



**HAL**  
open science

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

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

### ► To cite this version:

Manon Le Goff, E. Le Ferrec, Claire Mayer, Virginie Mimouni, Dominique Lagadic-Gossmann, et al.. Microalgal carotenoids and phytosterols regulate biochemical mechanisms involved in human health and disease prevention. *Biochimie*, 2019, 167, pp.106-118. 10.1016/j.biochi.2019.09.012 . hal-02302332

**HAL Id: hal-02302332**

**<https://univ-lemans.hal.science/hal-02302332>**

Submitted on 19 Mar 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## 1 **Abstract**

2

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

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

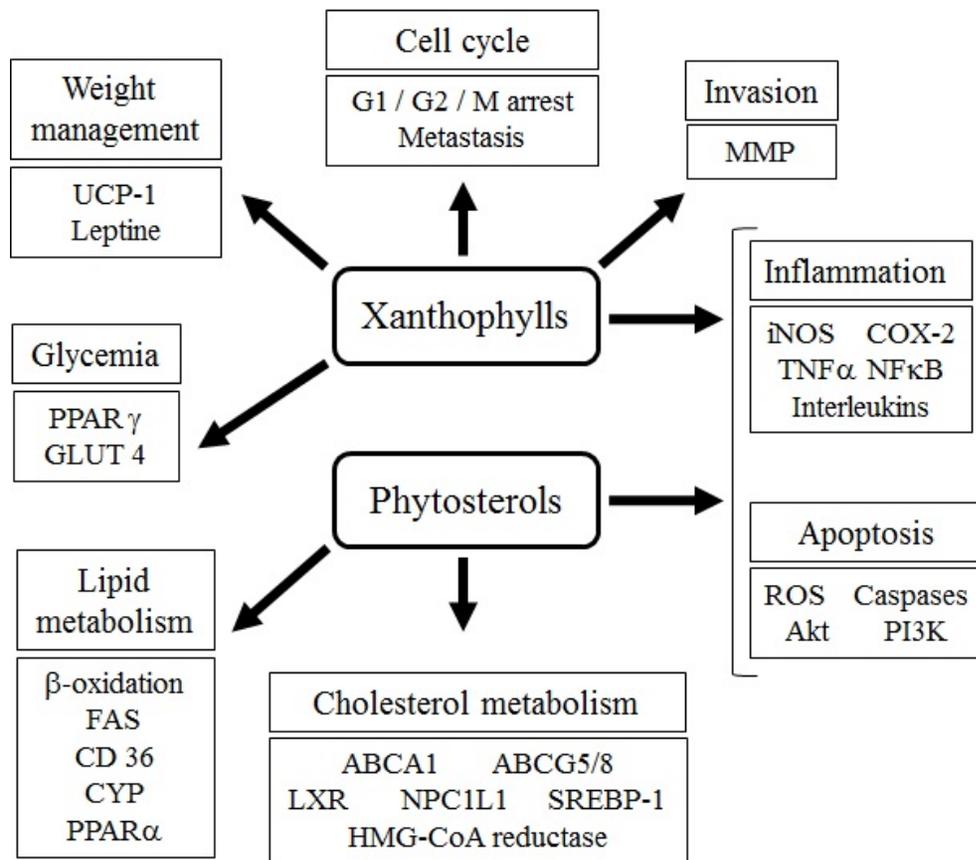
12 This review explains how these microalgal molecules modulate cell signaling pathways  
13 involved in carbohydrate and lipid metabolisms, inflammation, apoptosis, invasion and  
14 metastasis. Xanthophylls and phytosterols are involved in the reduction of inflammatory  
15 markers in relation with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

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

26 esterification. Their action are also related with the modulation of PPARs and sterol  
27 regulatory element-binding protein-1 activities.

28

Journal Pre-proof



1 **BIOCHIMIE conflict of interest declaration and author agreement form**

2 *Title of Paper:* **Microalgal carotenoids and phytosterols regulate biochemical mechanisms**  
3 **involved in human health and disease prevention**

4 *Author (s):* **Manon Le Goff, Eric Le Ferrec, Claire Mayer, Virginie Mimouni, Dominique**  
5 **Lagadic-Gossmann, Benoît Schoefs, Lionel Ulmann**

6

7 *Please delete one of the following two lines:*

8 We have no conflict of interest to declare.

9 ~~We have a competing interest to declare (please describe below):~~

10 This statement is to certify that all Authors have seen and approved the manuscript being submitted,  
11 and agree to the submission to *BIOCHIMIE*. We warrant that the article is the Authors' original work.  
12 We warrant that the article has not received prior publication, is not under consideration for  
13 publication elsewhere, and will not be submitted for publication elsewhere, in whole or in part, while  
14 under consideration for publication in *BIOCHIMIE*. On behalf of all Co-Authors, the corresponding  
15 Author shall bear full responsibility for the submission.

16 We attest to the fact that all Authors listed on the title page have contributed significantly to the  
17 work, have read the manuscript, attest to the validity and legitimacy of the data and their  
18 interpretation, and agree to its submission to *BIOCHIMIE*. We further attest that no other person has  
19 fulfilled the requirements for authorship as stated in the Elsevier Authorship-factsheet  
20 (2017\_ETHICS\_AUTH02 - attached), but is not included in the list of authors, and that no other  
21 person has contributed substantially to the writing of the manuscript but is not included either  
22 among the authors or in the acknowledgements.

23 All authors agree that no modification to the author list can be made without the written acceptance  
24 of all authors and the formal approval of the Editor-in-Chief. All authors accept that the Editor-in-  
25 Chief's decisions over acceptance or rejection or in the event of any breach of the Principles of  
26 Ethical Publishing in *BIOCHIMIE* being discovered, of retraction are final.

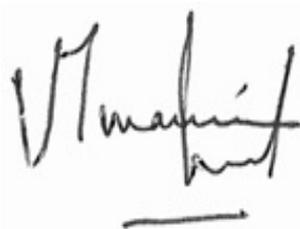
27

28 On behalf of all authors (*delete line if not appropriate*)

29 *Corresponding Author : Lionel ULMANN*

30 *3 september 2019*

31



A handwritten signature in black ink, appearing to read 'L. Ulmann', with a horizontal line underneath it.

32

33

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

36

37 **Manon Le Goff<sup>1</sup>, Eric Le Ferrec<sup>2</sup>, Claire Mayer<sup>1</sup>, Virginie Mimouni<sup>1</sup>, Dominique**  
38 **Lagadic-Gossmann<sup>2</sup>, Benoît Schoefs<sup>1</sup>, Lionel Ulmann<sup>1</sup>**

39

40 <sup>1</sup> EA 2160 Mer Molécules Santé - MIMMA, IUML FR-3473 CNRS, Le Mans Université,  
41 Laval F-53020, France

42 <sup>2</sup> Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail)  
43 - UMR\_S 1085, F-35000 Rennes, France

44

45 **Author contacts**

46 Manon Le Goff: [manon.le\\_goff@univ-lemans.fr](mailto:manon.le_goff@univ-lemans.fr)

47 Eric Le Ferrec: [eric.leferrec@univ-rennes1.fr](mailto:eric.leferrec@univ-rennes1.fr)

48 Claire Mayer: [Claire.Mayer@univ-lemans.fr](mailto:Claire.Mayer@univ-lemans.fr)

49 Virginie Mimouni: [Virginie.Mimouni@univ-lemans.fr](mailto:Virginie.Mimouni@univ-lemans.fr)

50 Dominique Lagadic-Gossmann: [dominique.lagadic@univ-rennes1.fr](mailto:dominique.lagadic@univ-rennes1.fr)

51 Benoît Schoefs: [Benoit.Schoefs@univ-lemans.fr](mailto:Benoit.Schoefs@univ-lemans.fr)

52 Lionel Ulmann: [Lionel.Ulmann@univ-lemans.fr](mailto:Lionel.Ulmann@univ-lemans.fr)

53

54 **\* Corresponding author:**

55 Lionel Ulmann

56 Mail: [Lionel.Ulmann@univ-lemans.fr](mailto:Lionel.Ulmann@univ-lemans.fr)

57 Tel: + 33-244-022-473 Fax: +33-243-594-958

58 EA 2160 Mer Molécules Santé - MIMMA

59 Le Mans Université

60 IUT - Dépt. Génie Biologique

61 Laval F-53020, France

62

63 **Declaration of interest:** none

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

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

92

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

Journal Pre-proof

95	<b>Contents</b>
96	
97	<b>1. Introduction</b>
98	<b>2. Bioactive molecules synthesized by microalgae</b>
99	2.1 Production of carotenoids by microalgae
100	2.2 Production of phytosterols by microalgae
101	2.3 Characteristics and synthesis of carotenoids
102	2.4 Characteristics and synthesis of phytosterols
103	<b>3. Bioavailability and absorption of carotenoids and phytosterols</b>
104	3.1 Carotenoids
105	3.1.1 Bioavailability
106	3.1.2 Mechanisms of absorption
107	3.2 Phytosterols
108	3.2.1 Bioavailability
109	3.2.2 Mechanisms of absorption
110	<b>4. Carotenoids and disease prevention</b>
111	4.1 Metabolic disease prevention
112	4.2 Anti-cancer and anti-angiogenic activities
113	4.3 Anti-inflammatory activities
114	4.4 Anti-oxidant activities
115	<b>5. Phytosterols and disease prevention</b>
116	5.1 Cholesterol-lowering activity and lipid metabolism
117	5.2 Anti-inflammatory activities
118	5.3 Anti-cancer activities
119	<b>6. Conclusion</b>

120	<b>7. Acknowledgements</b>
121	<b>8. Authors' contribution</b>
122	<b>9. References</b>

