Microalgal carotenoids and phytosterols regulate biochemical mechanisms involved in human health and disease prevention

Manon Le Goff, Eric Le Ferrec, Claire Mayer, Virginie Mimouni, Dominique Lagadic-Gossmann, Benoît Schoefs, Lionel Ulmann

Abstract

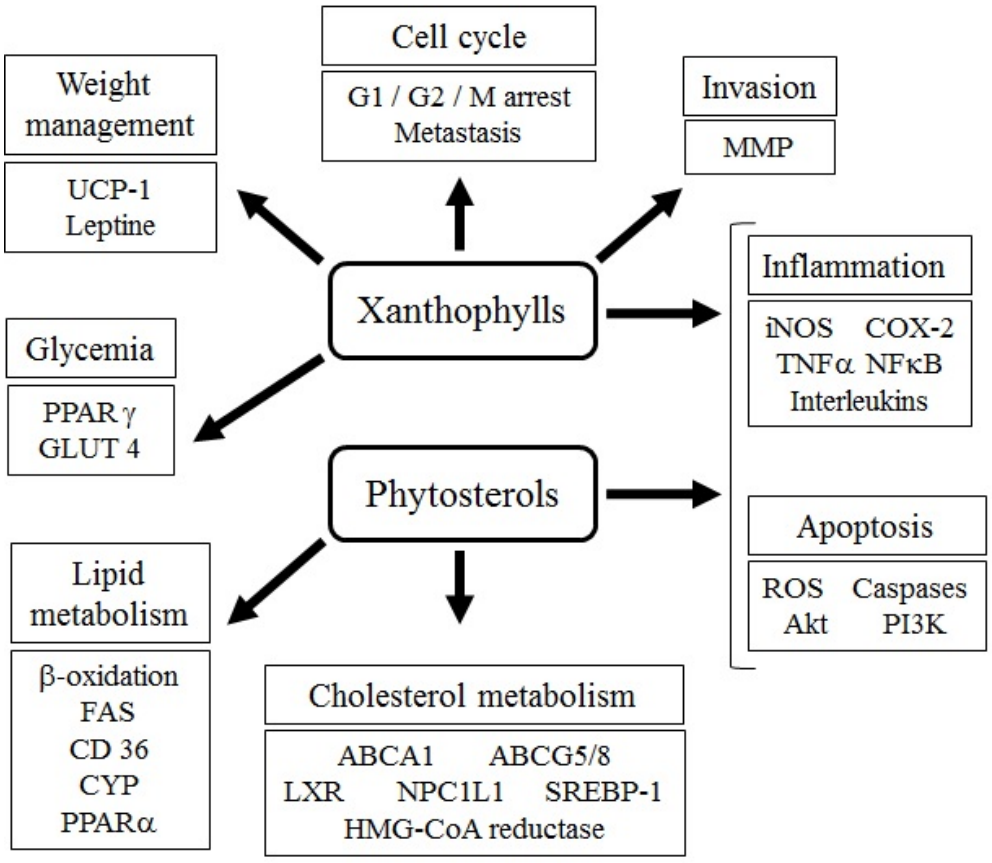
Microalgae are photosynthetic microorganisms that produce numerous bioactive molecules that can be used as food supplement to prevent chronic disease installation. Indeed, they produce phycobiliproteins, polysaccharides, lipids, carotenoids and sterolic compounds. The use of microalgae in human nutrition provide a mixture of these molecules with synergistic effect.

The aim of this review is to present the specific roles played by the xanthophylls, and specifically astaxanthin and fucoxanthin, two high added value carotenoids, and by microalgal phytosterols such as β-sitosterol, campesterol and stigmasterol on several cell mechanisms involved in the prevention of cardiometabolic diseases and cancers.

This review explains how these microalgal molecules modulate cell signaling pathways involved in carbohydrate and lipid metabolisms, inflammation, apoptosis, invasion and metastasis. Xanthophylls and phytosterols are involved in the reduction of inflammatory markers in relation with the regulation of the c-Jun N-terminal kinases and nuclear factor-kappa B signaling pathways, and suppression of production of pro-inflammatory mediators. Xanthophylls act on glucose and lipid metabolisms via both the upregulation of peroxisome proliferator-activated receptors (PPARs) and glucose transporters and its effects on the expression of enzymes involved in fatty acid synthesis and cholesterol metabolism. Their anti-cancer effects are related to the induction of intrinsic apoptosis due to down-regulation of key regulatory kinases. The anti-angiogenesis, anti-proliferative and anti-invasive effects are correlated with decreased production of endothelial growth factors and of matrix metalloproteinases.

Phytosterols have a major role on cholesterol absorption via modification of the activities of Niemann-Pick C1 like 1 and ATP-binding cassette transporters and on cholesterol

esterification. Their action are also related with the modulation of PPARs and sterol regulatory element-binding protein-1 activities.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Invasion
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Xanthophylls
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TNFα NFκB
Interleukins
Glycemia
PPARγ
GLUT 4
Phytosterols
Apoptosis
ROS Caspases
Akt PI3K
Lipid metabolism
β-oxidation
FAS
CD 36
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PPARα
Cholesterol metabolism
ABCA1 ABCG5/8
LXR NPC1L1 SREBP-1
HMG-CoA reductase

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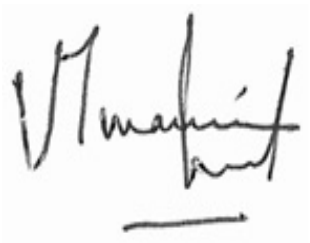

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Abbreviations: ABC: ATP-binding cassette, ACAT: acyl-CoA cholesterol acyltransferase, Adr: adrenic receptor, Akt: protein kinase B, CD36: cluster of differentiation 36, CPT: carnitine palmitoyl-transferase, COX: cyclooxygenase, CVD: cardiovascular disease, CYP: cytochrome P450, DMAPP: dimethylallyl pyrophosphate, DOXP: 1-deoxy-D-xylulose-5-phosphate, EGR: early growth factor, EPS: exopolysaccharides, ERK: extracellular signal-regulated kinase, FADD: Fas-associated protein with dead domain, FAS: fatty acid synthase, FGF: fibroblast growth factor, FGFR: fibroblast growth factor receptor, GGPP: geranyl geranyl pyrophosphate, GLUT: glucose transporter protein, GSH: glutathione, GSH-Px: glutathione peroxidase, GSK: glycogen synthase kinase, Hb: hemoglobin, HDL: high density lipoprotein, HepG2: liver hepatocellular cells, HL: human leukemia, HMG-CoA: hydroxyl methyl glutaryl CoA, HO: heme oxygenase, HUVEC: human umbilical vein endothelial cells, IKK: I kappa B kinase, IL: interleukin, iNOS: inductible nitric oxide synthase, IPP: isopentenyl pyrophosphate, IRS: insulin receptor substrate, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, LCAT: lecithin-cholesterol acyltransferase, LDL: low density lipoprotein, LPS: lipopolysaccharide, LXR: liver X receptor, MAPK: mitogen-activated protein kinase, MDA: malondialdehyde, MEP: methylerythrol phosphate, MMP: matrix metalloproteinase, MVA: mevalonate, NAFLD: nonalcoholic fatty liver disease, NF-κB: nuclear factor-kappa B, NO: nitric oxide, NPC1L1: Niemann-Pick C1 like 1, NQO: NADPH quinone oxidoreductase, Nrf2: nuclear factor erythroid-2 related factor 2, PC: prostate cancer, PGE: prostaglandin E, PI3K: phosphoinositide 3-kinase, PKA: protein kinase A, PKC: protein kinase C, PPAR: peroxisome proliferator-activated receptor, PS: polysaccharides, PUFA: polyunsaturated fatty acids, ROS**:** reactive oxygen species, RXR: retinoid X receptor, SCD: stearoyl CoA desaturase, S-EPS, sulfated exopolysaccharides, SERBP: sterol regulatory element-binding protein, SOD: superoxide dismutase, S-PS: sulfated polysaccharides, SR-B1: scavenger receptor class B type 1, StAR: steroidogenic acute regulatory protein, STAT: signal

transducer and activator of transcription, TNF: tumor necrosis factor, UCP: uncoupling protein, VEGF: vascular endothelial growth factor, VEGFR: vascular endothelial growth factor receptor, VLDL: very low density lipoprotein.

