophyceae), and golden algae (Chrysophyceae) (Borowitzka, 2018; Jacob-Lopes et al., 2019).

The application of microalgae biomass or its commodities as intermediate ingredients or final products touches on several industrial sectors related to bioenergy, animal feed, biofertilizers, food, pharmaceutical, nutraceuticals and cosmetics products (Rizwan et al., 2018; Sahni et al., 2019). However, the greatest exploration and application has been in the food, pharmaceutical, nutraceuticals products. This is because the presence of a diversity of bioactive metabolites in microalgae seems to be a promising

approach for the development of healthier, more functional and sustainable products (Barkia et al., 2019; Lafarga, 2019; Tang et al., 2020).

In the nutraceutical industries, Spirulina and Chlorella are the most important species to be marketed as healthy foods and nutritional supplements with several health benefits, including increased immune system activity, antitumor effects and the promotion of animal growth, due to their abundant proteins, vitamins, active polysaccharides and other important compounds (Camacho et al., 2019). In parallel, Haematococcus pluvialis and Dunaliella salina are produced at an industrial level to obtain carotenoids, especially astaxanthin (a potent antioxidant), and β-carotene (precursor to vitamin A), respectively, while phycocyanins are commercially produced from Arthrospira platensis (Spirulina) (Silva et al., 2020). However, other species need to be scientifically explored to increase the portfolio of microalgae and their bioproducts for industrial application.

3 MICROALGAE BIOPRODUCTS

Microalgae have been considered as alternative sustainable resources for high value bioproducts, such as proteins, lipids, polyunsaturated fatty acids, carbohydrates, minerals, vitamins, polysaccharides and pigments, among other compounds in constant exploration. These biomolecules from microalgae biomass have qualitative and quantitative characteristics that consolidate the biological, nutritional, and functional value of these microorganisms (Jacob-lopes et al., 2019; Dolganyuk et al., 2020). The cellular composition of each microalgae varies according to the species and their physiological responses to growing conditions (Jabri et al., 2021).

Phycobiliproteins, carotenoids and chlorophylls are the three major classes of bioactive photosynthetic pigments in microalgae, which are responsible for the colors red/blue, yellow/orange and green, respectively (Pagels et al., 2020). Carotenoids and chlorophylls are synthesized by all microalgae species, but only the Cyanophyta (blue-green algae), Cryptophyta (Cryptomonas) and Rhodophyta (red algae) divisions synthesize phycobiliproteins (Nwoba et al., 2020).

In addition to their potential application these pigments in aquaculture and in the food and pharmaceutical industries, many of these compounds have been related to beneficial health effects, which are mainly due to their antioxidant properties, anticancer, anti-inflammatory, anti-obesity, neuroprotective activities, among other health promoting benefits (Galasso et al., 2019). Thus, natural pigments are currently one of the most

commercialized products from microalgae, renewing the interest in exploring these substances in microalgae biomass (Silva et al., 2020).

3.1 CAROTENOIDS

Carotenoids are the most abundant and widely distributed pigments on earth, second only to chlorophylls. In the last compilation by Yabuzaki (2021), it is estimated that more than 1200 natural carotenoids have been characterized in different sources, including species of the plant kingdom, animal kingdom, and microorganisms, as microalgae.

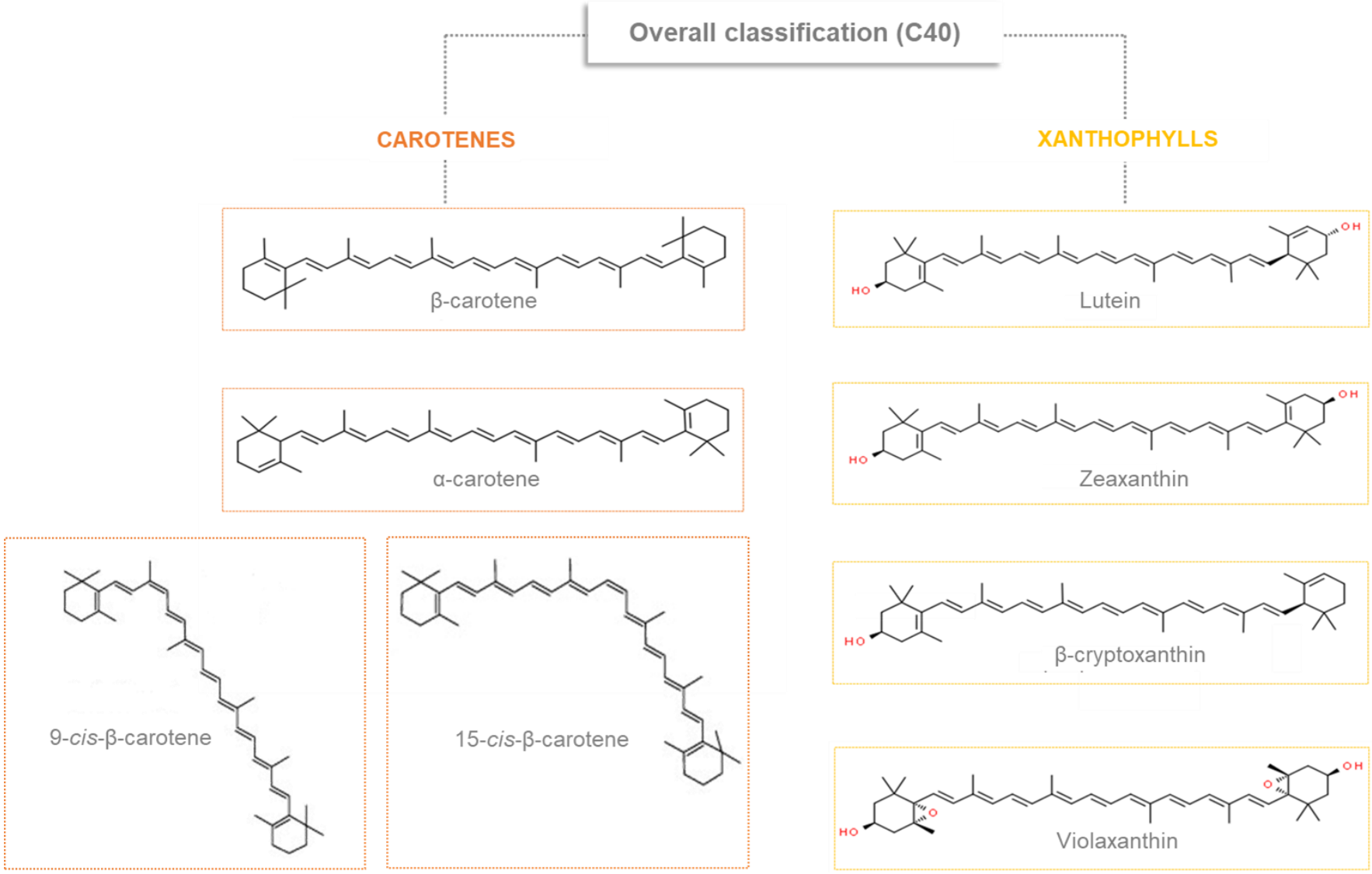
Chemically, they are classified according to the number of carbons that constitute their molecules into C30, C40, C45, and C50 carotenoid backbones. However, most carotenoids have C40-skeletons (i.e., tetraterpenes/tetraterpenoids). They are compounds fat-soluble and consist of a symmetrical structure with a set of conjugated double bonds (CDBs), which constitutes its chromophore, and which generates a π electron resonance system that moves throughout the polyene chain (Britton, 1995). Due to this structural characteristic, they absorb light in the visible region of the electromagnetic spectrum, with strong absorption in the 400 to 500 nm region (Mercadante, 2008). In turn, this part of the highly unsaturated structure is responsible for the functions, color (yellow/orange) and activities of carotenoids, such as chemical reactivity, molecular form, and activity in energy transfer processes. Also, they can be classified as acyclic (e.g., lycopene, ζ-carotene) or cyclic when they have at their ends one (monocyclic) or two (dicyclic) terminal groups in the form of rings (e.g., γ-carotene, δ-carotene) (Rodriguez-Amaya, 2015). Regarding their geometric configuration, carotenoid molecules can exist in two forms: denominated trans or cis, equivalent to E and Z. This characteristic depended exclusively on the disposition of substituent groups, specifically those that constitute a continuation of the polyene chain, about that double bond. In general, isomers (all-E)- usually have long, linear and rigid molecules, while their Z- counterparts have bent structures (Britton, 1995).