Journal Pre-proof

## 1. Introduction

123  
124  
125 For more than twenty five years, worldwide production of oilseed crops – soybean, peanut,  
126 rapeseed, sunflower, olive or coprah – has been increasing in order to answer the strong  
127 population growth in many countries in which food access is not easy, thereby resulting in  
128 caloric deficits and therefore precarious health status [1]. Recently, there has been an  
129 increased interest for the use of microalgae as new sources of bioactive molecules for animal  
130 and human nutrition, but also due to the growing awareness and search for healthier foods [2].  
131 Microalgae are able to produce numerous bioactive phytochemicals which major compounds  
132 are phycobiliproteins, polysaccharides, carotenoids and lipids [3]. Other molecules such as  
133 phenolic and sterolic compounds are also produced by microalgae. All these molecules are  
134 known to provide health benefits when they are used as food supplement for human nutrition.  
135 For this use, molecules have to be extracted from fresh, frozen, dried or freeze-dried  
136 microalgal biomass. Several extraction techniques can be used such as mechanical treatments,  
137 solvents, pressurized extraction, ultrasounds, pulsed electric field [4], resonance frequency [5]  
138 and microwaves. With the exception of polar carotenoids such as crocetin, carotenoids, as  
139 hydrophobic pigments, are extracted with organic solvents such as acetone, diethyl ether,  
140 ethanol and methanol [6]. Numerous studies have been conducted to optimize these extraction  
141 methods, by the use of combined methods with green solvents. For example, in the green  
142 microalga *Chlorella vulgaris*, it has been shown that the extraction of carotenoids was  
143 enhanced by the use of a mixture of ethanol and 2-methyltetrahydrofuran, a green solvent,  
144 coupled with high temperature (110°C) [7]. Specifically for the extraction of astaxanthin, a  
145 well-known bioactive carotenoid, it has been reported in the red phase *Haematococcus*  
146 *pluvialis*, that the use of acetone and ethanol, combined with mechanical pre-treatment to  
147 disrupt the hard double wall red cysts, was able to extract around 90% of total astaxanthin

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

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

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

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

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

194

## 195 **2. Bioactive molecules synthesized by microalgae**

196

197 Bioactive compounds are molecules that have functional properties for human health. Several  
198 of these functional ingredients could be used in food and pharmaceutical industries such as  
199 carotenoids, sterols, polyphenols or polyunsaturated fatty acids (PUFA). Microalgae have the  
200 advantage to produce these compounds but also other molecules such as vitamins, enzymes or  
201 PS that can also be used for commercial use. Microalgal primary metabolism, such as  
202 described in diatoms, requires different subcellular compartments as chloroplast,  
203 peroxisomes, mitochondria and cytoplasm involved in the Calvin-Benson cycle with the  
204 synthesis of glyceraldehyde-3-phosphate, fatty acid oxidation, the Krebs cycle and glycolysis  
205 and neoglucogenesis, respectively [21]. To produce all the molecules that can be used for  
206 nutrition and health, the microalgal metabolism requires light as the essential energy source  
207 but also water, CO<sub>2</sub>, and nutrients as nitrogen and phosphorus (Figure 3).

208

## 209 2.1 Production of carotenoids by microalgae

210 The fat-soluble carotenoids are divided into carotenes and xanthophylls. Total carotenoids in  
211 the different microalgae, range from 0.02 (*Scenedesmus obliquus*) to 291 mg/g dry weight  
212 (*Dunaliella salina*). Carotenes are represented by  $\alpha$ - and  $\beta$ -carotenes.  $\alpha$ -Carotene is present in  
213 *Chlorophyceae* and *Cryptophyceae* while  $\beta$ -carotene is present in all classes of microalgae [6].  
214 In addition to carotenes, *Chlorophytae* (*Chlorophyceae* and *Prasinophyceae*) produce lutein  
215 and siphonaxanthin as major xanthophylls, and minor pigments or pigments limited to some  
216 specific groups, as antheraxanthin, astaxanthin, canthaxanthin, prasinoxanthin, neoxanthin,  
217 violaxanthin, or/and zeaxanthin [22]. In addition to  $\beta$ -carotene, the *Bacillariophyceae* and  
218 *Prymnesiophyceae* groups produce mainly diadinoxanthin, diatoxanthin, and fucoxanthin  
219 (Table 1). Concerning fucoxanthin, its production in diatoms range from 0.07 to 26.6 mg/g  
220 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  $\beta$ -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

245 100% of stigmasterol while in *Asterionella glacialis*, the level of  $\beta$ -sitosterol is 95%.  
246 Campesterol is mainly found in the diatoms *Odontella aurita* and *Chaetoceros* sp. with levels  
247 from 18 to 38% of total sterols. A summary of the main classes of microalgae producing  
248 campesterol,  $\beta$ -sitosterol and stigmasterol, is given in Table 1. More detailed information  
249 concerning the main microalgal producers, are reported in an exhaustive review written by  
250 Volkman [29] and in Mimouni *et al.* [30].

251

### 252 2.3 Characteristics and synthesis of carotenoids

253 Carotenoids belong to the family of terpenoid compounds and has more than 750 members  
254 [31]. Carotenoids are represented by carotenes that are true hydrocarbons without any  
255 substituent in their structure, and by xanthophylls or oxycarotenoids, that contain oxygen  
256 atoms [32]. Carotenoids are C<sub>30</sub>-C<sub>50</sub> molecules characterized by an extended network of  
257 conjugated double bonds forming the chromophore, which absorbs visible light in the violet-  
258 green region. A consequence of the presence of double bonds is the abundant number of  
259 carotenoid isomers [33]. Thus, carotenoids may adopt several 3D-configurations that are  
260 important for their biological properties. For instance, *cis*-isomers of fucoxanthin have been  
261 reported to be more efficient than all-*trans*-isomers in human cancer lines [31]. Another  
262 consequence of the extended network of conjugated double bonds resides in their capacity to  
263 act as antioxidants [31]. Although the carotenoid diversity in microalgae is very large and in  
264 many cases specific of taxa, in addition to the regular carotenoids also found in land plants,  
265 microalgae can produce molecular species with unique chemical structures [34]. It is out of  
266 the scope of this review to describe here the different types of carotenoids present in  
267 microalgae; therefore, for more information, readers are directed towards excellent reviews on  
268 that specific topic [34].

269 Fucoxanthin (Figure 1A) is a carotenoid present in the chloroplast of most of the algae  
270 belonging to the Heterokonta. In the photosynthetic apparatus, fucoxanthin is associated to  
271 pigments and has an essential role in photon capture for photosynthesis. Like any carotenoid,  
272 fucoxanthin exhibits a polyene chain with a conjugated carbonyl, an epoxide and a hydroxyl  
273 groups. The presence of an allenic bond makes the structure of fucoxanthin unique (Figure  
274 1A). Altogether, these features confer to fucoxanthin a strong antioxidant capacity as well as a  
275 nonusual color for a carotenoid i.e. khaki color.

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

286 The first step in the carotenogenesis is the synthesis of isopentenyl pyrophosphate (IPP)  
287 through the mevalonate (MVA) pathway or the 1-deoxy-D-xylulose-5-phosphate (DOXP)  
288 pathway, that will be converted into geranyl geranyl pyrophosphate (GGPP) [40]. The  
289 carotenoid synthesis requires a condensing enzyme, the phytoene synthase, to produce the  
290 condensation of two molecules of GGPP to yield phytoene [41]. From this molecule, all the  
291 carotenoids will be synthesized, among which  $\beta$ -carotene, astaxanthin, lutein and  
292 xanthophylls as violaxanthin, diatoxanthin, and fucoxanthin through the xanthophyll cycles  
293 [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  $\beta$ -sitosterol, and campesterol (Table 2). All microalgae contain sterols dominated with a  $\Delta^5$   
309 double bond and no methyl group on C4. Stigmasterol and  $\beta$ -sitosterol (Figure 2) are C29  
310 sterols (C29:2 and C29:1, respectively), and their systematic names are 24 $\alpha$ -ethylcholesta-  
311 5,22E-dien-3 $\beta$ -ol and 24 $\beta$ -ethylcholest-5-en-3 $\beta$ -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  $\beta$ -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 24 $\alpha$ -methylcholest-5-en-3 $\beta$ -ol.  
317 Campesterol is more specifically found in chlorophytes [29].

318 Two metabolic pathways are involved in their biosynthesis. The MVA pathway and the  
319 methylerythritol phosphate (MEP) pathway [44]. The existence of these two metabolic  
320 pathways have been proved in numerous microalgae [45]. Microalgal-derived phytosterols are  
321 reported as 4-desmethyl- $\Delta$ 5-sterols, 4-desmethyl- $\Delta$ 7-sterols, 4-methyl-sterols and  
322 dihydroxylated sterols [46].