Keywords: Microalgae - Xanthophylls - Phytosterols - Chronic disease prevention - Cell signaling pathway regulation

Contents

1. Introduction

For more than twenty five years, worldwide production of oilseed crops – soybean, peanut, rapeseed, sunflower, olive or coprah – has been increasing in order to answer the strong population growth in many countries in which food access is not easy, thereby resulting in caloric deficits and therefore precarious health status [1]. Recently, there has been an increased interest for the use of microalgae as new sources of bioactive molecules for animal and human nutrition, but also due to the growing awareness and search for healthier foods [2]. Microalgae are able to produce numerous bioactive phytochemicals which major compounds are phycobiliproteins, polysaccharides, carotenoids and lipids [3]. Other molecules such as phenolic and sterolic compounds are also produced by microalgae. All these molecules are known to provide health benefits when they are used as food supplement for human nutrition.

For this use, molecules have to be extracted from fresh, frozen, dried or freeze-dried microalgal biomass. Several extraction techniques can be used such as mechanical treatments, solvents, pressurized extraction, ultrasounds, pulsed electric field [4], resonance frequency [5] and microwaves. With the exception of polar carotenoids such as crocetin, carotenoids, as hydrophobic pigments, are extracted with organic solvents such as acetone, diethyl ether, ethanol and methanol [6]. Numerous studies have been conducted to optimize these extraction methods, by the use of combined methods with green solvents. For example, in the green microalga *Chlorella vulgaris*, it has been shown that the extraction of carotenoids was enhanced by the use of a mixture of ethanol and 2-methyltetrahydrofuran, a green solvent, coupled with high temperature (110°C) [7]. Specifically for the extraction of astaxanthin, a well-known bioactive carotenoid, it has been reported in the red phase *Haematococcus pluvialis*, that the use of acetone and ethanol, combined with mechanical pre-treatment to disrupt the hard double wall red cysts, was able to extract around 90% of total astaxanthin

avoiding any degradation [8]. In this microalga, it has also been shown that the use of different types of 1-ethyl-3-methylimidazolium-based ionic liquids was efficient for cell disruption [9], and supercritical carbon dioxide extraction combined with temperature and pressure was able to extract more than 98% of total astaxanthin [10]. For an overview of the different techniques used for the extraction of microalgal hydrophobic molecules, see the review by Mubarak *et al* [11].

One major interest for proposing microalgae as food supplement is that they are able to provide a mixture of all these molecules, with an expected synergistic effect. Indeed, some of these microalgal chemicals can be used to counteract the inflammation and oxidative stress that are associated with diseases such as cardiovascular diseases, aging or cancer, notably due to damages caused by reactive oxygen species (ROS) to lipids, proteins and nucleic acids. For example, it is well-known that the blue photosynthetic pigment phycocyanin, a phycobiliprotein produced by the cyanobacteria *Arthrospira* sp., also called *Spirulina*, has a real potential in cholesterol metabolism regulation and in the inhibition of lipid peroxidation. Recently, it has been shown that a phycocyanin rich extract from *Spirulina*, protects against fibrosis during nonalcoholic steatohepatitis, with a decrease in superoxide anion, nitric oxide (NO) and thiobarbituric reactive substances [12]. A wide diversity of polysaccharides (PS) are produced by marine microalgae [13]. PS and exopolysaccharides (EPS) can be found also as sulfated derivates (S-PS and S-EPS, respectively). It has been reported that these different molecular species produced by numerous cyanobacteria and microalgae exhibit antiviral and antibacterial activities [14]. PS and EPS, sulfated or not, have also been shown to have anti-inflammatory and immunomodulatory properties, and other biological activities such as antioxidant, free radical scavenging, anti-tumor, and against vascular muscle cell proliferation. Finally, they have preventive properties of cardiovascular diseases (CVD), such as anti-lipidemic, anti-glycemic, anti-coagulant and anti-thrombotic activities [13]. Phenolic

compounds produced by microalgae accentuate their antioxidant properties. Indeed, numerous microalgae are able to produce phenolic acids such as caffeic and chlorogenic acids, that, in association with 13-cis-retinoic acid, are known to have high antioxidant activity by preventing lipid peroxidation [15].

In clinical studies, the use of whole microalgae as food supplement have shown their efficiency. Indeed, the use of *Dunaliella* sp. (0.56-3 g/day) afforded an antioxidant protection and counteracted diabetes and hyperlipidemia installation. The administration of *Arthrospira* sp. in diabetes, dyslipidemia and ischemic heart disease patients led to significant decreases in blood cholesterol, low density lipoproteins (LDL) and very low density lipoproteins (VLDL), triacyglycerols and lipid peroxidation (malondialdehyde, MDA) levels with an improvement in total antioxidant status [16]. In high fat-fed rats, it has been reported that the use of freeze-dried *Odontella aurita*, a marine diatom, had a preventive role in dyslipidemia, oxidative stress and platelet aggregation [17] with better results than those observed with fish oil [18], as earlier reported with the microalga *Chlorella pyrenoidosa* [19]. In diabetic rats, the microalga *Isochrysis galbana* has been reported to decrease blood levels of glucose, triacylglycerols and cholesterol [20].

To better understand the role played by microalgal compounds, the aim of this review will focus on the effects of microalgal-derived molecules, specifically carotenoids (Figure 1) and phytosterols (Figure 2), regarding the regulation of biochemical mechanisms involved in chronic diseases such as inflammation, cardiovascular diseases and cancer. Informations concerning the ability of microalgae to produce these molecules will be also given.

2. Bioactive molecules synthesized by microalgae

Bioactive compounds are molecules that have functional properties for human health. Several of these functional ingredients could be used in food and pharmaceutical industries such as carotenoids, sterols, polyphenols or polyunsaturated fatty acids (PUFA). Microalgae have the advantage to produce these compounds but also other molecules such as vitamins, enzymes or PS that can also be used for commercial use. Microalgal primary metabolism, such as described in diatoms, requires different subcellular compartments as chloroplast, peroxisomes, mitochondria and cytoplasm involved in the Calvin-Benson cycle with the synthesis of glyceraldehyde-3-phosphate, fatty acid oxidation, the Krebs cycle and glycolysis and neoglucogenesis, respectively [21]. To produce all the molecules that can be used for nutrition and health, the microalgal metabolism requires light as the essential energy source but also water, CO_2, and nutrients as nitrogen and phosphorus (Figure 3).

2.1 Production of carotenoids by microalgae

The fat-soluble carotenoids are divided into carotenes and xanthophylls. Total carotenoids in the different microalgae, range from 0.02 (*Scenedesmus obliquus*) to 291 mg/g dry weight (*Dunaliella salina*). Carotenes are represented by α- and β-carotenes. α-Carotene is present in *Chlorophyceae* and *Cryptophyceae* while β-carotene is present in all classes of microalgae [6]. In addition to carotenes, *Chlorophytae (Chlorophyceae and Prasinophyceae)* produce lutein and siphonaxanthin as major xanthophylls, and minor pigments or pigments limited to some specific groups, as antheraxanthin, astaxanthin, canthaxanthin, prasinoxanthin, neoxanthin, violaxanthin, or/and zeaxanthin [22]. In addition to β-carotene, the *Bacillariophyceae* and *Prymnesiophyceae* groups produce mainly diadinoxanthin, diatoxanthin, and fucoxanthin (Table 1). Concerning fucoxanthin, its production in diatoms range from 0.07 to 26.6 mg/g dry weight (the highest level being produced in *Mallomonas* sp.) compared to 2.19 mg/g dry

221 weight in the haptophyte *Isochrysis galbana*, [6]. Astaxanthin is produced by the green
222 microalga *Haematococcus pluvialis* when during its red phase can accumulate up to 5% of its
223 dry weight [23]. In *Chlorella zofingiensis*, another freshwater green microalga, it has been
224 shown that under stress conditions (high light irradiance, salt stress and low nitrogen), the
225 content of astaxanthin can reach 5.32 to 6.02 mg/g dry weight of biomass [24].