Based on their composition, carotenoids are subdivided into two groups, based on the functional groups; carotenes, which contain only the parent hydrocarbon chain without any functional group, such as α-carotene, β-carotene and lycopene, while those that contain at least one chemical function with oxygen, such as ketone (astaxanthin, canthaxanthin), hydroxyl (lutein and zeaxanthin), glycosylated (myxoxanthophyll), methoxy (spirilloxanthin) or epoxide groups (violaxanthin, neoxanthin, fucoxanthin), are

called xanthophylls, as seen in Figure 1 (Rodriguez-Concepcion et al., 2018). In addition to these important features, changes in the structure of the molecule can occur through cyclization, hydrogenation, epoxidation, dehydrogenation, the introduction of oxygen-containing groups, migration of double bonds, rearrangement, shortening or chain extension, or combinations, resulting in an arrangement of structures (Rodriguez-Amaya, 2016).

Apocarotenoids are another subclass of terpenoids that are formed from the reduction of the C40 structure by the oxidative cleavage of carotenoids. β-carotene and zeaxanthin are precursors of the main apocarotenoids described to date, including bixin, crocetin, abscisic acid, strigolactone and mycoradicin (Beltran and Stange, 2016). A recent study reported for the first

Figure 1 - Structures of carotenes and xanthophylls.



time the presence of 29 different apocarotenoids, including various apocarotenoid fatty acid esters, in different species of microalgae (Zoccali et al., 2019).

Constant advances in research and development of natural compounds from biotechnological processes, boost investigations on the composition of carotenoids in different strains of microalgae (Rodrigues et al., 2015; Patias et al., 2017; Di Lena et al., 2019; Nörnberg et al., 2021). Very recently, the occurrence of carotenoid esters in microalgae has also been reported (Maroneze et al., 2019). Carotenoid profiles vary widely between species, and the ability of microalgae to accumulate carotenoids with unique structures is well reported in the literature. Among these structures are xanthophylls with allenic (C═C═C), acetylenic (C≡C), glycosylated structures and ketocarotenoids (Takaichi, 2011). In addition, structures with chromophore more than 11 CDBs are synthesized solely from microalgae, such as echinenone and canthaxanthin, which have 12 and 13 CDBs, respectively, and have no equivalent in the plant kingdom (Rodrigues et al., 2015). Some of these structures are shown in Figure 2.

They are bioactive compounds with properties that result in biological functions beneficial to human health, classified as phytochemicals capable of modulating metabolic processes essential to cell health due to their protective action on cellular components against oxidative damage (Bohn, 2019; Khalid et al., 2019). In turn, three mechanisms have been proposed for the removal of radicals by carotenoids: electron transfer, allylic hydrogen abstraction, and addition to the conjugated double bond system (Fiedor and Burda, 2014). In addition, carotenoids also have excellent physical deactivation capabilities for singlet oxygen ($^1O_2$) through physical or chemical quenching (El-Agamey et al., 2004).

According to Rodrigues et al., (2012), the potential antioxidant effects of carotenoids are strictly related to their chemical structure, in which the number of CDBs that make up the chromophore is the most influential feature in the carotenoid's ability to reduce reactive oxygen species. Thus, it is suggested a greater antioxidant activity for microalgae carotenoids compared to conventional sources, due to the presence of exclusive carotenoids, which have a bathochromic effect, such as canthaxanthin (13 CDBs), myxoxanthophyll (12 CDBs), and echinenone (12 CDBs) (Patias et al., 2017).