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

325

### 326 **3. Bioavailability and absorption of carotenoids and phytosterols**

327

#### 328 3.1 Carotenoids

329

##### 330 3.1.1 Bioavailability

331 The absorption of hydrophobic molecules as carotenoids, need different steps as follows:  
332 release from food matrix, lipid emulsion, solubilization into mixed micelles, uptake by  
333 enterocytes and secretion into lymphatic system [47]. Moreover, the increase of diet fat seems  
334 to enhance the absorption of carotenoids [48]. Carotenoid bioavailability depends on  
335 numerous dietary factors such as the source, food matrix, food processing or lipid levels but  
336 also depends on host-related factors, e.g. diseases, life-style habits age or genetic variations  
337 [49]. It is difficult to compare bioavailabilities of carotenoids from different plant sources as  
338 vegetables, fruits or microalgae because different *in vivo* and *in vitro* approaches have been  
339 used, even if high correlations have been found, thus stressing that estimating *in vitro*  
340 bioaccessibility (solubility/ micellarization) can be indicative of the amount available for

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

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

358

### 359 3.1.2 Mechanisms of absorption

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

365 membrane transporters as scavenger receptor class B type 1 (SR-B1), cluster of differentiation  
366 36 (CD36) and Niemann-Pick C1-like 1 (NPC1L1) [55]. Carotenoids are transported by  
367 lipoproteins with a specific accumulation of  $\beta$ -carotene in chylomicrons and very low density  
368 lipoproteins (VLDL), while xanthophylls are more specifically incorporated into LDL and  
369 high density lipoproteins (HDL) [40] (Figure 5). Beside liver, the main tissue target of  $\beta$ -  
370 carotene in which it will be accumulated is adipose tissue [56].

371

## 372 3.2 Phytosterols

373

### 374 3.2.1 Bioavailability

375 Campesterol,  $\beta$ -sitosterol and stigmasterol are the most common phytosterols, with a chemical  
376 structure resembling cholesterol one. As human cells do not synthesize them, phytosterol  
377 levels and activities depend on plant origin diet [57]. In human serum, phytosterol levels are  
378 hundred times lower than cholesterol ones, in a range of 3-20 mg/L. This can be partially  
379 explained by the fact that less than 10% of phytosterols are absorbed by comparison with the  
380 levels of absorbed cholesterol that are around 50-60% [58]. However, due to the poor  
381 solubility and bioavailability of free phytosterols, a minimum intake of 2-3 g/days is  
382 necessary for a cholesterol-lowering effect [27]. In various animal species, it has been  
383 reported an absorption ranging from 0% in rabbit to 4% in rat; and in human subjects fed with  
384 240 to 320 mg of sitosterol, the estimated absorption was from 1.5% to 5% [59].

385

### 386 3.2.2 Mechanisms of absorption

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

397

#### 398 4. Carotenoids and disease prevention

399

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

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

417

#### 418 4.1 Metabolic disease prevention

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

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

441 Fucoxanthin is also recognized to have an anti-obesity activity. Indeed, several mechanisms  
442 are regulated by this carotenoid in relation with weight loss and lipid metabolism. It has thus  
443 been reported that fucoxanthin decreased the mRNA expression of the fatty acid synthase  
444 (FAS) and inhibited the adipocyte uptake of glucose by reducing the phosphorylation of the  
445 insulin receptor substrate 1 (IRS-1). Its anti-obesity effects would also be due to the  
446 stimulation of the uncoupling protein-1 (UCP-1) expression in white adipose tissue.  
447 Thermogenesis and lipolysis are increased by the UCP-1 stimulation and also by the increased  
448 mRNA expression of the  $\beta$ 3-adrenergic receptor (Adrb3) [70]. This anti-obesity action is also  
449 related to a decrease in plasma leptin levels and to a down-regulation of the liver stearoyl-  
450 CoA desaturase-1 (SCD1), a rate limiting step in the saturated and monounsaturated fatty  
451 acids. This alteration of fatty acid composition has been proposed to stem from leptin  
452 signaling in mice [71]. This anti-obesity effect is also due to the action of fucoxanthin and its  
453 derivatives on lipid metabolism. Fucoxanthin has been reported to regulate the hydroxyl methyl  
454 glutaryl CoA (HMG-CoA), acyl-CoA cholesterol acyltransferase (ACAT), lecithin-  
455 cholesterol acyltransferase (LCAT) and carnitine palmitoyl-transferase (CPT-1) activities as  
456 well as sterol regulatory element-binding protein-1 (SREBP-1) [70].

457

458 4.2 Anti-cancer and anti-angiogenic activities

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

470 In relation with these observations, molecular targets of astaxanthin have been proposed that  
471 would explain its role in cancer prevention. The activation of the caspase-mediated  
472 mitochondrial apoptotic pathway would be due to the inhibition of the NF- $\kappa$ B signaling  
473 pathway and to the down-regulation of the key regulatory kinases, I kappa B kinase (IKK $\beta$ )  
474 and glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) [72]. The anti-proliferative and anti-angiogenic  
475 effects of astaxanthin have been related to the inhibition of the JAK-2 (Janus kinase-2)/STAT-  
476 3 (signal transducers and activators of transcription-3) signaling with an inhibition of STAT-3  
477 phosphorylation and its nuclear translocation leading to down-regulation of STAT-3 target  
478 genes involved in cell proliferation (cyclin D1), invasion (MMP) and angiogenesis (vascular  
479 endothelial growth factor (VEGF) and its receptor VEGFR-2) [73]. Among the signaling  
480 pathways regulating cell survival, the phosphoinositide 3-kinase PI3K/Akt has an important  
481 role. Astaxanthin decreased the phosphorylation of AKT to induce apoptosis. Other molecular  
482 targets such as mitogen-activated protein kinases (MAPKs), PPAR $\gamma$  and nuclear factor

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

485 Fucoxanthin seems to have a better anti-cancer activity compared with lycopene,  $\beta$ -carotene  
486 or astaxanthin. Indeed, in the human leukemia HL-60 cell line, only fucoxanthin induced high  
487 levels of DNA fragmentation. In this study, the authors stated that the apoptosis induced by  
488 fucoxanthin was related to the generation of ROS, leading to cytotoxicity involving the  
489 cleavage of caspases-3 and -9 [75]. In prostate cancer cell lines, fucoxanthin and its  
490 metabolite fucoxanthinol have been reported to inhibit cell-growth rate [76] and to induce  
491 apoptosis in prostate cancer PC-3 cells by activating caspase-3 [77]. Fucoxanthin and  
492 fucoxanthinol have also been reported to induce cell-cycle arrest in numerous human tumor  
493 cell lines by modulating expression of various molecules and signal transduction pathways  
494 [78]. In osteosarcoma, they induced G<sub>1</sub> cell cycle arrest by reducing the expression of cyclin-  
495 dependent kinases. In this bone tumor, fucoxanthin and fucoxanthinol also induced apoptosis  
496 with a reduced expression of survivin, an X-linked inhibitor of apoptosis protein, and of the  
497 anti-apoptotic genes *Bcl-2* and *Bcl-xL*. The resulting apoptosis is associated with caspase  
498 activation and with an inhibition of the Akt pathway. Migration and invasion inhibitions have  
499 been associated with the reduced MMP-1 expression and the activator protein-1 signal [79].  
500 Studies reported in human umbilical vein endothelial cells (HUVEC) have demonstrated the  
501 anti-angiogenic activity of fucoxanthin and fucoxanthinol. Indeed, it has been shown a  
502 suppression of the growth of microvessels [80] and a down-regulation of the fibroblast growth  
503 factor-2 (FGF-2), its receptor FGFR-1 as well as of the factor of transcription early growth  
504 response protein 1 (EGR-1). In this study, it has also been shown that fucoxanthin hampered  
505 the phosphorylation of signaling proteins such as extracellular signal-regulated kinases (ERK)  
506 and Akt [81].

507

## 508 4.3 Anti-inflammatory activities

509 The anti-inflammatory effects of astaxanthin have been reported in numerous *in vitro* and *in*  
510 *vivo* studies on lipopolysaccharide (LPS)-induced inflammatory reactions, with suppression of  
511 the production of NO, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), interleukin (IL)-1 $\beta$  and tumor necrosis factor  
512 alpha (TNF- $\alpha$ ), and prevention of the expression of inducible nitric oxide synthase (iNOS)  
513 and cyclooxygenase (COX)-2. All these results have been related with the inhibition of the  
514 NF- $\kappa$ B signaling pathway. The same results have been observed in LPS-stimulated murine  
515 macrophages, in which fucoxanthin was able to reduce levels of pro-inflammatory molecules  
516 such as NO, PGE<sub>2</sub>, IL-1 $\beta$ /6, TNF- $\alpha$ , by the suppression of the NF- $\kappa$ B but also by the  
517 inhibition of the MAPK pathways signaling [75].

518

## 519 4.4 Anti-oxidant activities

520 Astaxanthin scavenges free radicals and other antioxidants to protect lipids from peroxidation.  
521 It inhibits H<sub>2</sub>O<sub>2</sub>-mediated activation of the transcription factor NF- $\kappa$ B that is involved in the  
522 expression of heme oxygenase-1 (HO-1) and iNOS engaged against oxidative stress [82];  
523 subsequently, the production of pro-inflammatory cytokines was blocked through the increase  
524 of tyrosine phosphatase-1 expression [83]. In brain, the use of astaxanthin has been reported  
525 to protect from oxidative stress with a decreased level of MDA and NO and increased  
526 activities of catalase and of superoxide dismutase (SOD) and higher glutathione (GSH) levels  
527 [84]. It also protects the steroidogenesis from oxidative stress with a prevention in the down-  
528 regulation of the steroidogenic acute regulatory protein (StAR), a protein involved in the  
529 transport of cholesterol. Indeed, during oxidative stress, the protein kinase A (PKA) pathway  
530 is attenuated, leading to the suppression of the expression of StAR; administration of  
531 astaxanthin acts on the PKA pathway, thereby restoring the StAR expression [85].