226

227 2.2 Production of phytosterols by microalgae

228 Phytosterols are important structural components of membranes and are involved in the
229 regulation of membrane fluidity and permeability; they also act as hormonal precursors
230 involved in signal transductions in organisms [25]. They are found in all microalgal species.
231 In microalgal oil extracts, the phytosterol content has been reported as ranging from 7 to 34 g
232 per kg (0.7-3.4%) when issued from *Isochrysis galbana*, *Nannochloropsis* sp. and
233 *Phaeodactylum tricornutum* [26]. Recently, *Diacronema lutheri* (syn. *Pavlova lutheri*),
234 *Tetraselmis* sp. and *Nannochloropsis* sp. have been identified as the highest phytosterol
235 producers, with content ranging from 0.4% to 2.6% dry weight biomass, and reaching 5.1%,
236 depending on nutrients, salinity and cultivation duration [27]. According to the results
237 reported by Volkman [28] in the genus *Chlorella*, a freshwater green alga (*Chlorophyceae*),
238 the highest levels of sterols (expressed as percentage of total sterols) are represented by
239 campesterol (23-31%) and by stigmasterol (56-72%). In the class of the *Prasinophyceae*,
240 campesterol range from 34 to 99% of total sterols in *Tetraselmis* sp. The haptophyte
241 (*Prymnesiophyceae*) *Diacronema lutheri* is characterized by levels of β-sitosterol ranging
242 from 23 to 73% of total sterols and by levels of campesterol and stigmasterol about 16-18%
243 and 10-31%, respectively. In diatoms (*Bacillariophyceae*), a large variety of sterols can be
244 found, depending on species. Indeed the diatom *Thalassiosira pseudonana* is characterized by

100% of stigmasterol while in *Asterionella glacialis*, the level of β-sitosterol is 95%. Campesterol is mainly found in the diatoms *Odontella aurita* and *Chaetoceros* sp. with levels from 18 to 38% of total sterols. A summary of the main classes of microalgae producing campesterol, β-sitosterol and stigmasterol, is given in Table 1. More detailed information concerning the main microalgal producers, are reported in an exhaustive review written by Volkman [29] and in Mimouni *et al*. [30].

2.3 Characteristics and synthesis of carotenoids

Carotenoids belong to the family of terpenoid compounds and has more than 750 members [31]. Carotenoids are represented by carotenes that are true hydrocarbons without any substituent in their structure, and by xanthophylls or oxycarotenoids, that contain oxygen atoms [32]. Carotenoids are C30-C50 molecules characterized by an extended network of conjugated double bonds forming the chromophore, which absorbs visible light in the violet-green region. A consequence of the presence of double bonds is the abundant number of carotenoid isomers [33]. Thus, carotenoids may adopt several 3D-configurations that are important for their biological properties. For instance, *cis*-isomers of fucoxanthin have been reported to be more efficient than all-*trans*-isomers in human cancer lines [31]. Another consequence of the extended network of conjugated double bonds resides in their capacity to act as antioxidants [31]. Although the carotenoid diversity in microalgae is very large and in many cases specific of taxa, in addition to the regular carotenoids also found in land plants, microalgae can produce molecular species with unique chemical structures [34]. It is out of the scope of this review to describe here the different types of carotenoids present in microalgae; therefore, for more information, readers are directed towards excellent reviews on that specific topic [34].

Fucoxanthin (Figure 1A) is a carotenoid present in the chloroplast of most of the algae belonging to the Heterokonta. In the photosynthetic apparatus, fucoxanthin is associated to pigments and has an essential role in photon capture for photosynthesis. Like any carotenoid, fucoxanthin exhibits a polyene chain with a conjugated carbonyl, an epoxide and a hydroxyl groups. The presence of an allenic bond makes the structure of fucoxanthin unique (Figure 1A). Altogether, these features confer to fucoxanthin a strong antioxidant capacity as well as a nonusual color for a carotenoid i.e. khaki color.

Astaxanthin (Figure 1B) is a ketocarotenoid accumulating under stress conditions [35] in several green microalgae [36], the most famous being *Haematococcus pluvialis* [37]. In contrast to fucoxanthin and most of other carotenoids, astaxanthin accumulates in the cytoplasm within lipid/carotenoid droplets. As fucoxanthin, astaxanthin exhibits a polyene chain with an epoxide and a hydroxyl groups on each end cycle. The astaxanthin that accumulates in microalgae is usually esterified with fatty acids [38], which might be interesting from the nutritional point of view [39]. Altogether, these features confer to astaxanthin a very strong antioxidant capacity, a red color and a potential for nutrition. The main characteristics of fucoxanthin and astaxanthin, with their microalgal location and role, are reported in Table 2.

The first step in the carotenogenesis is the synthesis of isopentenyl pyrophosphate (IPP) through the mevalonate (MVA) pathway or the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, that will be converted into geranyl geranyl pyrophosphate (GGPP) [40]. The carotenoid synthesis requires a condensing enzyme, the phytoene synthase, to produce the condensation of two molecules of GGPP to yield phytoene [41]. From this molecule, all the carotenoids will be synthesized, among which β-carotene, astaxanthin, lutein and xanthophylls as violaxanthin, diatoxanthin, and fucoxanthin through the xanthophyll cycles [32].

294

295 2.4 Characteristics and synthesis of phytosterols

296 Phytosterols are tetracyclic cyclopenta phenanthrene structures (ring A, B, C, and D)
297 associated with an aliphatic chain on the carbon 17 (ring D) that can be analyzed by
298 chromatography techniques and detected with numerous methods [42]. They are the end
299 products of isoprenoid synthesis, derived from IPP and dimethylallyl pyrophosphate
300 (DMAPP). Phytosterols are essential components of membranes [43], controlling fluidity,
301 permeability or activities of membrane-bound enzymes [44]. In microalgae, there is a wide
302 range of structures due to the great diversity of microalgal classes, genera and species,
303 combined with a long evolutionary history of most microalgae [29]. Some sterols can be
304 restricted to few microalgal classes while others are widespread. A large diversity of sterols
305 are found in microalgae. For a review, it can be consulted a very interesting work in this field
306 [29]. The aim of our review is to focus on some phytosterols that are known to have benefits
307 on human health. In this context, here will be presented some characteristics of stigmasterol,
308 β-sitosterol, and campesterol (Table 2). All microalgae contain sterols dominated with a Δ5
309 double bond and no methyl group on C4. Stigmasterol and β-sitosterol (Figure 2) are C29
310 sterols (C29:2 and C29:1, respectively), and their systematic names are 24α-ethylcholesta-
311 5,22E-dien-3β-ol and 24β-ethylcholest-5-en-3β-ol, respectively. One of the characteristics of
312 C29 sterols is that they have a 24-ethyl substituent while C28 sterols have a 24-methyl group.
313 Even if stigmasterol and β-sitosterol are commonly associated with higher plants, they can
314 also be found in numerous microalgal species such as diatoms, chlorophytes, chrysophytes,
315 haptophytes and freshwater eustigmatophytes [44]. Campesterol (Figure 2) is a C28 sterol
316 with one double bond (C28:1) and its systematic name is 24α-methylcholest-5-en-3β-ol.
317 Campesterol is more specifically found in chlorophytes [29].

Two metabolic pathways are involved in their biosynthesis. The MVA pathway and the methylerythritol phosphate (MEP) pathway [44]. The existence of these two metabolic pathways have been proved in numerous microalgae [45]. Microalgal-derived phytosterols are reported as 4-desmethyl-Δ5-sterols, 4-desmethyl-Δ7-sterols, 4-methyl-sterols and dihydroxylated sterols [46].

In Figure 4 is reported a short view of the two common metabolic pathways involved in the synthesis of microalgal carotenoids and sterols.

3. Bioavailability and absorption of carotenoids and phytosterols

3.1 Carotenoids

3.1.1 Bioavailability

The absorption of hydrophobic molecules as carotenoids, need different steps as follows: release from food matrix, lipid emulsion, solubilization into mixed micelles, uptake by enterocytes and secretion into lymphatic system [47]. Moreover, the increase of diet fat seems to enhance the absorption of carotenoids [48]. Carotenoid bioavailability depends on numerous dietary factors such as the source, food matrix, food processing or lipid levels but also depends on host-related factors, e.g. diseases, life-style habits age or genetic variations [49]. It is difficult to compare bioavailabilities of carotenoids from different plant sources as vegetables, fruits or microalgae because different *in vivo* and *in vitro* approaches have been used, even if high correlations have been found, thus stressing that estimating *in vitro* bioaccessibility (solubility/ micellarization) can be indicative of the amount available for

uptake in the gastro-intestinal tract *in vivo* [50]. Moreover, there is a lack of information concerning microalgal sources. Nevertheless, below will be presented some reported results with different plant sources.