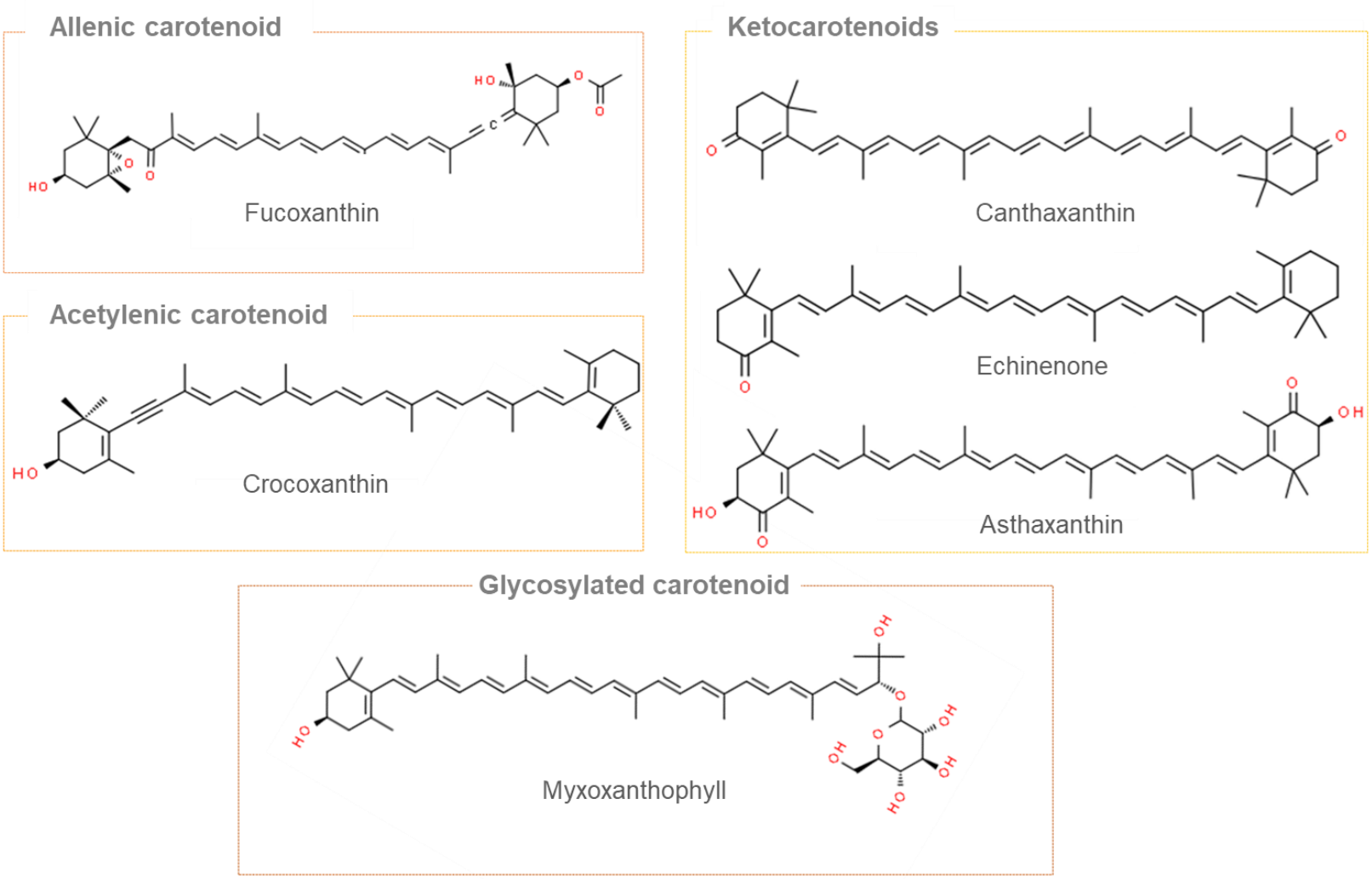
In photosynthetic organisms such as microalgae, carotenoids are associated with the light harvest photosynthesis complex, thus reducing the excess energy required in photosynthesis, transferring the absorbed energy to chlorophylls (Varela et al., 2015). Additionally, they are considered essential molecules for the survival of microalgae, as

they protect cells from reactive oxygen species generated during photosynthesis and high light intensity and also dissipate excess light as heat through the xanthophyll cycle (Hu et al., 2018).

In fact, carotenoids are essential pigments for all photosynthetic organisms; however, they also have nutritional importance related to their unique properties and potential therapeutic effects, biological activities and beneficial effects on health. A relevant property of carotenoids for human nutrition is their use as precursors of vitamin A. However, the ability of carotenoids to exert pro-vitamin is restricted to structures with at least one unsubstituted β ring with an 11-carbon polyene chain (Nascimento et al., 2019). Also, due to the protective effect exerted on the cells, these compounds are associated with the modulation of other pathologies, such as reducing the risk of developing chronic diseases such as cancer, cardiovascular diseases, cataracts and macular degeneration (Rodriguez-Concepcion et al., 2018; Eggersdorfer and Wyss, 2018).

Among the most explored carotenoids, lutein is known for its protective role against macular degeneration of the eye, making the ingestion of this xanthophyll very important, as it cannot be synthesized by humans (Becerra et al., 2020). Astaxanthin, a carotenoid found in microalgae, is a potent ketocarotenoid with well-known antioxidant properties, together with

Figure 2 – Different classes of xanthophylls.



prevention and the treatment of cancers, chronic inflammatory diseases, diabetes, obesity, cardiovascular diseases and neurodegenerative diseases (Fakhri et al., 2018; Xia et al., 2020). β-carotene is provitamin A and is also a potent antioxidant with cardioprotective effects (Grune et al., 2010).