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

537

## 538 **5. Phytosterols and disease prevention**

539

### 540 **5.1 Cholesterol-lowering activity and lipid metabolism**

541 Phytosterols have been reported to interact with the cholesterol absorption through inhibitory  
542 mechanisms. Among suggested mechanisms, it has been proposed the release of cholesterol  
543 from mixed micelles, the modification of the gene expression of NPC1L1 and of ABCG5 and  
544 ABCG8 transporters, or the decrease of the intestinal cholesterol esterification rate [88]. The  
545 selective binding of sitosterol to the ABCG5 and ABCG8 transporters involved in the  
546 regulation of liver and intestinal sterol absorption and secretion, is related to blood cholesterol  
547 lowering [89]. Microalgal phytosterols as campesterol and  $\beta$ -sitosterol are also involved in  
548 cholesterol level decrease through the down-regulation of hepatic HMG-CoA reductase and  
549 the stimulation of the LDL-C receptors, thus facilitating the removal of plasma cholesterol  
550 [90]. Moreover,  $\beta$ -sitosterol has a competing activity with NPC1L1 [91]. LXRs are nuclear  
551 receptors involved in the regulation of lipid metabolism. Even if the main function of LXRs is  
552 the regulation of cholesterol metabolism, their activation inhibits inflammation, autoimmune  
553 reactions and atherogenesis [92]. The treatment of intestinal cells with phytosterols, like  
554 campesterol and  $\beta$ -sitosterol, would lead to an increase in the expression of LXR target genes,  
555 suggesting a ligand action of these molecules on LXRs. Specifically, phytosterols increase the  
556 ABCA1 expression and decrease cholesterol absorption [93]. In rat, it has been shown that the

557 incorporation of  $\beta$ -sitosterol into liver membranes, decreased its fluidity while an increase in  
558 liver desaturases ( $\Delta 9$ ,  $\Delta 6$  and  $\Delta 5$  desaturases) was observed, probably as a compensatory  
559 mechanism for the decreased fluidity [94] [57].

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

569 In human pancreatic islet and in the INS-1 insulinoma cell line, the use of stigmasterol  
570 prevents the increase of both cholesterol and ROS levels induced by glucolipototoxicity. In this  
571 study, stigmasterol has been shown to decrease free cholesterol levels resulting from  
572 glucolipototoxicity, with a real potential to protect pancreatic cells during diabetes progression  
573 [96].

574 Recently, in mice fed high-fat western-style diet, it has been reported that stigmasterol and  $\beta$ -  
575 sitosterol were able to attenuate the nonalcoholic fatty liver disease (NAFLD) and to alter  
576 lipid metabolism. Administered at a dose corresponding to what is suggested for human, it has  
577 been shown an amelioration of high fat diet-induced fatty liver, including liver total lipid,  
578 triacylglycerol and cholesterol levels. Moreover, both phytosterols were able to decrease the  
579 intestinal bile acid levels. Analyses of gene expressions have shown a prevention of the  
580 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 PPAR $\alpha$  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- $\alpha$ , IL-6, IL-10) [98].  $\beta$ -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- $\kappa$ B, 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- $\kappa$ B 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].  $\beta$ -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-

604 231 cells, this phytosterol has been shown to inhibit G0/G1 cell cycle associated with  
605 induction of apoptosis. The use of  $\beta$ -sitosterol indeed induced the depolarization of  
606 mitochondrial membrane potential and also increased the *Bax/Bcl-2* ratio in this human breast  
607 cancer line [104]. The Fas/CD95 apoptotic pathway in human breast cancer has also been  
608 suggested to be affected by  $\beta$ -sitosterol. Indeed, its incorporation in cell membranes induced  
609 an increase in Fas protein levels, and caspase-8 activity, resulting in an inhibition of tumor  
610 cell growth [105].

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

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

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

629

## 630 **6. Conclusion**

631

632 The results reported in this review explain how microalgae could be promising food  
633 supplement for health and disease prevention. The whole cell can be used in nutrition to  
634 provide a mixture of molecules that possess preventive effects towards several chronic  
635 diseases; on the other hand, extracted molecules could also be proposed for targeting specific  
636 effects. In this review, the effects of microalgal  $\omega$ 3 PUFA have not been discussed but their  
637 high amounts contained in microalgae help in the efficiency of these microorganisms in  
638 cardiometabolic disease prevention and anti-tumoral activities. Fish oils provide high levels of  
639  $\omega$ 3 PUFA but the advantage of microalgae is that they also provide molecules such as  
640 carotenoids and sterols that have obvious health beneficial effects. At present, few microalgae  
641 can be used in human nutrition because cytotoxicity tests must be conducted prior to  
642 agreement by food authorities. However, as emphasized in this review, extracted molecules  
643 might be directly used for nutrition in order to act specifically on cell signaling pathways and  
644 thus, might be considered not only as preventive agents but also as therapeutic agents.

645

## 646 **7. Acknowledgements**

647

648 This work was jointly funded by the Collectivités Locales Mayennaises and the French  
649 Ministère de l'Enseignement Supérieur de la Recherche et de l'Innovation.

650

## 651 **8. Authors' contribution**

652

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

661

## 662 **9. References**

663

- 664 [1] R. L. Naylor, 'Oil crops, aquaculture, and the rising role of demand: A fresh perspective on food  
665 security', *Glob. Food Secur.*, vol. 11, pp. 17–25, Dec. 2016.
- 666 [2] A. Molino *et al.*, 'Microalgae Characterization for Consolidated and New Application in Human  
667 Food, Animal Feed and Nutraceuticals', *Int. J. Environ. Res. Public. Health*, vol. 15, no. 11, p. 2436,  
668 Nov. 2018.
- 669 [3] R. H. Liu, 'Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action', *J.*  
670 *Nutr.*, vol. 134, no. 12, p. 3479S–3485S, Dec. 2004.
- 671 [4] V. Vinayak *et al.*, 'Diatom Milking: A Review and New Approaches', *Mar. Drugs*, vol. 13, no. 5, pp.  
672 2629–2665, May 2015.
- 673 [5] V. Vinayak, V. Kumar, M. Kashyap, K. B. Joshi, R. Gordon, and B. Schoefs, 'Fabrication of  
674 resonating microfluidic chamber for biofuel production in diatoms (Resonating device for biofuel  
675 production)', in *2016 3rd International Conference on Emerging Electronics (ICEE)*, 2016, pp. 1–6.
- 676 [6] M. Morançais, J.-L. Mouget, and J. Dumay, 'Proteins and Pigments', in *Microalgae in Health and*  
677 *Disease Prevention*, I. A. Levine and J. Fleurence, Eds. Academic Press, 2018, pp. 145–175.
- 678 [7] E. Damergi, J.-P. Schwitzguébel, D. Refardt, S. Sharma, C. Holliger, and C. Ludwig, 'Extraction of  
679 carotenoids from *Chlorella vulgaris* using green solvents and syngas production from residual  
680 biomass', *Algal Res.*, vol. 25, pp. 488–495, 2017.

- 681 [8] A. Molino *et al.*, 'Extraction of astaxanthin from microalga *Haematococcus pluvialis* in red phase  
682 by using generally recognized as safe solvents and accelerated extraction', *J. Biotechnol.*, vol.  
683 283, pp. 51–61, Oct. 2018.
- 684 [9] S.-A. Choi *et al.*, 'High-efficiency cell disruption and astaxanthin recovery from *Haematococcus*  
685 *pluvialis* cyst cells using room-temperature imidazolium-based ionic liquid/water mixtures',  
686 *Bioresour. Technol.*, vol. 274, pp. 120–126, Feb. 2019.
- 687 [10] G. D. Sanzo *et al.*, 'Supercritical Carbon Dioxide Extraction of Astaxanthin, Lutein, and Fatty Acids  
688 from *Haematococcus pluvialis* Microalgae', *Mar. Drugs*, vol. 16, no. 9, p. 334, Sep. 2018.
- 689 [11] M. Mubarak, A. Shaija, and T. V. Suchithra, 'A review on the extraction of lipid from microalgae  
690 for biodiesel production', *Algal Res.*, vol. 7, pp. 117–123, Jan. 2015.
- 691 [12] M. Coué *et al.*, 'Spirulina Liquid Extract Protects against Fibrosis Related to Non-Alcoholic  
692 Steatohepatitis and Increases Ursodeoxycholic Acid', *Nutrients*, vol. 11, no. 1, p. 194, Jan. 2019.
- 693 [13] M. F. de Jesus Raposo, A. M. M. B. de Morais, and R. M. S. C. de Morais, 'Bioactivity and  
694 Applications of Polysaccharides from Marine Microalgae', in *Polysaccharides: Bioactivity and*  
695 *Biotechnology*, K. G. Ramawat and J.-M. Mérillon, Eds. Cham: Springer International Publishing,  
696 2015, pp. 1683–1727.
- 697 [14] M. F. de J. Raposo *et al.*, 'Bioactivity and Applications of Sulphated Polysaccharides from Marine  
698 Microalgae', *Mar. Drugs*, vol. 11, no. 1, pp. 233–252, Jan. 2013.
- 699 [15] E. M. Marinova and N. V. L. Yanishlieva, 'Inhibited Oxidation of Lipids II: Comparison of the  
700 Antioxidative Properties of Some Hydroxy Derivatives of Benzoic and Cinnamic Acids', *Lipid Fett*,  
701 vol. 94, no. 11, pp. 428–432, Jan. 1992.
- 702 [16] M. F. de J. Raposo and A. M. M. B. de Morais, 'Microalgae for the prevention of cardiovascular  
703 disease and stroke', *Life Sci.*, vol. 125, pp. 32–41, Mar. 2015.
- 704 [17] A. Haimeur *et al.*, 'The role of *Odontella aurita*, a marine diatom rich in EPA, as a dietary  
705 supplement in dyslipidemia, platelet function and oxidative stress in high-fat fed rats', *Lipids*  
706 *Health Dis.*, vol. 11, no. 1, p. 147, Oct. 2012.
- 707 [18] A. Haimeur *et al.*, 'Fish Oil and Microalga Omega-3 as Dietary Supplements: A Comparative Study  
708 on Cardiovascular Risk Factors in High-Fat Fed Rats', *Lipids*, vol. 51, no. 9, pp. 1037–1049, Sep.  
709 2016.
- 710 [19] J.-Y. Cherng and M.-F. Shih, 'Preventing dyslipidemia by *Chlorella pyrenoidosa* in rats and  
711 hamsters after chronic high fat diet treatment', *Life Sci.*, vol. 76, no. 26, pp. 3001–3013, May  
712 2005.
- 713 [20] K. Nuño, A. Villarruel-López, A. M. Puebla-Pérez, E. Romero-Velarde, A. G. Puebla-Mora, and F.  
714 Ascencio, 'Effects of the marine microalgae *Isochrysis galbana* and *Nannochloropsis oculata* in  
715 diabetic rats', *J. Funct. Foods*, vol. 5, no. 1, pp. 106–115, Jan. 2013.
- 716 [21] Heydarizadeh Parisa *et al.*, 'Response of CO<sub>2</sub>-starved diatom *Phaeodactylum tricornutum* to light  
717 intensity transition', *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 372, no. 1728, p. 20160396, Sep. 2017.
- 718 [22] D. B. Stengel, S. Connan, and Z. A. Popper, 'Algal chemodiversity and bioactivity: Sources of  
719 natural variability and implications for commercial application', *Biotechnol. Adv.*, vol. 29, no. 5,  
720 pp. 483–501, Sep. 2011.
- 721 [23] R. Sathasivam, R. Radhakrishnan, A. Hashem, and E. F. Abd\_Allah, 'Microalgae metabolites: A  
722 rich source for food and medicine', *Saudi J. Biol. Sci.*, vol. 26, no. 4, pp. 709–722, 2019.
- 723 [24] D. Pelah, A. Sintov, and E. Cohen, 'The Effect of Salt Stress on the Production of Canthaxanthin  
724 and Astaxanthin by *Chlorella zofingiensis* Grown Under  
725 Limited Light Intensity', *World J. Microbiol. Biotechnol.*, vol. 20, no. 5, pp. 483–486, Jul. 2004.
- 726 [25] W. A. Stirk *et al.*, 'Hormone profiles in microalgae: Gibberellins and brassinosteroids', *Plant*  
727 *Physiol. Biochem.*, vol. 70, pp. 348–353, Sep. 2013.
- 728 [26] E. Ryckebosch, C. Bruneel, R. Termote-Verhalle, K. Muylaert, and I. Foubert, 'Influence of  
729 extraction solvent system on extractability of lipid components from different microalgae  
730 species', *Algal Res.*, vol. 3, pp. 36–43, Jan. 2014.
- 731 [27] X. Luo, P. Su, and W. Zhang, 'Advances in Microalgae-Derived Phytosterols for Functional Food  
732 and Pharmaceutical Applications', *Mar. Drugs*, vol. 13, no. 7, pp. 4231–4254, Jul. 2015.