According to the literature, more than 750 carotenoid species have been identified and only 40 are consumed, the most abundant being β-carotene, lycopene, lutein, β-cryptoxanthin, α-carotene and zeaxanthin. Astaxanthin and cantaxanthin are absorbed in subjects fed with diets rich in sea food [49]. It has been reported that vegetable and fruit carotene bioavailability was ranged from 1.5 to 39%; concerning xanthophylls, this bioavailability was of 4 to 59% [51]. Carotenoids from microalgae have also been reported to be bioavailable for nutrition studies. Indeed, a study has been conducted in rats with diets containing *Spirulina platensis*, *Haematococcus pluvialis* or *Botryococcus braunii*, providing 200 µmol/L of β-carotene, astaxanthin or lutein, respectively [52]. After 15 days of diet, plasma levels of these carotenoids range from 255 to 485 nmol/L, the highest level being obtained for astaxanthin, probably due to astaxanthin esterification, which can increase astaxanthin absorption [53]. Moreover, in a previous similar nutritional study, it was also reported that the peak level of individual carotenoids in plasma was observed 2 hours after administration of microlagal biomass [54].

3.1.2 Mechanisms of absorption

When carotenoids are released from the food matrix, they are incorporated into lipid droplets and then in mixed micelles after action of pancreatic lipases and bile salts that are absorbed in the enterocytes. For a long time, it has been thought that carotenoids were absorbed by passive diffusion but recently it has been proposed the role of proteins involved in their uptake but also in their secretion. Indeed, the uptake of carotenoids are captured by the apical

membrane transporters as scavenger receptor class B type 1 (SR-B1), cluster of differentiation 36 (CD36) and Niemann-Pick C1-like 1 (NPC1L1) [55]. Carotenoids are transported by lipoproteins with a specific accumulation of β-carotene in chylomicrons and very low density lipoproteins (VLDL), while xanthophylls are more specifically incorporated into LDL and high density lipoproteins (HDL) [40] (Figure 5). Beside liver, the main tissue target of β-carotene in which it will be accumulated is adipose tissue [56].

3.2 Phytosterols

3.2.1 Bioavailability

Campesterol, β-sitosterol and stigmasterol are the most common phytosterols, with a chemical structure resembling cholesterol one. As human cells do not synthesize them, phytosterol levels and activities depend on plant origin diet [57]. In human serum, phytosterol levels are hundred times lower than cholesterol ones, in a range of 3-20 mg/L. This can be partially explained by the fact that less than 10% of phytosterols are absorbed by comparison with the levels of absorbed cholesterol that are around 50-60% [58]. However, due to the poor solubility and bioavailability of free phytosterols, a minimum intake of 2-3 g/days is necessary for a cholesterol-lowering effect [27]. In various animal species, it has been reported an absorption ranging from 0% in rabbit to 4% in rat; and in human subjects fed with 240 to 320 mg of sitosterol, the estimated absorption was from 1.5% to 5% [59].

3.2.2 Mechanisms of absorption

Intestinal uptake of phytosterols takes place after their incorporation into mixed micelles. Then, sterols are released from the micelles and transported into the enterocyte via the NPC1L1 transporter [60]. Once incorporated, the absorption is inhibited by ATP-binding cassette (ABC) efflux transporters as ABCG5 and ABCG8 that are involved in the secretion of phytosterols in the inter-intestinal lumen [61]. In the enterocytes, contrary to cholesterol, phytosterols are not esterified, resulting in a lower incorporation into the nascent chylomicrons which enter the lymphatic system and then blood circulation. Then, phytosterols are taken up by liver in which there are metabolized. However, based upon a higher rate of bile excretion of phytosterols compared to cholesterol, this can also explain the low serum levels of these molecules [62].

4. Carotenoids and disease prevention

In microalgae, β-carotene, astaxanthin and fucoxanthin are among the most interesting compounds based upon their antioxidant functions. Among the carotenoids, β-carotene is responsible for the production of retinol and retinoic acid, thus activating numerous transcription factors such as retinoid X receptor (RXR) and peroxisome proliferator-activated receptors (PPARs), and hormone receptors [56]. *In vitro* and *in vivo* studies have reported that β-carotene was also involved in the reduction of angiogenesis through decreasing new formed blood vessels and suppression of cell proliferation and migration. The expression of matrix metalloproteinases (MMP)-2 and -9 was downregulated, and a decrease of the pro-inflammatory cytokine levels has also been observed [63]. Concerning diabetes, serum β-carotene levels have been reported to be inversely correlated with levels of HbA1c that are linked to impaired insulin sensitivity.

In the following sections, only the effects of xanthophylls on biochemical mechanisms in disease prevention and health benefits will be detailed, and more specifically the role played by astaxanthin and fucoxanthin, two microalgal high added value products. Indeed, these molecules have been proposed to have benefits on human health through antioxidant activity, hypolipidemic, anti-inflammatory and hypotensive effects, and through the improvement of endothelial function [64]. A summary of the role of xanthophylls is given in Figure 6.

4.1 Metabolic disease prevention

Astaxanthin, one of the most important carotenoids that can be extracted from the microalga *Haematococcus pluvialis*, has a real anti-hypertensive effect. This protective effect has been reported in SHR and Zucker rats with a systolic blood pressure lowering action [65] [66]. It has been proposed that this effect could be mediated by NO-related mechanisms and by the activity of the renin-angiotensin system. Astaxanthin has also been shown to have a great potential in diabetes prevention and treatment. In the db/db mouse model, treatment with astaxanthin induced a decrease of glucose tolerance with attenuation of blood glucose levels and serum insulin enhancement. Moreover, due to its antioxidant properties, astaxanthin was reported to help in the preservation of pancreatic β-cell function [67]. In high fat diet-fed mice, it has been shown that this xanthophyll was able to reduce ROS production, lipid accumulation, and the hepatic expression of endoplasmic reticulum stress and inflammatory markers. These reductions have been related to reduced c-Jun N-terminal kinase 1 (JNK1) and nuclear factor-kappa B (NF-κB) cell signaling pathways, leading to the conclusion that astaxanthin might be used as a treatment for insulin resistant patients [68]. This carotenoid has also been shown to bind to PPARγ, decrease glycemia and triglyceridemia and improve serum HDL-cholesterol and adiponectin levels, thus resulting in an anti-hyperglycemic effect [41].

Still concerning the carbohydrate metabolism regulation, the xanthophyll fucoxanthin, like astaxanthin, has the ability to regulate glycemia and insulin levels in diabetic/obese mice but it also upregulates the mRNA levels of the glucose transporter 4 protein (GLUT4), known to be involved in the uptake of glucose. The translocation of GLUT4 from intracellular vesicles to plasma membrane would result from the increased phosphorylation levels of protein kinase B (Akt) and from the induction of PPARγ coactivator-1α [69].

Fucoxanthin is also recognized to have an anti-obesity activity. Indeed, several mechanisms are regulated by this carotenoid in relation with weight loss and lipid metabolism. It has thus been reported that fucoxanthin decreased the mRNA expression of the fatty acid synthase (FAS) and inhibited the adipocyte uptake of glucose by reducing the phosphorylation of the insulin receptor substrate 1 (IRS-1). Its anti-obesity effects would also be due to the stimulation of the uncoupling protein-1 (UCP-1) expression in white adipose tissue. Thermogenesis and lipolysis are increased by the UCP-1 stimulation and also by the increased mRNA expression of the β3-adrenergic receptor (Adrb3) [70]. This anti-obesity action is also related to a decrease in plasma leptin levels and to a down-regulation of the liver stearoyl-CoA desaturase-1 (SCD1), a rate limiting step in the saturated and monounsaturated fatty acids. This alteration of fatty acid composition has been proposed to stem from leptin signaling in mice [71]. This anti-obesity effect is also due to the action of fucoxanthin and its derivates on lipid metabolism. Fucoxanthin has been reported to regulate the hydroxyl methyl glutaryl CoA (HMG-CoA), acyl-CoA cholesterol acyltransferase (ACAT), lecithin-cholesterol acyltransferase (LCAT) and carnitine palmitoyl-transferase (CPT-1) activities as well as sterol regulatory element-binding protein-1 (SREBP-1) [70].