Currently, the main application of microalgae carotenoids is in the food industry as a pigment, especially β-carotene and astaxanthin. However, due to their important bioactive properties and biologicals, pharmaceutical and nutraceutical applications are also applied (Jacob-Lopes et al., 2019). Due to this wide range of applications, the global carotenoid market is projected to reach US$2.0 billion by the year 2022. Where astaxanthin, β-carotene, lutein, fucoxanthin, zeaxanthin have the largest market shares to their wider applications. Additionally, world market projections show that in 2022 astaxanthin will reach the value of US$ 426.9 million, β-carotene US$ 572.78 million and lutein US$ 357.7 million (Gupta et al., 2021).

Along with these factors, the production of carotenoids from microalgae is continuously growing. It has become one of the most successful activities in the biotechnology sector since natural and controlled sources of carotenoid production are highly desirable due to their aspects positive economic and environmental (Khan et al., 2018). As previously mentioned, currently, Dunaliella salina and Haematococcus pluvialis are the main producers of β-carotene and astaxanthin respectively, representing 90% of the carotenoids present in the composition of these biomasses (Rammuni et al., 2019). In parallel, Muriellopsis sp., Chlorella zofingiensis, and Scenedesmus almeriensis can be explored for lutein production (Saha et al., 2020). In this context, studies are constantly being elucidated in the search for alternative microalgae species that can supplement the production of natural carotenoids.

3.2 CHLOROPHYLLS

Chlorophylls are green pigments that occur in the plastids of most plants, certain bacteria, abundantly in green algae, and also in cyanobacteria. In these organisms, chlorophylls participate in the biosynthesis of complex biomolecules ($C_6H_{12}O_6$) from simpler molecules (CO_2 and H_2O) through the process of photosynthesis . They are the most abundant and widely distributed class of pigments in nature. Due to their light energy capture and electron transfer functions in photosynthetic organisms, chlorophyll pigments are considered essential compounds in photosynthesis (Hosikian et al., 2010).

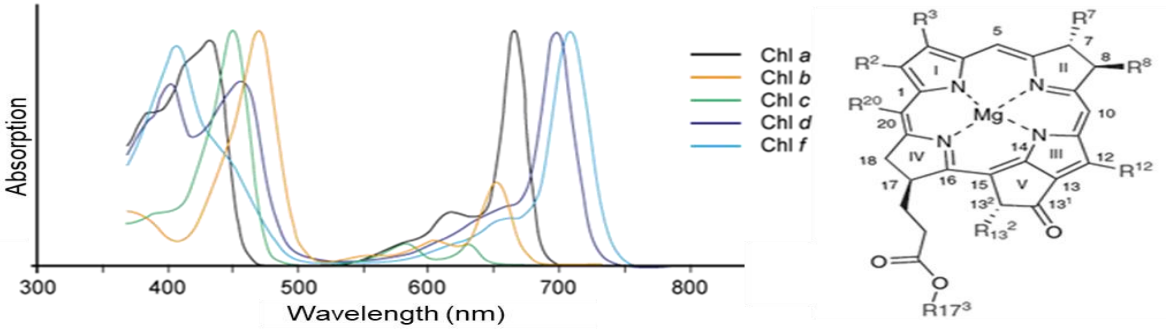
They are complex organic molecules, chemically classified within the group of porphyrins. In general, chlorophylls comprise a tetrapyrrole macrocycle system, linked by methylene bridges (-CH-) and an Mg^{2+} ion inside, coordinated to the rings by 4 nitrogen atoms. This structure also contains at C-17, a propionic acid chain esterified with phytol diterpene alcohol (a 20-carbon hydrocarbon chain), giving the molecule a hydrophobic character. However, there are exceptions to these characteristics (Roca et al., 2016).

In turn, chlorophylls constitute a large and diverse family of molecules similar to each other, called chlorophyll a, b, c (c_1, c_2 e c_3), d and f (Figure 3) (Pérez-Gálvez et al., 2017). All chlorophyll species are reported in microalgae, and except for the first two, the others are not found in higher plants (Zepka et al., 2019). Structurally, chlorophyll molecules differ from each other due to the degree of saturation of the pyrrole rings and their terminal groups, which alters the absorption of these pigments (Pareek et al., 2017). According to their degree of saturation, they are subdivided into three main classes: phytoporphyrins with a completely unsaturated macrocycle, such as chlorophylls c; bacteriochlorins present in the bacteriochlorophylls; finally the chlorins which comprise the most common chlorophylls (Roca et al., 2016).

As shown in Figure 3, chlorophyll a, chlorophyll d, and chlorophyll f have approximately equal absorption intensities in blue, red, and green. On the other hand, chlorophyll c phytoporphyrins absorb weakly in red and more intensely around 450 nm. Specifically, chlorophyll a being a blue/green pigment with maximum absorption of 660-665 nm and chlorophyll b being a green/yellow with maximum absorption of 642-652 nm (Solymosi and Mysliwa-Kurdziel, 2017).

Chlorophylls a and b are widely distributed in nature and are the best-known types. Structurally, chlorophylls a has a methyl group (CH_3) on carbon C-7, whereas chlorophyll b (Figure 3) has an aldehyde group (CHO). Chlorophyll a occurs as a ubiquitous pigment in all microalgae species, unlike chlorophyll b which is present in large concentrations in Chlorophyta and Euglenophyta which are similar to higher plants. Chlorophyll d is present in Rhodophyta and some species of Cyanophyta. While chlorophylls c are present in freshwater

Figure 3 - Chlorophyll structures and their absorption spectra.