- 733 [28] J. K. Volkman, 'A review of sterol markers for marine and terrigenous organic matter', *Org.*  
734 *Geochem.*, vol. 9, no. 2, pp. 83–99, Jan. 1986.
- 735 [29] J. K. Volkman, 'Sterols in Microalgae', in *The Physiology of Microalgae*, M. A. Borowitzka, J.  
736 Beardall, and J. A. Raven, Eds. Cham: Springer International Publishing, 2016, pp. 485–505.
- 737 [30] V. Mimouni, A. Couzinet-Mossion, L. Ulmann, and G. Wielgosz-Collin, 'Lipids From Microalgae', in  
738 *Microalgae in Health and Disease Prevention*, I. A. Levine and J. Fleurence, Eds. Academic Press,  
739 2018, pp. 109–131.
- 740 [31] H. Gateau, K. Solymosi, J. Marchand, and B. Schoefs, 'Carotenoids of Microalgae Used in Food  
741 Industry and Medicine', 2017. [Online]. Available:  
742 <https://www.ingentaconnect.com/contentone/ben/mrmc/2017/00000017/00000013/art00005>.  
743 [Accessed: 23-Apr-2019].
- 744 [32] K. J. M. Mulders, P. P. Lamers, D. E. Martens, and R. H. Wijffels, 'Phototrophic pigment  
745 production with microalgae: biological constraints and opportunities', *J. Phycol.*, vol. 50, no. 2,  
746 pp. 229–242, Apr. 2014.
- 747 [33] P. Heydarizadeh *et al.*, 'Plastids of Marine Phytoplankton Produce Bioactive Pigments and Lipids',  
748 *Mar. Drugs*, vol. 11, no. 9, pp. 3425–3471, Sep. 2013.
- 749 [34] E. S. Egeland, 'Carotenoids', in *The Physiology of Microalgae*, M. A. Borowitzka, J. Beardall, and J.  
750 A. Raven, Eds. Cham: Springer International Publishing, 2016, pp. 507–563.
- 751 [35] Y. Lemoine and B. Schoefs, 'Secondary ketocarotenoid astaxanthin biosynthesis in algae: a  
752 multifunctional response to stress', *Photosynth. Res.*, vol. 106, no. 1–2, pp. 155–177, Nov. 2010.
- 753 [36] J. Kopecky, B. Schoefs, K. Loest, D. Stys, and O. Pulz, 'Microalgae as a source for secondary  
754 carotenoid production: a screening study', *Algological Studies*, pp. 153–168, 2000.
- 755 [37] A. Solovchenko and K. Neverov, 'Carotenogenic response in photosynthetic organisms: a colorful  
756 story', *Photosynth. Res.*, vol. 133, no. 1–3, pp. 31–47, Sep. 2017.
- 757 [38] B. Schoefs, N.-E. Rmiki, J. Rachadi, and Y. Lemoine, 'Astaxanthin accumulation in *Haematococcus*  
758 requires a cytochrome P450 hydroxylase and an active synthesis of fatty acids', *FEBS Lett.*, vol.  
759 500, no. 3, pp. 125–128, Jul. 2001.
- 760 [39] Y. Lemoine, N. E. Rmiki, A. Creach, J. Rachidi, and B. Schoefs, 'Cytoplasmic accumulation of  
761 astaxanthin by the green alga *Haematococcus pluvialis* (floc) in Plant Cell Organelles- Selected  
762 topics', in *Plant Cell Organelles- Selected topics*, B. Schoefs (ed.), Ed. Research Signpost Publ.,  
763 Kerala, India. (ISBN: 978-81-308-0104-9), 2008, pp. 251–284.
- 764 [40] M. F. de J. Raposo *et al.*, 'Carotenoids from Marine Microalgae: A Valuable Natural Source for the  
765 Prevention of Chronic Diseases', *Mar. Drugs*, vol. 13, no. 8, pp. 5128–5155, Aug. 2015.
- 766 [41] R. Sathasivam and J.-S. Ki, 'A Review of the Biological Activities of Microalgal Carotenoids and  
767 Their Potential Use in Healthcare and Cosmetic Industries', *Mar. Drugs*, vol. 16, no. 1, p. 26, Jan.  
768 2018.
- 769 [42] M. J. Lagarda, G. García-Llatas, and R. Farré, 'Analysis of phytosterols in foods', *Nutraceuticals*  
770 *Anal.*, vol. 41, no. 5, pp. 1486–1496, Aug. 2006.
- 771 [43] M.-A. Hartmann, 'Plant sterols and the membrane environment', *Trends Plant Sci.*, vol. 3, no. 5,  
772 pp. 170–175, May 1998.
- 773 [44] J. Volkman, 'Sterols in microorganisms', *Appl. Microbiol. Biotechnol.*, vol. 60, no. 5, pp. 495–506,  
774 Jan. 2003.
- 775 [45] M. Lohr, J. Schwender, and J. E. W. Polle, 'Isoprenoid biosynthesis in eukaryotic phototrophs: A  
776 spotlight on algae', *Plant Sci.*, vol. 185–186, pp. 9–22, Apr. 2012.
- 777 [46] S. W. Rampen, B. A. Abbas, S. Schouten, and J. S. Sinninghe Damste, 'A comprehensive study of  
778 sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom  
779 productivity', *Limnol. Oceanogr.*, vol. 55, no. 1, pp. 91–105, Jan. 2010.
- 780 [47] R. K. Saini, S. H. Nile, and S. W. Park, 'Carotenoids from fruits and vegetables: Chemistry, analysis,  
781 occurrence, bioavailability and biological activities', *Food Res. Int.*, vol. 76, pp. 735–750, Oct.  
782 2015.
- 783 [48] L. Yonekura and A. Nagao, 'Intestinal absorption of dietary carotenoids', *Mol. Nutr. Food Res.*,  
784 vol. 51, no. 1, pp. 107–115, Jan. 2007.