4.2 Anti-cancer and anti-angiogenic activities

The anti-cancer effects of astaxanthin have been studied in numerous cancers in murine models in which it has been reported a significant lower occurrence of cancer, an anti-proliferative activity, a reduction of metastasis induced by stress, and a reduction of weights and sizes of tumors [41]. The pro-cell death effect of astaxanthin could go through the activation of a caspase-dependent mitochondrial apoptotic pathway; this would involve a downregulation of the expression of anti-apoptotic proteins (eg. *Bcl-2*, *p-Bad*, and surviving), and an upregulation of pro-apoptotic proteins (*Bax* and *Bad*), with a release of cytochrome c from mitochondria and cleavage of ADP-ribose polymerase [72]. In oral cancer, astaxanthin has been shown to inhibit invasion through a decrease in the mRNA and protein levels of overexpressed MMP-2 and -9, and through an increase of the protein levels of their endogenous inhibitors [73].

In relation with these observations, molecular targets of astaxanthin have been proposed that would explain its role in cancer prevention. The activation of the caspase-mediated mitochondrial apoptotic pathway would be due to the inhibition of the NF-κB signaling pathway and to the down-regulation of the key regulatory kinases, I kappa B kinase (IKKβ) and glycogen synthase kinase-3β (GSK-3β) [72]. The anti-proliferative and anti-angiogenic effects of astaxanthin have been related to the inhibition of the JAK-2 (Janus kinase-2)/STAT-3 (signal transducers and activators of transcription-3) signaling with an inhibition of STAT-3 phosphorylation and its nuclear translocation leading to down-regulation of STAT-3 target genes involved in cell proliferation (cyclin D1), invasion (MMP) and angiogenesis (vascular endothelial growth factor (VEGF) and its receptor VEGFR-2) [73]. Among the signaling pathways regulating cell survival, the phosphoinositide 3-kinase PI3K/Akt has an important role. Astaxanthin decreased the phosphorylation of AKT to induce apoptosis. Other molecular targets such as mitogen-activated protein kinases (MAPKs), PPARγ and nuclear factor

erythroid-2 related factor 2 (Nrf-2) are regulated by astaxanthin. For a detailed review see Zhang and Wang [74].

Fucoxanthin seems to have a better anti-cancer activity compared with lycopene, β-carotene or astaxanthin. Indeed, in the human leukemia HL-60 cell line, only fucoxanthin induced high levels of DNA fragmentation. In this study, the authors stated that the apoptosis induced by fucoxanthin was related to the generation of ROS, leading to cytotoxicity involving the cleavage of caspases-3 and -9 [75]. In prostate cancer cell lines, fucoxanthin and its metabolite fucoxanthinol have been reported to inhibit cell-growth rate [76] and to induce apoptosis in prostate cancer PC-3 cells by activating caspase-3 [77]. Fucoxanthin and fucoxanthinol have also been reported to induce cell-cycle arrest in numerous human tumor cell lines by modulating expression of various molecules and signal transduction pathways [78]. In osteosarcoma, they induced G_1 cell cycle arrest by reducing the expression of cyclin-dependent kinases. In this bone tumor, fucoxanthin and fucoxanthinol also induced apoptosis with a reduced expression of survivin, an X-linked inhibitor of apoptosis protein, and of the anti-apoptotic genes *Bcl-2* and *Bcl-xL*. The resulting apoptosis is associated with caspase activation and with an inhibition of the Akt pathway. Migration and invasion inhibitions have been associated with the reduced MMP-1 expression and the activator protein-1 signal [79]. Studies reported in human umbilical vein endothelial cells (HUVEC) have demonstrated the anti-angiogenic activity of fucoxanthin and fucoxanthinol. Indeed, it has been shown a suppression of the growth of microvessels [80] and a down-regulation of the fibroblast growth factor-2 (FGF-2), its receptor FGFR-1 as well as of the factor of transcription early growth response protein 1 (EGR-1). In this study, it has also been shown that fucoxanthin hampered the phosphorylation of signaling proteins such as extracellular signal-regulated kinases (ERK) and Akt [81].

4.3 Anti-inflammatory activities

The anti-inflammatory effects of astaxanthin have been reported in numerous *in vitro* and *in vivo* studies on lipopolysaccharide (LPS)-induced inflammatory reactions, with suppression of the production of NO, prostaglandin E_2 ($PGE_{2)}$, interleukin (IL)-1β and tumor necrosis factor alpha (TNF-α), and prevention of the expression of inductible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2. All these results have been related with the inhibition of the NF-κB signaling pathway. The same results have been observed in LPS-stimulated murine macrophages, in which fucoxanthin was able to reduce levels of pro-inflammatory molecules such as NO, PGE_2, IL-1β/6, TNF-α, by the suppression of the NF-κB but also by the inhibition of the MAPK pathways signaling [75].

4.4 Anti-oxidant activities

Astaxanthin scavenges free radicals and other antioxidants to protect lipids from peroxidation. It inhibits H_2O_2-mediated activation of the transcription factor NF-κB that is involved in the expression of heme oxygenase-1 (HO-1) and iNOS engaged against oxidative stress [82]; subsequently, the production of pro-inflammatory cytokines was blocked through the increase of tyrosine phosphatase-1 expression [83]. In brain, the use of astaxanthin has been reported to protect from oxidative stress with a decreased level of MDA and NO and increased activities of catalase and of superoxide dismutase (SOD) and higher glutathione (GSH) levels [84]. It also protects the steroidogenesis from oxidative stress with a prevention in the down-regulation of the steroidogenic acute regulatory protein (StAR), a protein involved in the transport of cholesterol. Indeed, during oxidative stress, the protein kinase A (PKA) pathway is attenuated, leading to the suppression of the expression of StAR; administration of astaxanthin acts on the PKA pathway, thereby restoring the StAR expression [85].

In vivo studies have shown that fucoxanthin exhibit antioxidant activities through the increase of catalase, SOD and glutathione peroxidase (GSH-Px) activities, as well as the plasmatic expression of Nrf2 and NADPH quinone oxidoreductase 1 (NQO1) [86]. The increased Nrf2 protein accumulation is accompanied by an enhancement of ERK, p38 phosphorylation and of HO-1expression [87].

5. Phytosterols and disease prevention

5.1 Cholesterol-lowering activity and lipid metabolism

Phytosterols have been reported to interact with the cholesterol absorption through inhibitory mechanisms. Among suggested mechanisms, it has been proposed the release of cholesterol from mixed micelles, the modification of the gene expression of NPC1L1 and of ABCG5 and ABCG8 transporters, or the decrease of the intestinal cholesterol esterification rate [88]. The selective binding of sitosterol to the ABCG5 and ABCG8 transporters involved in the regulation of liver and intestinal sterol absorption and secretion, is related to blood cholesterol lowering [89]. Microalgal phytosterols as campesterol and β-sitosterol are also involved in cholesterol level decrease through the down-regulation of hepatic HMG-CoA reductase and the stimulation of the LDL-C receptors, thus facilitating the removal of plasma cholesterol [90]. Moreover, β-sitosterol has a competing activity with NPC1L1 [91]. LXRs are nuclear receptors involved in the regulation of lipid metabolism. Even if the main function of LXRs is the regulation of cholesterol metabolism, their activation inhibits inflammation, autoimmune reactions and atherogenesis [92]. The treatment of intestinal cells with phytosterols, like campesterol and β-sitosterol, would lead to an increase in the expression of LXR target genes, suggesting a ligand action of these molecules on LXRs. Specifically, phytosterols increase the ABCA1 expression and decrease cholesterol absorption [93]. In rat, it has been shown that the

incorporation of β-sitosterol into liver membranes, decreased its fluidity while an increase in liver desaturases (Δ9, Δ6 and Δ5 desaturases) was observed, probably as a compensatory mechanism for the decreased fluidity [94] [57].