Pigment	R2	R3	R7	R8	7-8 bond	R12	$R13^2$	17-18 bond	R20	$R\ 17^3$
Chl a	CH_3	$CH{=}CH_2$	CH_3	C_2H_5	=	CH_3	$COOCH_3$	–	H	phytol
Chl b	CH_3	$CH{=}CH_2$	CHO	C_2H_5	=	CH_3	$COOCH_3$	–	H	phytol
Chl c1	CH_3	$CH{=}CH_2$	CH_3	C_2H_5	=	CH_3	$COOCH_3$	=	H	–CH = CH-COOH
Chl c2	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$	=	CH_3	$COOCH_3$	=	H	–CH = CH-COOH
Chl c3	CH_3	$CH{=}CH_2$	$COOCH_3$	$CH{=}CH_2$	=	CH_3	$COOCH_3$	=	H	–CH = CH-COOH
Chl d	CH_3	CHO	CH_3	C_2H_5	=	CH_3	$COOCH_3$	–	H	phytol
Chl f	CHO	C_2H_5	CH_3	C_2H_5	=	CH_3	$COOCH_3$	–	H	phytol

diatoms (Zepka et al., 2019). Likewise, chlorophyll f was found in a Cyanophyta (Acaryochloris ssp.) (Chen et al., 2010).

Chlorophylls are molecules are highly unstable and may undergo changes in their structures with the formation of derivative compounds. The greatest instability of chlorophyll molecules comprises the hydrocarbon side chain, which can be removed by enzymes (chlorophylases) and/or acidic conditions, thus changing the molecular polarity, which makes the molecule hydrophilic. While Mg^{2+} can be removed when exposed to acid and thermal treatment, which evidences the formation of degradation compounds with different colors such as light green, brown green and olive green (Zepka et al., 2019). Alkaline conditions, exposure to light and oxygen, also negatively affect its stability, inducing changes in its structure (Simpson et al., 2012). Furthermore, chlorophyll molecules can undergo hydroxylation reactions in their structures, in which a series of complex hydroxylated chlorophyll derivatives are formed. These compounds may be a consequence of the natural metabolism of these microorganisms, or due to the

extraction method applied to obtain these compounds (Fernandes et al., 2017; Chen et al., 2017). However, aiming its application, the stability of chlorophylls against degradation can be increased by de-esterification of chlorophyll and complexation with copper ions in place of magnesium ion (Viera et al., 2019). Great advances have been made in understanding the biological functions of chlorophylls and their derivatives, in which studies prove that these compounds are related to several biological activities, including healing, antimutagenic, anti-inflammatory, antimutagenicity, free radical scavenging capacity, nutraceutical properties and ability to inhibit calcium oxalate crystallization. These and other important biological activities of chlorophylls and their derivatives are clarified in previous works (Lanfer-Marquez et al., 2005; Pérez-Gálvez et al., 2017; Pareek et al., 2017).

Like carotenoids, chlorophylls are also of great commercial importance. For many years, they have been used as food additives, mainly as colorants, in the food industry due to the demand for natural sources of pigments (Fernandes et al., 2020). In addition to this application, these molecules are also used in various industrial sectors such as pharmaceutical, cosmetic, and especially in the food industry, with a recent appeal to produce nutraceutical and functional products due to their possible health benefits (Viera et al., 2019; Silva et al., 2020).

Commercial sources of chlorophylls include Chlorella and Arthrospira platensis (Spirulina) microalgae and plant sources such as Enteromorpha (Chlorophyta) and Ulva (Chlorophyta) (Simpson et al., 2012). In turn, chlorophylls produced on a commercial scale are currently obtained mainly from superior plants such as stinging nettle, corn, alfalfa, or spinach. However, these matrices consist of chlorophyll content often less (< 1 %) than that found in microalgae species (~ 7 %) (Sarkar et al., 2020).

Thus, these microorganisms, especially green microalgae (Chlorophyta) and other species of cyanobacteria (Cyanophyta), become an alternative source for the biological synthesis of these compounds, as they have chlorophylls in their structures as the predominant pigments and can be cultivated in continuous systems in contrast to vegetable sources (Pagels et al., 2020).

4 BIOACCESSIBILITY AND BIOAVAILABILITY

Carotenoids and chlorophylls are not synthesized by humans and therefore are obtained from the diet or via supplementation (Pérez-Gálvez et al., 2017; Dias et al., 2018). In turn, to assume some protagonism at the biological level, these bioactive

compounds need to be absorbed by the organism. For this to occur, these biomolecules must be bioaccessible and are susceptible to absorption in the enterocytes, involved in the chylomicrons, reaching the target tissue and finally being able to exert beneficial actions to the health (Fernandez-Garcia et al., 2012; Kopec and Failla, 2018). Thus, one of the primary current knowledge to establish the real contribution of carotenoids and chlorophylls to human health is the bioaccessibility and bioavailability study.

The concept of bioaccessibility has evolved over the years and is currently the most pertinent definition refers to the sequence of events that occur during the digestive transformation of food into compounds that can be assimilated by the body (Fernández-García et al., 2012). These steps include the release and transfer of fat-soluble compounds from the food matrix to mixed micelles during digestion, which is a necessary preliminary process by which the compound becomes accessible for apical absorption by the intestinal mucosa (Kopec and Failla, 2018). The bioaccessibility of a compound added its absorption/assimilation, pre-systemic metabolism, transport, tissue distribution and bioactivity is defined as bioavailability. It represents the fraction of the ingested component available for use in physiological functions or stored in the human body (Gallardo-Guerrero et al., 2008; Saini et al., 2015). Regardless of terminology, these phases are complementary, since the ability to release biocompounds from the food matrix and transfer to mixed micelles (measured by bioaccessibility tests) is highly correlated with the absorption and your systemic distribution (bioavailability). In other words, the bioavailability of biocompounds is largely dependent on the bioaccessible fraction (Kopec and Failla, 2018).

Carotenoids and chlorophylls are lipophilic compounds and therefore are digested and absorbed in the same way as lipids. In this sense, they first need to be released from the food matrix, solubilized in particles of lipid emulsion, solubilization into pancreatic lipases and bile salts and formation of mixed micelles, movement across the microvilli, uptake by intestinal mucosal cells, incorporated into chylomicrons and enters in the lymphatic system and circulation (Ferruzzi and Blakeslee, 2007; Saini et al., 2015). As only the amount of micellarized compound is considered bioaccessible, the release of matrix and micellarization are the critical steps towards the bioavailability of carotenoids and chlorophylls.