- 785 [49] C. Desmarchelier and P. Borel, 'Overview of carotenoid bioavailability determinants: From  
786 dietary factors to host genetic variations', *Food Bioact. Evid. Health Benefits Underst. Mech.*, vol.  
787 69, pp. 270–280, Nov. 2017.
- 788 [50] T. Bohn *et al.*, 'Mind the gap—deficits in our knowledge of aspects impacting the bioavailability  
789 of phytochemicals and their metabolites—a position paper focusing on carotenoids and  
790 polyphenols', *Mol. Nutr. Food Res.*, vol. 59, no. 7, pp. 1307–1323, Jul. 2015.
- 791 [51] J. M. Carbonell-Capella, M. Buniowska, F. J. Barba, M. J. Esteve, and A. Frígola, 'Analytical  
792 Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits  
793 and Vegetables: A Review', *Compr. Rev. Food Sci. Food Saf.*, vol. 13, no. 2, pp. 155–171, Mar.  
794 2014.
- 795 [52] A. Ranga Rao, V. Baskaran, R. Sarada, and G. A. Ravishankar, 'In vivo bioavailability and  
796 antioxidant activity of carotenoids from microalgal biomass — A repeated dose study', *Food Res.*  
797 *Int.*, vol. 54, no. 1, pp. 711–717, Nov. 2013.
- 798 [53] J.-P. Yuan, J. Peng, K. Yin, and J.-H. Wang, 'Potential health-promoting effects of astaxanthin: A  
799 high-value carotenoid mostly from microalgae', *Mol. Nutr. Food Res.*, vol. 55, no. 1, pp. 150–165,  
800 Jan. 2011.
- 801 [54] A. Ranga Rao, R. L. Raghunath Reddy, V. Baskaran, R. Sarada, and G. A. Ravishankar,  
802 'Characterization of Microalgal Carotenoids by Mass Spectrometry and Their Bioavailability and  
803 Antioxidant Properties Elucidated in Rat Model', *J. Agric. Food Chem.*, vol. 58, no. 15, pp. 8553–  
804 8559, Aug. 2010.
- 805 [55] E. Reboul, 'Absorption of Vitamin A and Carotenoids by the Enterocyte: Focus on Transport  
806 Proteins', *Nutrients*, vol. 5, no. 9, pp. 3563–3581, Sep. 2013.
- 807 [56] A. Dembinska-Kiec, 'Carotenoids: risk or benefit for health', *Carotenoids Diet. Lipids*, vol. 1740,  
808 no. 2, pp. 93–94, May 2005.
- 809 [57] N. Shahzad *et al.*, 'Phytosterols as a natural anticancer agent: Current status and future  
810 perspective', *Biomed. Pharmacother.*, vol. 88, pp. 786–794, Apr. 2017.
- 811 [58] R. E. Ostlund *et al.*, 'Gastrointestinal absorption and plasma kinetics of soy delta5-phytosterols  
812 and phytostanols in humans', *Am. J. Physiol.-Endocrinol. Metab.*, vol. 282, no. 4, pp. E911–E916,  
813 Apr. 2002.
- 814 [59] D. Kritchevsky and S. C. Chen, 'Phytosterols—health benefits and potential concerns: a review',  
815 *Nutr. Res.*, vol. 25, no. 5, pp. 413–428, May 2005.
- 816 [60] H. Gylling *et al.*, 'Plant sterols and plant stanols in the management of dyslipidaemia and  
817 prevention of cardiovascular disease', *Atherosclerosis*, vol. 232, no. 2, pp. 346–360, Feb. 2014.
- 818 [61] T. Sudhop, D. Lütjohann, and K. von Bergmann, 'Sterol transporters: targets of natural sterols  
819 and new lipid lowering drugs', *Pharmacol. Ther.*, vol. 105, no. 3, pp. 333–341, Mar. 2005.
- 820 [62] T. Sudhop *et al.*, 'Comparison of the hepatic clearances of campesterol, sitosterol, and  
821 cholesterol in healthy subjects suggests that efflux transporters controlling intestinal sterol  
822 absorption also regulate biliary secretion', *Gut*, vol. 51, no. 6, pp. 860–863, Dec. 2002.
- 823 [63] C. Guruvayoorappan and G. Kuttan, 'β-Carotene Inhibits Tumor-Specific Angiogenesis by Altering  
824 the Cytokine Profile and Inhibits the Nuclear Translocation of Transcription Factors in B16F-10  
825 Melanoma Cells', *Integr. Cancer Ther.*, vol. 6, no. 3, pp. 258–270, Sep. 2007.
- 826 [64] B. Kulczyński, A. Gramza-Michałowska, J. Kobus-Cisowska, and D. Kmiecik, 'The role of  
827 carotenoids in the prevention and treatment of cardiovascular disease – Current state of  
828 knowledge', *J. Funct. Foods*, vol. 38, pp. 45–65, Nov. 2017.
- 829 [65] G. Hussein *et al.*, 'Antihypertensive and neuroprotective effects of astaxanthin in experimental  
830 animals', *Biol. Pharm. Bull.*, vol. 28, no. 1, pp. 47–52, Jan. 2005.
- 831 [66] H. G. Preuss, B. Echard, E. Yamashita, and N. V. Perricone, 'High Dose Astaxanthin Lowers Blood  
832 Pressure and Increases Insulin Sensitivity in Rats: Are These Effects Interdependent?', *Int. J. Med.*  
833 *Sci.*, vol. 8, no. 2, pp. 126–138, Feb. 2011.
- 834 [67] K. Uchiyama, Y. Naito, G. Hasegawa, N. Nakamura, J. Takahashi, and T. Yoshikawa, 'Astaxanthin  
835 protects beta-cells against glucose toxicity in diabetic db/db mice', *Redox Rep. Commun. Free*  
836 *Radic. Res.*, vol. 7, no. 5, pp. 290–293, 2002.

- 837 [68] S. Bhuvaneshwari, B. Yogalakshmi, S. Sreeja, and C. V. Anuradha, 'Astaxanthin reduces hepatic  
838 endoplasmic reticulum stress and nuclear factor- $\kappa$ B-mediated inflammation in high fructose and  
839 high fat diet-fed mice', *Cell Stress Chaperones*, vol. 19, no. 2, pp. 183–191, Mar. 2014.
- 840 [69] S. Nishikawa, M. Hosokawa, and K. Miyashita, 'Fucoxanthin promotes translocation and  
841 induction of glucose transporter 4 in skeletal muscles of diabetic/obese KK-A(y) mice',  
842 *Phytomedicine Int. J. Phytother. Phytopharm.*, vol. 19, no. 5, pp. 389–394, Mar. 2012.
- 843 [70] M. A. Gammone and N. D'Orazio, 'Anti-Obesity Activity of the Marine Carotenoid Fucoxanthin',  
844 *Mar. Drugs*, vol. 13, no. 4, pp. 2196–2214, Apr. 2015.
- 845 [71] F. Beppu, M. Hosokawa, M.-J. Yim, T. Shinoda, and K. Miyashita, 'Down-regulation of hepatic  
846 stearoyl-CoA desaturase-1 expression by fucoxanthin via leptin signaling in diabetic/obese KK-  
847 A(y) mice', *Lipids*, vol. 48, no. 5, pp. 449–455, May 2013.
- 848 [72] K. Kavitha, J. Kowshik, T. K. K. Kishore, A. B. Baba, and S. Nagini, 'Astaxanthin inhibits NF- $\kappa$ B and  
849 Wnt/ $\beta$ -catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic  
850 apoptosis in a hamster model of oral cancer', *Biochim. Biophys. Acta*, vol. 1830, no. 10, pp. 4433–  
851 4444, Oct. 2013.
- 852 [73] J. Kowshik, A. B. Baba, H. Giri, G. Deepak Reddy, M. Dixit, and S. Nagini, 'Astaxanthin inhibits  
853 JAK/STAT-3 signaling to abrogate cell proliferation, invasion and angiogenesis in a hamster model  
854 of oral cancer', *PLoS One*, vol. 9, no. 10, p. e109114, 2014.
- 855 [74] L. Zhang and H. Wang, 'Multiple Mechanisms of Anti-Cancer Effects Exerted by Astaxanthin',  
856 *Mar. Drugs*, vol. 13, no. 7, pp. 4310–4330, Jul. 2015.
- 857 [75] K.-N. Kim *et al.*, 'Fucoxanthin inhibits the inflammatory response by suppressing the activation of  
858 NF- $\kappa$ B and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages', *Eur. J. Pharmacol.*,  
859 vol. 649, no. 1–3, pp. 369–375, Dec. 2010.
- 860 [76] E. Kotake-Nara, M. Kushiro, H. Zhang, T. Sugawara, K. Miyashita, and A. Nagao, 'Carotenoids  
861 affect proliferation of human prostate cancer cells', *J. Nutr.*, vol. 131, no. 12, pp. 3303–3306,  
862 Dec. 2001.
- 863 [77] E. Kotake-Nara, M. Terasaki, and A. Nagao, 'Characterization of apoptosis induced by fucoxanthin  
864 in human promyelocytic leukemia cells', *Biosci. Biotechnol. Biochem.*, vol. 69, no. 1, pp. 224–227,  
865 Jan. 2005.
- 866 [78] Y. Satomi, 'Antitumor and Cancer-preventative Function of Fucoxanthin: A Marine Carotenoid',  
867 *Anticancer Res.*, vol. 37, no. 4, pp. 1557–1562, Apr. 2017.
- 868 [79] T. Rokkaku *et al.*, 'Anticancer effects of marine carotenoids, fucoxanthin and its deacetylated  
869 product, fucoxanthinol, on osteosarcoma', *Int. J. Oncol.*, vol. 43, no. 4, pp. 1176–1186, Oct. 2013.
- 870 [80] T. Sugawara, K. Matsubara, R. Akagi, M. Mori, and T. Hirata, 'Antiangiogenic activity of brown  
871 algae fucoxanthin and its deacetylated product, fucoxanthinol', *J. Agric. Food Chem.*, vol. 54, no.  
872 26, pp. 9805–9810, Dec. 2006.
- 873 [81] P. Ganesan, K. Matsubara, T. Sugawara, and T. Hirata, 'Marine algal carotenoids inhibit  
874 angiogenesis by down-regulating FGF-2-mediated intracellular signals in vascular endothelial  
875 cells', *Mol. Cell. Biochem.*, vol. 380, no. 1–2, pp. 1–9, Aug. 2013.
- 876 [82] M. A. Gammone, G. Riccioni, and N. D'Orazio, 'Marine Carotenoids against Oxidative Stress:  
877 Effects on Human Health', *Mar. Drugs*, vol. 13, no. 10, pp. 6226–6246, Oct. 2015.
- 878 [83] L. Speranza *et al.*, 'Astaxanthin treatment reduced oxidative induced pro-inflammatory cytokines  
879 secretion in U937: SHP-1 as a novel biological target', *Mar. Drugs*, vol. 10, no. 4, pp. 890–899,  
880 Apr. 2012.
- 881 [84] M. M. Al-Amin *et al.*, 'The antioxidant effect of astaxanthin is higher in young mice than aged: a  
882 region specific study on brain', *Metab. Brain Dis.*, vol. 30, no. 5, pp. 1237–1246, Oct. 2015.
- 883 [85] J.-Y. Wang *et al.*, 'Astaxanthin protects steroidogenesis from hydrogen peroxide-induced  
884 oxidative stress in mouse Leydig cells', *Mar. Drugs*, vol. 13, no. 3, pp. 1375–1388, Mar. 2015.
- 885 [86] A. W. Ha, S. J. Na, and W. K. Kim, 'Antioxidant effects of fucoxanthin rich powder in rats fed with  
886 high fat diet', *Nutr. Res. Pract.*, vol. 7, no. 6, pp. 475–480, Dec. 2013.