As phytosterols are supposed to be metabolized in the same way as cholesterol in intestinal lumen, the effect of campest-5-en-3-one (campestenone), an oxidized derivate of campesterol, has been studied on lipid metabolism in rats. It has been reported that campestenone increased the activities and mRNA expressions of enzymes involved in liver β-oxidation; it also reduced visceral fat weight, triacylglycerol and cholesterol levels in serum and liver, as well as the activities and mRNA expression of enzymes involved in fatty acid synthesis [95]. In this study, it has also been reported an activation of human PPARα and a decreased mRNA level of SREBP-1. All these results are in favor of an effect of dietary campesterol and its oxidized derivate to prevent CVD by improving obesity and dyslipidemia.

In human pancreatic islet and in the INS-1 insulinoma cell line, the use of stigmasterol prevents the increase of both cholesterol and ROS levels induced by glucolipotoxicity. In this study, stigmasterol has been shown to decrease free cholesterol levels resulting from glucolipotoxicity, with a real potential to protect pancreatic cells during diabetes progression [96].

Recently, in mice fed high-fat western-style diet, it has been reported that stigmasterol and β-sitosterol were able to attenuate the nonalcoholic fatty liver disease (NAFLD) and to alter lipid metabolism. Administered at a dose corresponding to what is suggested for human, it has been shown an amelioration of high fat diet-induced fatty liver, including liver total lipid, triacylgycerol and cholesterol levels. Moreover, both phytosterols were able to decrease the intestinal bile acid levels. Analyses of gene expressions have shown a prevention of the decreased mRNA expression of HMG-CoA reductase in liver by both phytosterols. Only

581 stigmasterol was reported to suppress lipogenesis-related genes such as SCD1 and FAS, while
582 both increased the expression of PPARα and CD36. The hepatic expressions of the
583 cytochrome P450s CYP7A1, CYP8B1 and CYP27A1 were also increased [35].

584

585 5.2 Anti-inflammatory activities

586 Ergosterol and peroxide-derived ergosterol from microalga such as *Chlorella vulgaris* and
587 *Dunaliella tertiolecta* have been shown to reduce the LPS-induced inflammatory response
588 through the reduction of pro-inflammatory cytokines (TNF-α, IL-6, IL-10) [98]. β-sitosterol,
589 has been shown to have anti-inflammatory activity in murine macrophages. Indeed, treatment
590 with this phytosterol inhibited both the STAT1 pathway and the translocation of NF-κB, two
591 pro-inflammatory signal transduction pathways, which were supposed to be mediated by the
592 activation of the tyrosine phosphatase SHP-1 [99]. Stigmasterol has been described to have
593 anti-osteoarthritic properties with inhibition of pro-inflammatory and matrix degradation
594 mediators involved in cartilage degradation in part via the NF-κB pathway [100]. In LPS-
595 induced innate immune responses in mice, stigmasterol has also been reported to decrease
596 fever response, lung inflammation, transaminase activities and liver damages [101].

597

598 5.3 Anti-cancer activities

599 Numerous studies have reported that phytosterols exhibit bioactivities against tumours. Thus,
600 stigmasterol isolated from the microalga *Navicula incerta* induces toxicity in HepG2 cells and
601 triggers apoptosis via up-regulation of *Bax* and down-regulation of *Bcl-2* [102]. β-sitosterol is
602 also involved in the increase of the *Bax*/*Bcl*-2 ratio and in the activation of caspase-3, thus
603 enhancing apoptosis and inhibiting proliferation in human leukemic cells [103]. In MDA-MB-

231 cells, this phytosterol has been shown to inhibit GO/G1 cell cycle associated with induction of apoptosis. The use of β-sitosterol indeed induced the depolarization of mitochondrial membrane potential and also increased the *Bax*/*Bcl*-2 ratio in this human breast cancer line [104]. The Fas/CD95 apoptotic pathway in human breast cancer has also been suggested to be affected by β-sitosterol. Indeed, its incorporation in cell membranes induced an increase in Fas protein levels, and caspase-8 activity, resulting in an inhibition of tumor cell growth [105].

As shown in macrophages, another mechanism of the anti-cancer activity of phytosterols might occur through the increase of antioxidant enzymes, like the manganese SOD and the GSH-Px, resulting in a protection of cells from damage caused by ROS and depending on the estrogen/PI3K pathway [106].

As phospholipids interact with cholesterol in cell membranes, the incorporation of phytosterols into membranes could modify their structure and thus cell signaling [107]. Indeed, lipid rafts, where sterols are highly concentrated, regulate phosphorylation chain reactions, and the incorporation of phytosterols might lead to beneficial changes in signal transduction [108]. To explain the role of β-sitosterol on the inhibition of tumor growth, the signaling pathways involving the protein kinase C (PKC) and sphingomyelin cycle have been investigated. *In vivo* and *in vitro* studies did not report any effect of β-sitosterol on the PKC pathway and on the phospholipase C activity. However, the *in vitro* studies showed an activation of the sphingomyelin cycle, in cells supplemented with β-sitosterol, resulting in an increased production of ceramide, a major cell second messenger promoting cell cycle arrest and apoptosis [57]. *In vitro* studies have further shown the role of β-sitosterol in the reduction of sphingomyelin levels via the activation of sphingomyelinase leading to an increase in

ceramide [109]. A summary of the role of the main microalgal phytosterols is reported in Figure 6.

6. Conclusion

The results reported in this review explain how microalgae could be promising food supplement for health and disease prevention. The whole cell can be used in nutrition to provide a mixture of molecules that possess preventive effects towards several chronic diseases; on the other hand, extracted molecules could also be proposed for targeting specific effects. In this review, the effects of microalgal ω3 PUFA have not been discussed but their high amounts contained in microalgae help in the efficiency of these microorganisms in cardiometabolic disease prevention and anti-tumoral activities. Fish oils provide high levels of ω3 PUFA but the advantage of microalgae is that they also provide molecules such as carotenoids and sterols that have obvious health beneficial effects. At present, few microalgae can be used in human nutrition because cytotoxicity tests must be conducted prior to agreement by food authorities. However, as emphasized in this review, extracted molecules might be directly used for nutrition in order to act specifically on cell signaling pathways and thus, might be considered not only as preventive agents but also as therapeutic agents.

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8. Authors' contribution

MLG and ELF contributed to the redaction of the section related to cancer prevention ; CM and VM contributed to the section related to cardiovascular disease prevention ; DLG thoroughly read the manuscript and added more information, notably related to underlying mechanisms, when necessary, besides checking for correct English usage; BS contributed to the redaction of the section related to the carotenoid production, and also to the revision of English language; LU supervised the redaction of this review and compiled all the reported informations, was responsible of the different sections, created all figures and tables and wrote the sections in relation with the illustrations.

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Figure 1. Chemical structures of the main high added value bioactive xanthophylls produced by microalgae.

A: fucoxanthin, B: astaxanthin

Figure 2. Chemical structures of bioactive phytosterols produced by microalgae.

A: stigmasterol, B: β-sitosterol, C: campesterol

Figure 3. Synthesis of bioactive compounds by microalgae.

CH: chloroplast, CW: cell wall, ER: endoplasmic reticulum, LD: lipid droplet, MT: mitochondria, N, nucleus.

Figure 4. Common metabolic pathways of microalgal carotenoid and sterol syntheses [32] [45] [110]**.**

DMAPP: dimethylallyl pyrophosphate, DOXP / MEP: 1-deoxy-D-xylulose-5-phosphate / methylerythrol phosphate, GGPP: geranyl geranyl pyrophosphate, IPP: isopentenyl pyrophosphate, MVA: mevalonate.

Figure 5. Intestinal uptake and secretion pathways of carotenoids and phytosterols [55] [88] [111] [112]**.**

ABC: ATP-binding cassette, ACAT2: acyl-coenzyme A cholesterol acyltransferase 2, ApoB-48: apolipoprotein B48, CD36: cluster of differentiation 36, FA: fatty acid, HDL: high density lipoprotein, LXR: liver X receptor, MTP: mitochondrial transfer protein, NPC1L1: Niemann-Pick C1-like 1, PS-E: esterified phytosterol, SR-B1: scavenger receptor class B type 1, TAG: triacylglycerol.

Figure 6. The roles of microalgal xanthophylls and phytosterols in the regulation of biochemical parameters involved in chronic diseases.