According to Carbonell-Capella et al. (2014), bioaccessibility is generally assessed by mimicking gastric and small intestinal digestion, followed or not by Caco-2 cell uptake, one of the most widespread models for mimicking the absorption of

bioactive compounds by human intestinal epithelium (Viera et al., 2018). In parallel, the bioavailability is evaluated through the following steps: gastrointestinal digestion, absorption, metabolism, tissue distribution and bioactivity (Carbonell-Capella et al., 2014). Bioavailability is best determined by extremely controlled studies in humans. In these trials, the bioavailable content of a lipophilic compound is usually monitored for its increase in the TAG-rich fraction of blood plasma after its ingestion (Chung et al., 2004; Kopec et al., 2017). However, due to some factors such as complexity, long duration and high cost of analysis, and often inconclusive results due to the great variation in inter-individual responses, its use becomes limited. Animal models can also be used to estimate bioavailability, but they have limitations mainly related to physiological differences in relation to humans, costs and ethical considerations (Rodriguez-Amaya, 2015). In this sense, protocols that simulate in vitro digestion have been developed and have been improved and successfully applied for the initial screening of bioaccessibility and bioavailability of biocompounds from different matrices (Garrett et al., 1999; Failla and Chitchumronchokchai, 2004; Minekus et al., 2014; Rodrigues et al., 2017). Typically, these protocols are used to measure bioaccessibility and are known to mimic the physiological conditions and events that occur in the human gastrointestinal tract during the digestion process. Basically, they comprise the oral, gastric and small intestine phases. Despite not including factors related to the individual, the in vitro methodologies do not have ethical commitments, are relatively simple, rapid, inexpensive, reproducible, and valid (Rodriguez-Amaya, 2015).

The bioaccessibility/bioavailability of carotenoids and chlorophyll can be positively or negatively influenced by a complexity of factors such as food matrix interferents (e. g., constituents of the cell walls of the matrix, carbohydrates, proteins, fibers, minerals, and lipids), processing characteristics, structural physicochemical properties of molecules, and the physiological issues, genetic aspects related to the host, among others (Ferruzzi and Blakeslee 2007; Sy et al., 2012; Desmarchelier and Borel, 2017; Xavier and Mercadante, 2019). All these factors are closely related to the bioaccessibility and bioavailability of carotenoids and chlorophylls and should be considered in any study evaluating the absorption metabolism of these compounds.

The bioaccessibility and bioavailability of carotenoids are widely evaluated in different food sources, emphasizing mainly on fruits and vegetables (Reboul et al. 2006; O'Connell et al., 2007; Chitchumroonchokchai and Failla, 2017; Petry and Mercadante,

2017; Rodrigues et al., 2017; Murador et al., 2021; Nascimento et al., 2021). In parallel, chlorophylls have few studies found in the literature, although a significant advance has taken place in recent years (Ferruzzi and Blakeslee, 2007; Gallardo-Guerrero et al., 2008; Gandul-Rojas et al., 2009; Chen and Roca, 2018a; Chen and Roca, 2019). In turn, a wide range of bioaccessibility and bioavailability values can be found in the literature for the same compound. The factors mentioned above, intrinsic to the food matrix or to the compound itself, and the different methods used to assess these parameters are closely related to the high variability of the data. However, regardless of the values found in the literature, it can be considered that the absorption of carotenoids and chlorophylls by the body is considered relatively low, mainly due to factors related to the bioaccessibility of these compounds. Of these factors, it is considered that natural physical (cell wall) and chemical (interaction with other components) barriers of the food matrix and the physicochemical characteristics of the compounds are probably the main aggravating (Sy et al., 2012; Desmarchelier and Borel, 2017). Jointly to these points, the low presence of lipids in food matrices can be harmful to the absorption of these compounds since these molecules are promoters of solubilization and micellarization of lipophilic compounds such as chlorophylls and carotenoids (Failla et al., 2014; Xavier and Mercadante, 2019). Aiming at these aspects, strategies to promote increased bioaccessibility of carotenoids and chlorophylls are often being adopted, including pre-processing of foods (rupture the cell walls of the food matrix) and increase the content of lipids co-ingested with their compounds. Some of these strategies can be seen in Table 1.

To date, little is known about the bioaccessibility/bioavailability of microalgae carotenoids. As can be seen in Table 1, most of the works available so far in the literature focus only on specific carotenoids, disregarding the carotenoid profile in its entirety. Available reports are restricted to the bioaccessibility of β-carotene, α-carotene, lutein, zeaxanthin, antheraxanthin, violaxanthin, astaxanthin and fucoxanthin. In parallel, only Nascimento et al. (2021) investigated the total and individual bioaccessibility of all carotenoids present in the biomass of the microalgae Scenedesmus obliquus. Furthermore, investigations are restricted to just a few species of microalgae, which include Scenedesmus almeriensis, Chlorella ellipsoidea, Chlorella vulgaris, Chlamydomonas reinhardtii, Phaeodactylum tricornutum, Nannochloropsis sp., Spirulina platensis, Haematococcus pluvialis, Nitzschia laevis, Botryococcus braunii, and Scenedesmus obliquus. Considering the diversity and a large number of existing

microalgae species, few studies have been carried out so far, including few investigations using in vivo experimental models (Yu et al., 2012; Rao et al., 2013; Gille et al., 2018). Among the reports, most aim to determine the carotenoid bioaccessible and bioavailable content by simulating the human gastrointestinal environment through an in vitro digestion process.

The transfer of carotenoids from the matrix into micelles is a critical step that determines the extent of absorption of these compounds. In microalgae, factors influencing these aspects mainly include the structural rigidity of the microalgae cell. Therefore, strategies to increase the bioaccessibility of such biocompounds have been adopted through processes that disrupt cellular structures, promoting their dispersal in the gastrointestinal tract (Table 1). Among the

Table 1. Bioaccessibility and bioavailability of microalgae carotenoids.