- 887 [87] C.-L. Liu, Y.-T. Chiu, and M.-L. Hu, 'Fucoxanthin enhances HO-1 and NQO1 expression in murine  
888 hepatic BNL CL.2 cells through activation of the Nrf2/ARE system partially by its pro-oxidant  
889 activity', *J. Agric. Food Chem.*, vol. 59, no. 20, pp. 11344–11351, Oct. 2011.
- 890 [88] H. Gylling, P. Simonen, H. Gylling, and P. Simonen, 'Phytosterols, Phytostanols, and Lipoprotein  
891 Metabolism', *Nutrients*, vol. 7, no. 9, pp. 7965–7977, Sep. 2015.
- 892 [89] M. Luz Fernandez and S. Vega-López, 'Efficacy and Safety of Sitosterol in the Management of  
893 Blood Cholesterol Levels', *Cardiovasc. Drug Rev.*, vol. 23, no. 1, pp. 57–70, Mar. 2005.
- 894 [90] X. Luo, P. Su, W. Zhang, X. Luo, P. Su, and W. Zhang, 'Advances in Microalgae-Derived  
895 Phytosterols for Functional Food and Pharmaceutical Applications', *Mar. Drugs*, vol. 13, no. 7, pp.  
896 4231–4254, Jul. 2015.
- 897 [91] J. Chen, R. Jiao, Y. Jiang, Y. Bi, and Z.-Y. Chen, 'Algal Sterols are as Effective as  $\beta$ -Sitosterol in  
898 Reducing Plasma Cholesterol Concentration', *J. Agric. Food Chem.*, vol. 62, no. 3, pp. 675–681,  
899 Jan. 2014.
- 900 [92] S. D. Lee and P. Tontonoz, 'Liver X receptors at the intersection of lipid metabolism and  
901 atherogenesis', *Atherosclerosis*, vol. 242, no. 1, pp. 29–36, Sep. 2015.
- 902 [93] R. Komati *et al.*, 'Ligands of Therapeutic Utility for the Liver X Receptors', *Molecules*, vol. 22, no.  
903 1, p. 88, Jan. 2017.
- 904 [94] A. B. Awad and C. S. Fink, 'Phytosterols as Anticancer Dietary Components: Evidence and  
905 Mechanism of Action', *J. Nutr.*, vol. 130, no. 9, pp. 2127–2130, Sep. 2000.
- 906 [95] I. Ikeda *et al.*, 'Campest-5-en-3-one, an oxidized derivative of campesterol, activates PPAR $\alpha$ ,  
907 promotes energy consumption and reduces visceral fat deposition in rats', *Biochim. Biophys.*  
908 *Acta BBA - Gen. Subj.*, vol. 1760, no. 5, pp. 800–807, May 2006.
- 909 [96] M. G. Ward, G. Li, V. C. Barbosa-Lorenzi, and M. Hao, 'Stigmasterol prevents glucolipototoxicity  
910 induced defects in glucose-stimulated insulin secretion', *Sci. Rep.*, vol. 7, Aug. 2017.
- 911 [97] S. Feng *et al.*, 'Intake of stigmasterol and  $\beta$ -sitosterol alters lipid metabolism and alleviates  
912 NAFLD in mice fed a high-fat western-style diet', *Biochim. Biophys. Acta BBA - Mol. Cell Biol.*  
913 *Lipids*, vol. 1863, no. 10, pp. 1274–1284, Oct. 2018.
- 914 [98] M. Caroprese, M. Albenzio, M. G. Ciliberti, M. Francavilla, and A. Sevi, 'A mixture of phytosterols  
915 from *Dunaliella tertiolecta* affects proliferation of peripheral blood mononuclear cells and  
916 cytokine production in sheep', *Vet. Immunol. Immunopathol.*, vol. 150, no. 1, pp. 27–35, Nov.  
917 2012.
- 918 [99] M. Valerio and A. B. Awad, ' $\beta$ -Sitosterol down-regulates some pro-inflammatory signal  
919 transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in J774A.1  
920 murine macrophages', *Int. Immunopharmacol.*, vol. 11, no. 8, pp. 1012–1017, Aug. 2011.
- 921 [100] O. Gabay *et al.*, 'Stigmasterol: a phytosterol with potential anti-osteoarthritic properties',  
922 *Osteoarthritis Cartilage*, vol. 18, no. 1, pp. 106–116, Jan. 2010.
- 923 [101] A. O. Antwi, D. D. Obiri, N. Osafo, A. D. Forkuo, and L. B. Essel, 'Stigmasterol inhibits  
924 lipopolysaccharide-induced innate immune responses in murine models', *Int.*  
925 *Immunopharmacol.*, vol. 53, pp. 105–113, Dec. 2017.
- 926 [102] Y.-S. Kim, X.-F. Li, K.-H. Kang, B. Ryu, and S. K. Kim, 'Stigmasterol isolated from marine  
927 microalgae *Navicula incerta* induces apoptosis in human hepatoma HepG2 cells', *BMB Rep.*, vol.  
928 47, no. 8, pp. 433–438, Aug. 2014.
- 929 [103] C. Park *et al.*, ' $\beta$ -Sitosterol Induces Anti-proliferation and Apoptosis in Human Leukemic U937  
930 Cells through Activation of Caspase-3 and Induction of Bax/Bcl-2 Ratio', *Biol. Pharm. Bull.*, vol.  
931 30, no. 7, pp. 1317–1323, 2007.
- 932 [104] S. S. Vundru, R. K. Kale, and R. P. Singh, ' $\beta$ -sitosterol induces G1 arrest and causes  
933 depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells',  
934 *BMC Complement. Altern. Med.*, vol. 13, no. 1, p. 280, Oct. 2013.
- 935 [105] A. B. Awad, M. Chinnam, C. S. Fink, and P. G. Bradford, ' $\beta$ -Sitosterol activates Fas signaling in  
936 human breast cancer cells', *Phytomedicine*, vol. 14, no. 11, pp. 747–754, Nov. 2007.
- 937 [106] M. Vivancos and J. J. Moreno, ' $\beta$ -Sitosterol modulates antioxidant enzyme response in RAW  
938 264.7 macrophages', *Free Radic. Biol. Med.*, vol. 39, no. 1, pp. 91–97, Jul. 2005.

- 939 [107] K. Hąc-Wydro, P. Wydro, A. Jagoda, and J. Kapusta, 'The study on the interaction between  
940 phytosterols and phospholipids in model membranes', *Chem. Phys. Lipids*, vol. 150, no. 1, pp. 22–  
941 34, Nov. 2007.
- 942 [108] V. R. Ramprasath and A. B. Awad, 'Role of Phytosterols in Cancer Prevention and Treatment',  
943 *J. AOAC Int.*, vol. 98, no. 3, pp. 735–738, Jun. 2015.
- 944 [109] A. B. Awad, S. L. Barta, C. S. Fink, and P. G. Bradford, 'β-Sitosterol enhances tamoxifen  
945 effectiveness on breast cancer cells by affecting ceramide metabolism', *Mol. Nutr. Food Res.*, vol.  
946 52, no. 4, pp. 419–426, Apr. 2008.
- 947 [110] S. Takaichi, 'Carotenoids in Algae: Distributions, Biosyntheses and Functions', *Mar. Drugs*, vol.  
948 9, no. 6, pp. 1101–1118, Jun. 2011.
- 949 [111] E. Kotake-Nara and A. Nagao, 'Absorption and Metabolism of Xanthophylls', *Mar. Drugs*, vol.  
950 9, no. 6, pp. 1024–1037, Jun. 2011.
- 951 [112] L. Calpe-Berdiel, J. C. Escolà-Gil, and F. Blanco-Vaca, 'New insights into the molecular actions  
952 of plant sterols and stanols in cholesterol metabolism', *Atherosclerosis*, vol. 203, no. 1, pp. 18–  
953 31, Mar. 2009.
- 954

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

957 A: fucoxanthin, B: astaxanthin

958

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

960 A: stigmasterol, B:  $\beta$ -sitosterol, C: campesterol

961

962 **Figure 3. Synthesis of bioactive compounds by microalgae.**

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

965

966 **Figure 4. Common metabolic pathways of microalgal carotenoid and sterol syntheses**

967 [32] [45] [110].