ABC: ATP-binding cassette, Akt: protein kinase B, COX: cyclooxygenase, CYP: cytochrome P450, EGR: early growth factor, ERK: extracellular signal-regulated kinase, FAS: fatty acid synthase, FGF: fibroblast growth factor, FGFR: fibroblast growth factor receptor, GLUT: glucose transporter protein, GSH-Px: glutathione peroxidase, HO: heme oxygenase, IKK: I kappa B kinase, IL: interleukin, iNOS: inductible nitric oxide synthase, IRS: insulin receptor substrate, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, LXR: liver X receptor, MMP: matrix metalloproteinase, NF-κB: nuclear factor-kappa B, NO: nitric oxide, NPC1L1: Niemann-Pick C1 like 1, NQO: NADPH quinone oxidoreductase, Nrf2: nuclear factor erythroid-2 related factor 2, PI3K: phosphoinositide 3-kinase, PPAR: peroxisome proliferator-activated receptor, SCD: stearoyl CoA desaturase, SERBP: sterol regulatory element-binding protein, SOD: superoxide dismutase, STAT: signal transducer and activator of transcription, TNF: tumor necrosis factor, VEGF: vascular endothelial growth factor, VEGFR: vascular endothelial growth factor receptor.

Table 1.

Main microalgal producers of carotenoids and selected phytosterols [6,22,28,31].

Results are expressed in mg of total carotenoids per g dry weight of microalgal biomass * and in percentage of total sterols **. Main accessory carotenoids in bold.

Classes	**Carotenoids**	**Contents***	**Activities in human health**
Chlorophyceae *Prasinophyceae*	**α-, β-, γ-carotenes, lutein siphonaxanthin, siphonein,** antheraxanthin, astaxanthin, canthaxanthin, prasinoxanthin, neoxanthin, violaxanthin, zeaxanthin	0.02-291	Anti-cancer Anti-inflammatory Anti-oxidant Anti-obesity Cardiovascular
Bacillariophyceae *Prymnesiophyceae*	**β-carotene, fucoxanthin diatoxanthin, diadinoxanthin** cantaxanthin	0.11-26.6	

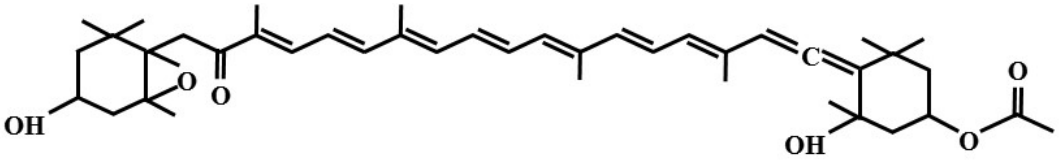
Classes	**Phytosterol**	**Contents****	**Activities in human health**
	Campesterol		
Chlorophyceae		2-48	Anti-angiogenic
Prasinophyceae		34-99	Anti-cancer Anti-oxidant
Bacillariophyceae		1-39	Cholesterol-lowering
Prymnesiophyceae		6-18	
	β-Sitosterol		
Chlorophyceae		1-33	Analgesic activity
Prasinophyceae		-	Anti-cancer Anti-inflammatory
Bacillariophyceae		1-95	Anti-mutagenic
Prymnesiophyceae		1-73	
	Stigmasterol		
Chlorophyceae		19-72	Anti-cancer
Prasinophyceae		-	Anti-oxidant Cholesterol-lowering
Bacillariophyceae		1-100	Hypoglycaemic
Prymnesiophyceae		10-47	

Table 2.

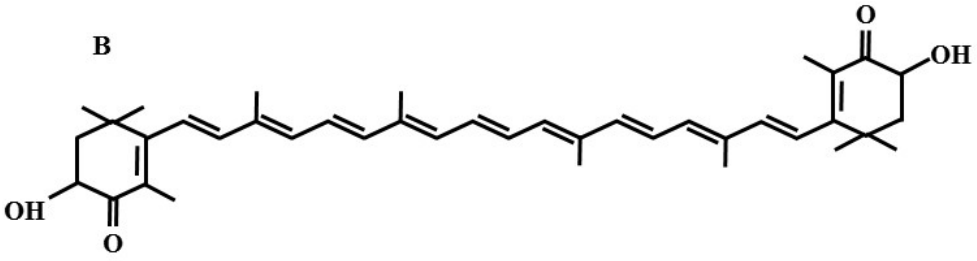
Main characteristics of selected bioactive molecules in microalgae [6,25,29,42,43].

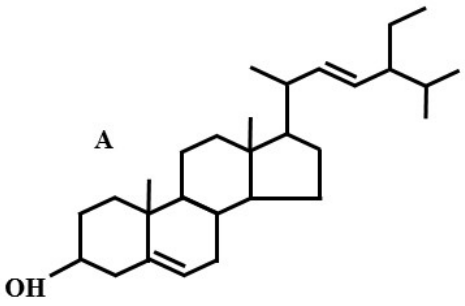
Carotenoids	**Chemical structure**	**Color**	**Detection Spectrophotometry (nm)**	**Location**	**Role**
Astaxanthin	$C_{40}H_{52}O_4$	Red	472 (methanol) 466 (hexane) 485 (chloroform)	Cytoplasm Lipid droplets	Photosynthetic apparatus
Fucoxanthin	$C_{42}H_{58}O_6$	Orange	449 (acetone) 453 (petroleum ether)	Chloroplast	Photosynthetic apparatus
Phytosterols	**Chemical structure**	**Systematic name**	**Detection Chromatography** (all sterols)	**Location**	**Role**
Campesterol	$C_{28}H_{48}O$	24α-methylcholest-5-en-3β-ol	Flame ionization detection Mass spectrometry UV 208 nm Diode array detection Evaporative light scattering detection	Membranes	Fluidity / Permability Brassinosteroid synthesis
β-Sitosterol	$C_{29}H_{50}O$	24β-ethylcholest-5-en-3β-ol		Membranes	Fluidity / Permeability Membrane-bound enzyme effector
Stigmasterol	$C_{29}H_{48}O$	24α-ethylcholesta-5,22E-dien-3β-ol		Membranes	Fluidity / Permeability Membrane-bound enzyme effector Cell proliferation