Carotenoid	Microalgae	Assay	Strategy	Reference
lutein, zeaxanthin, α-carotene, β-carotene	Scenedesmus almeriensis	in vitro	extraction and dispersion in oil	Granado-Lorencio et al., 2009
lutein	Chlorella vulgaris	in vitro and Caco-2 cells	microfluidization	Cha et al., 2011
zeaxanthin, antheraxanthin, β-carotene	Chlorella ellipsoedea	in vitro	microfluidization	Cha et al., 2012
zeaxanthin	Spirulina	in vivo	addition of fat	Yu et al., 2012
β-carotene, astaxanthin, lutein	Spirulina platensis	in vivo	biomass dispersed in oil	Rao et al., 2013
β-carotene, astaxanthin, lutein	Haematococcus pluvialis	in vivo	biomass dispersed in oil	Rao et al., 2013
β-carotene, astaxanthin, lutein	Botryococcus braunii	in vivo	biomass dispersed in oil	Rao et al., 2013
lutein, β-carotene	Chlorella vulgaris	in vitro	sonication	Gille et al., 2016
lutein, β-carotene	Chlamydomonas reinhardtii	in vitro	sonication	Gille et al., 2016
lutein, β-carotene	Chlorella vulgaris	in vitro	maceration biomass	Gille et al., 2018
fucoxanthin, zeaxanthin, β-carotene	Phaeodactylum tricornutum	in vitro	maceration biomass	Gille et al., 2018
lutein, β-carotene	Chlorella vulgaris	in vivo	maceration biomass	Gille et al., 2018
fucoxanthin, zeaxanthin, β-carotene	Phaeodactylum tricornutum	in vivo	maceration biomass	Gille et al., 2018
fucoxanthin, zeaxanthin, β-Carotene	Phaeodactylum tricornutum	in vitro and Caco-2 cells	sonication and cellulase digestion	Gille et al., 2019
astaxanthin	Haematococcus pluvialis	in vitro	extraction and nanoencapsulation	Zanoni et al., 2019
fucoxanthin	Nitzschia laevis	in vitro	extraction	Guo et al., 2020
violaxanthin, antheraxanthin, zeaxanthin, β-carotene	Nannochloropsis sp	in vitro	high pressure homogenization and oil dispersion	Bernaerts et al., 2020
zeaxanthin, β-carotene	Arthrospira platensis	in vitro	biomass dispersed in oil	Tudor et al., 2021
lutein	Chlorella pyrenoidosa	in vitro	biomass dispersed in oil	Tudor et al., 2021
Total carotenoids in biomass (20 compounds)	Scenedesmus obliquus	in vitro and Caco-2 cells	ultrasonication, extraction and dispersion in oil	Nascimento et al., 2021

strategies applied, more rudimentary techniques are included, such as maceration, and more sophisticated methods, such as microfluidization, high pressure homogenization and sonication. In addition, the obtainment of isolated extract of carotenoids was also used as an alternative to enhance the bioaccessibility of these bioactive compounds. Due to the clear contribution of lipids in the micellarization process of carotenoids, the addition of oil during digestion was also adopted, in order to increase the lipid fraction present in the microalgae biomass.

The work by Granado-Lorencio et al. (2009) was the first to investigate the bioaccessibility of microalgae carotenoids. The findings of this research demonstrated that the incorporation of carotenoids in supernatants (micellarization) from S. almeriensis, specifically the compounds lutein, zeaxanthin, and β-carotene, have extremely low bioaccessibility (<1%) when administered from lyophilized biomass. However, an increase in the transfer to the micellar phase was observed when the carotenoids are extracted from the biomass and added to the olive oil used as the vehicle. The oil extracts enriched with different concentrations of carotenoids reached relative incorporation values of 35-90% for lutein, 45-80% for zeaxanthin, 50-80% for α-carotene and 25-70% for β-carotene. The authors attributed the differences in micellar incorporation to the strong effect of the microalgae matrix, mainly to the high content of fibers, components that negatively impact the bioaccessibility of carotenoids (Desmarchelier and Borel, 2017).

Maceration based on structural breakdown by mechanical means was used by Gille et al. (2018) to improve the content of lutein, β-carotene, fucoxanthin and zeaxanthin bioavailable from C. vulgaris. and P. tricornutum. The sonication method based on the propagation of ultrasonic waves significantly improved the bioaccessibility of β-carotene and lutein of C. vulgaris, reaching values of 12.5% and 18%, respectively. For C. reinhardtii, however, bioaccessibility was not influenced by sonication. Values of approximately 10% for β-carotene and 20% for lutein were found from the biomass with and without sonication (Gille et al., 2016). For unprocessed P. tricornutum biomass, bioaccessibility values of 27% for β-carotene, 29% for zeaxanthin and 52% for fucoxanthin were found after in vitro digestion, which is further improved by sonication for β-carotene (75%) and fucoxanthin (62%). The use of cellulase during the digestion process also contributes positively to bioaccessibility levels, as it aids the improved degradation of microalgae cells. Combined with the significant bioaccessibility,

fucoxanthin was the most abundant xanthophyll in Caco-2 cells, followed by zeaxanthin, while β-carotene was not detected (Gille et al., 2019).

Microfluidization, a wet milling technique that creates fine emulsions from large particles by high pressure homogenization was used by Cha et al. (2011; 2012), demonstrating to be effective in increasing the bioaccessibility of carotenoids of C. vulgaris and C. Ellipsoidea. In the first study carried out with C. vulgaris, the authors demonstrated that only 25% of the lutein presented in the untreated biomass was micellarized. Cell disruption by microfluidization processes resulted in an increase of up to 3 times more efficiency in lutein micellarization (57-73%), depending on the pressures used. As well, the final content of lutein accumulated by intestinal Caco-2 cells was also higher with microfluidization. In the second study, the results obtained for C. ellipsoedea carotenoids showed relatively low bioaccessibility values (zeaxanthin, 2.60%; β-carotene, 1.69%). Also, approximately 95% of total carotenoids were not released from the matrix and micellarized. After microfluidization of the microalgae biomass, the micelle formation efficiency increased up to 12 times more, reaching bioaccessibility values of 32.60% for zeaxanthin and 18.19% for β-carotene. The antheraxanthin content decreased with microfluidization, and this epoxy xanthophyll did not show bioaccessibility as it was not detected after in vitro digestion.