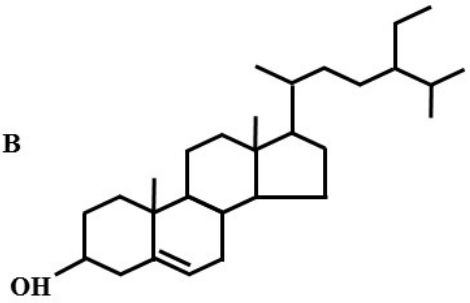
A

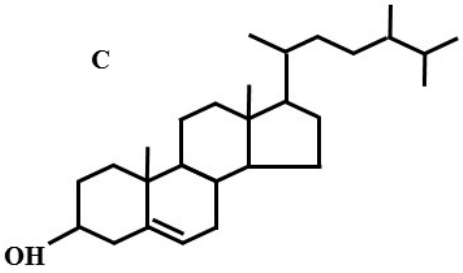


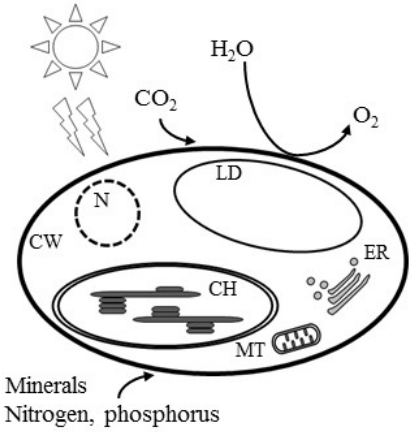
B



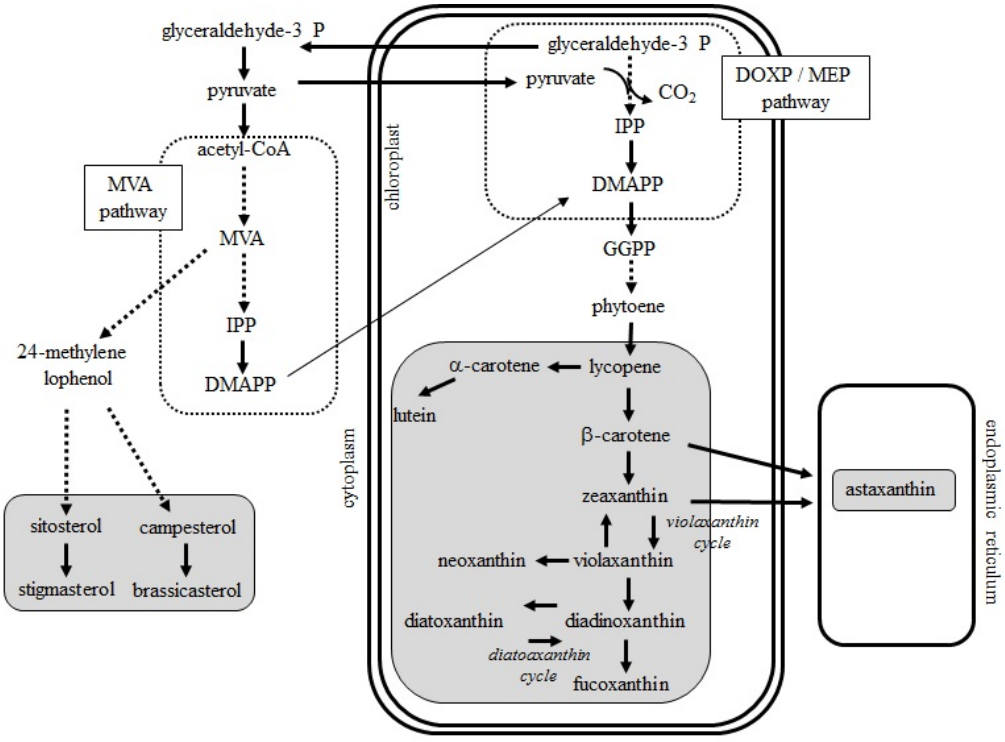


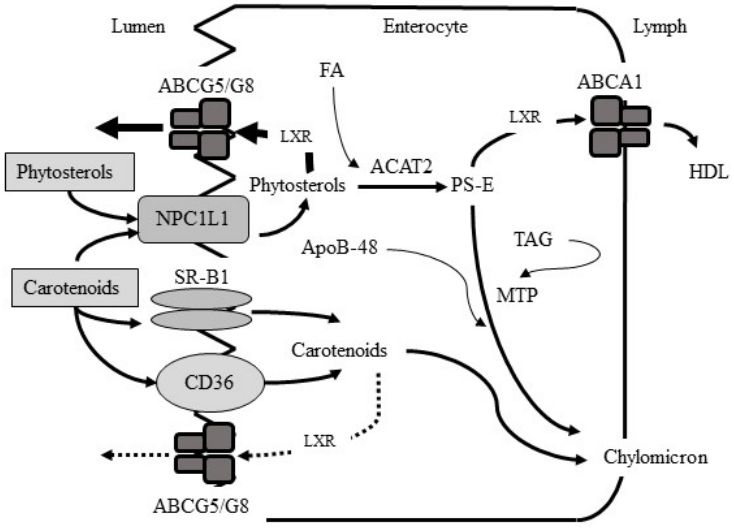


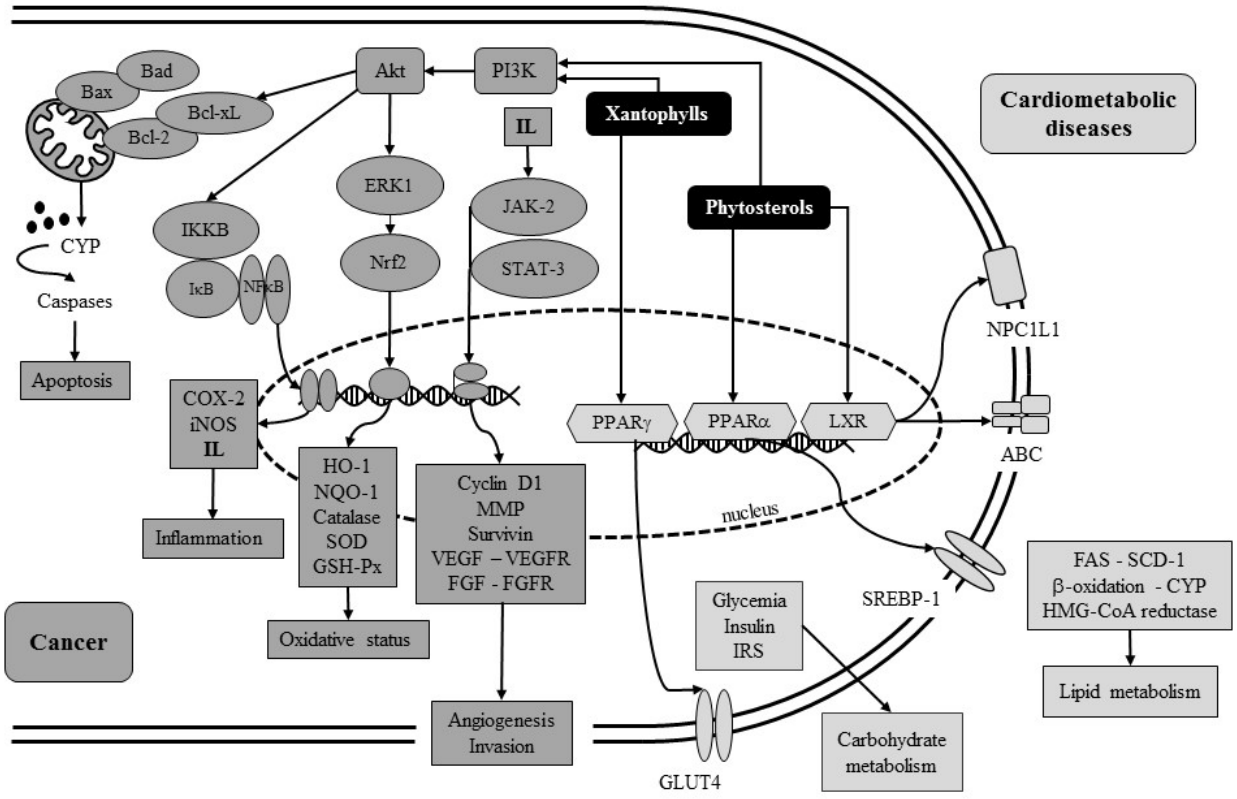




- Nitrogen compounds
  - Phycobiliproteins / Vitamins
- Phenolic compounds
  - Phenolic acids / Stilbenes
- Sterolic compounds
  - Sterols / Stanols
- Polysaccharides
  - Sulphated derived / β-glucans
- Carotenoids
  - Carotenes / Xantophylls
- Lipids
  - Glycerolipids / ω3 PUFA




Lumen
Enterocyte
Lymph
ABCG5/G8
FA
ABCA1
LXR
LXR
HDL
Phytosterols
ACAT2
Phytosterols
PS-E
NPC1L1
ApoB-48
TAG
MTP
Carotenoids
SR-B1
Carotenoids
CD36
LXR
Chylomicron
ABCG5/G8


Bad
Bax
Bcl-xL
Bcl-2
Akt
PI3K
IL
Xantophylls
Cardiometabolic diseases
ERK1
JAK-2
Phytosterols
IKKB
IκB
NFκB
Nrf2
STAT-3
CYP
Caspases
Apoptosis
NPC1L1
COX-2
iNOS
IL
PPARγ
PPARα
LXR
ABC
HO-1
NQO-1
Catalase
SOD
GSH-Px
Cyclin D1
MMP
Survivin
VEGF – VEGFR
FGF - FGFR
nucleus
Inflammation
FAS - SCD-1
β-oxidation - CYP
HMG-CoA reductase
Glycemia
Insulin
IRS
SREBP-1
Cancer
Oxidative status
Lipid metabolism
Angiogenesis
Invasion
GLUT4
Carbohydrate metabolism

Highlights

- Microalgae produce high added value molecules involved in human health and disease prevention
- Microalgal xantophylls and phytosterols regulate cell signaling pathways involved in chronic disease
- Astaxanthin and fucoxanthin exhibit anti-oxidant, hypolipidemic, anti-inflammatory and anti-tumoral activities
- Microalgal sterols regulate cholesterol and lipid metabolisms and possess anti-cancer activities