Yu et al. (2012), in an in vivo study with humans, observed an increase of zeaxanthin concentration in human serum from 0.06 to 0.15 μmol/L after ingesting a single dose of Spirulina with dietary fat. These results emphasize not only the high bioaccessibility but also the high bioavailability of zeaxanthin from this microalgae source.

The biomass dispersion strategy of S. platensis, H. pluvialis and B. braunii in oil positively impacted the bioavailability of β-carotene, astaxanthin and lutein in a healthy animal model. In this study in vivo conducted by Rao et al. (2013), it was observed that microalgae biomass could prevent lipid peroxidation by scavenging free radicals and hydroxy radicals in living cells and restoring the enzyme activity.

Nanoencapsulation, an entrapping technique of active ingredients in nanometer sized capsules, was used by Zanoni et al. (2019). In this study, the oleoresin of astaxanthin obtained from biomass of the H. pluvialis was successfully nanoencapsulated through the solvent emulsification-evaporation technique using a protein carrier, which increased the stability and bioaccessibility of astaxanthin to 76%.

The bioaccessibility of fucoxanthin varied according to the source from which it was digested in the study by Guo et al. (2020). The pure standard showed bioaccessibility values of 27.7%, while the fucoxanthin standard in emulsion with 5% oil was 27.5% bioaccessible. On the other hand, a greater total bioaccessibility for fucoxanthin (32.7%) was obtained from an extract of the microalgae Nitzschia laevis containing about 5.1% of fucoxanthin and 7% of lipids.

Bernaerts et al. (2020) reported low bioaccessibility for β-carotene, zeaxanthin, and antheraxanthin (1-6%) in the untreated biomass Nannochloropsis sp. In comparison, a threefold increase in the extent of bioaccessibility (8-16%) of the compounds was observed when cell disruption of biomass by high pressure homogenization and dispersion in oil was used. The violaxanthin present in the microalgae biomass was not detected in the bioaccessible fraction of any biomass analyzed.

Recently, the addition of organic cold-pressed coconut oil (Cocos nucifera L.) was used as a strategy to increase the bioaccessibility of carotenoids present in commercial biomass of A. platensis and C. pyrenoidosa (Tudor et al., 2021). Overall, the bioaccessibility of lutein from C. pyrenoidosa ranged from 17.77% to 19.19% associated with the type of bile source used in the in vitro digestion process (bovine bile or porcine bile) and addition or not of oil in the biomass. β-carotene from A. platensis showed bioaccessibility values from 18.94% to 20.29%, while zeaxanthin ranged from 24.68% to 42.82%. Lutein and β-carotene showed no significant difference in bioaccessibility about the bile source used; however, zeaxanthin was more bioaccessible in the presence of bovine bile. The addition of 5% coconut oil led to a significant increase in the bioaccessibility of zeaxanthin from A. platensis (from 37.2% to 42.8%); in contrast, it did not significantly alter the bioaccessibility of lutein and β-carotene.

In a study conducted with S. obliquus, it was observed that the bioaccessibility of carotenoids increases according to the degree of pre-release of the matrix to which they were submitted before the in vitro simulated digestion. Isolated extracts of carotenoids dispersed in oil showed 3 times more bioaccessible carotenoids than when digested from lyophilized biomass without treatment. At the same time, a pre-treatment with ultrasound in the wet biomass allowed an increase of up to 2 times in the bioaccessibility values. Also, this study demonstrated that carotenes and xanthophylls from S. obliquus are significantly absorbable by Caco-2 intestinal cells, reinforcing the importance of these microalgae as a potential source of carotenoids (Nascimento et al., 2021).

Although recognized as potential sources of these biocompounds, to the best of our knowledge, regrettably, there is no evidence of bioaccessibility and absorption of chlorophylls from microalgae. The available information on bioaccessibility and bioavailability of chlorophylls and their derivatives is limited and are based on in vitro assays with higher food matrix plants (Ferruzzi et al., 2001; Gallardo-Guerrero et al., 2008; Hayes et al., 2020), edible seaweeds (Chen and Roca, 2018a; Chen and Roca, 2018b; Chen and Roca, 2019) and some chlorophyll standards (Gandul-Rojas et al., 2009). In fact, these biomolecules were considered non-absorbable by our body, and only in the last few decades have studies proven the absorption of these compounds (Ferruzzi and Blakeslee, 2007). Consequently, there is currently a growing interest in expanding knowledge about the digestibility of chlorophyll pigments, which also drive studies in vivo (Fernandes et al., 2007; Hsu et al., 2014; Chao et al., 2018; Viera et al., 2018). However, there are still many gaps to be explored to increase the understanding of the metabolism and biodistribution of these phytochemicals in the human body.

5 FINAL CONSIDERATIONS

Despite numerous researches proving that microalgae are promising sources of a variety of carotenoids and chlorophylls, research focusing on the digestive behavior of these microalgae compounds is still limited. Specifically, many studies have demonstrated, mainly in vitro, the bioaccessibility and bioavailability of carotenoids from microalgae. However, studies with chlorophylls are not yet inexistent. Thus, the full exploitation of microalgae carotenoids and chlorophylls for food and health purposes requires an approach that includes the composition of these biomolecules in different microalgae species and digestive monitoring and their potential biological actions. For this, bioaccessibility and bioavailability studies are indispensable, along with strategies that can promote the accessibility and absorption of microalgae compounds. These approaches strongly contributed to the consolidation of microalgae biomass or their biocompounds in food and pharmaceutical formulations with nutritional and functional purposes.

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