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

971

972 **Figure 5. Intestinal uptake and secretion pathways of carotenoids and phytosterols [55]**

973 [88] [111] [112].

974 ABC: ATP-binding cassette, ACAT2: acyl-coenzyme A cholesterol acyltransferase 2, ApoB-  
975 48: apolipoprotein B48, CD36: cluster of differentiation 36, FA: fatty acid, HDL: high density  
976 lipoprotein, LXR: liver X receptor, MTP: mitochondrial transfer protein, NPC1L1: Niemann-  
977 Pick C1-like 1, PS-E: esterified phytosterol, SR-B1: scavenger receptor class B type 1, TAG:  
978 triacylglycerol.

979

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

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

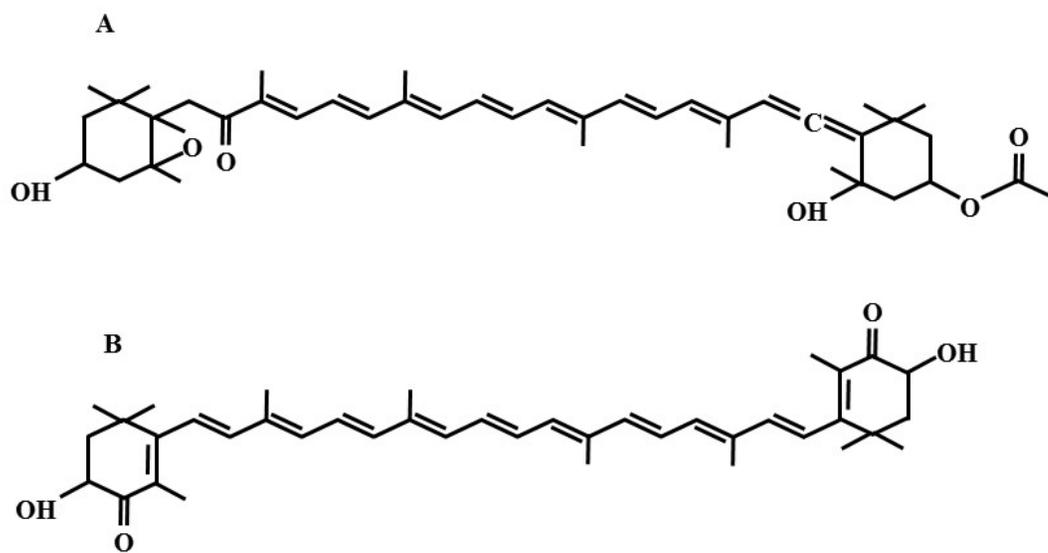
**Table 1.****Main microalgal producers of carotenoids and selected phytosterols [6,22,28,31].**

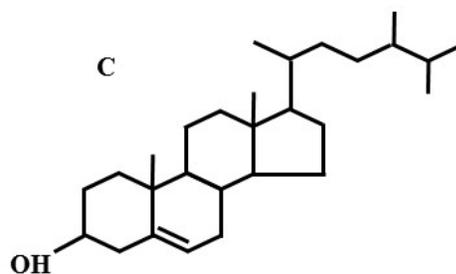
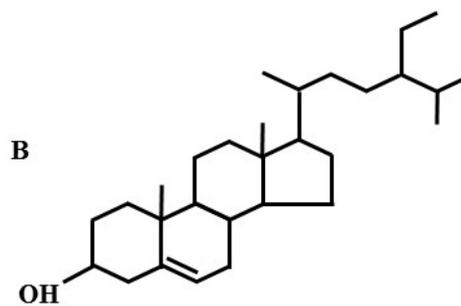
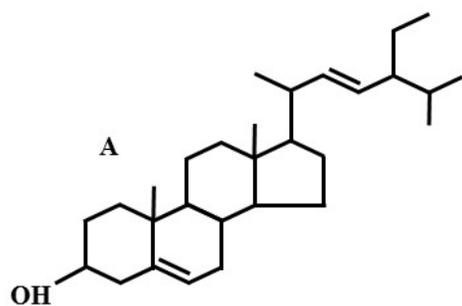
Results are expressed in mg of total carotenoids per g dry weight of microalgal biomass \* and in percentage of total sterols \*\*. Main accessory carotenoids in bold.

<b>Classes</b>	<b>Carotenoids</b>	<b>Contents*</b>	<b>Activities in human health</b>
<i>Chlorophyceae</i> <i>Prasinophyceae</i>	<b><math>\alpha</math>-, <math>\beta</math>-, <math>\gamma</math>-carotenes, lutein siphonaxanthin, siphonein,</b> antheraxanthin, astaxanthin, canthaxanthin, prasinoxanthin, neoxanthin, violaxanthin, zeaxanthin	0.02-291	Anti-cancer Anti-inflammatory Anti-oxidant Anti-obesity Cardiovascular
<i>Bacillariophyceae</i> <i>Prymnesiophyceae</i>	<b><math>\beta</math>-carotene, fucoxanthin diatoxanthin, diadinoxanthin</b> cantaxanthin	0.11-26.6	
<b>Classes</b>	<b>Phytosterol</b>	<b>Contents**</b>	<b>Activities in human health</b>
	Campesterol		
<i>Chlorophyceae</i> <i>Prasinophyceae</i>		2-48 34-99	Anti-angiogenic Anti-cancer Anti-oxidant
<i>Bacillariophyceae</i> <i>Prymnesiophyceae</i>		1-39 6-18	Cholesterol-lowering
	$\beta$ -Sitosterol		
<i>Chlorophyceae</i> <i>Prasinophyceae</i>		1-33 -	Analgesic activity Anti-cancer Anti-inflammatory
<i>Bacillariophyceae</i> <i>Prymnesiophyceae</i>		1-95 1-73	Anti-mutagenic
	Stigmasterol		
<i>Chlorophyceae</i> <i>Prasinophyceae</i>		19-72 -	Anti-cancer Anti-oxidant Cholesterol-lowering
<i>Bacillariophyceae</i> <i>Prymnesiophyceae</i>		1-100 10-47	Hypoglycaemic

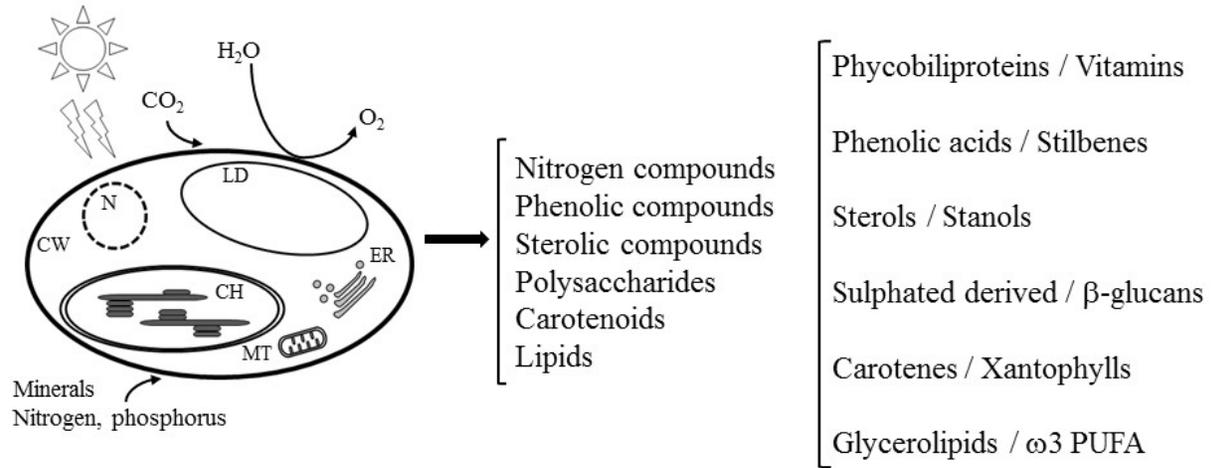
**Table 2.****Main characteristics of selected bioactive molecules in microalgae [6,25,29,42,43].**

<b>Carotenoids</b>	<b>Chemical structure</b>	<b>Color</b>	<b>Detection Spectrophotometry (nm)</b>	<b>Location</b>	<b>Role</b>
Astaxanthin	C <sub>40</sub> H <sub>52</sub> O <sub>4</sub>	Red	472 (methanol) 466 (hexane) 485 (chloroform)	Cytoplasm Lipid droplets	Photosynthetic apparatus
Fucoxanthin	C <sub>42</sub> H <sub>58</sub> O <sub>6</sub>	Orange	449 (acetone) 453 (petroleum ether)	Chloroplast	Photosynthetic apparatus
<b>Phytosterols</b>	<b>Chemical structure</b>	<b>Systematic name</b>	<b>Detection Chromatography (all sterols)</b>	<b>Location</b>	<b>Role</b>
Campesterol	C <sub>28</sub> H <sub>48</sub> O	24 $\alpha$ -methylcholest-5-en-3 $\beta$ -ol	Flame ionization detection Mass spectrometry	Membranes	Fluidity / Permability Brassinosteroid synthesis
$\beta$ -Sitosterol	C <sub>29</sub> H <sub>50</sub> O	24 $\beta$ -ethylcholest-5-en-3 $\beta$ -ol	UV 208 nm Diode array detection	Membranes	Fluidity / Permeability Membrane-bound enzyme effector
Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	24 $\alpha$ -ethylcholesta-5,22E-dien-3 $\beta$ -ol	Evaporative light scattering detection	Membranes	Fluidity / Permeability Membrane-bound enzyme effector Cell proliferation

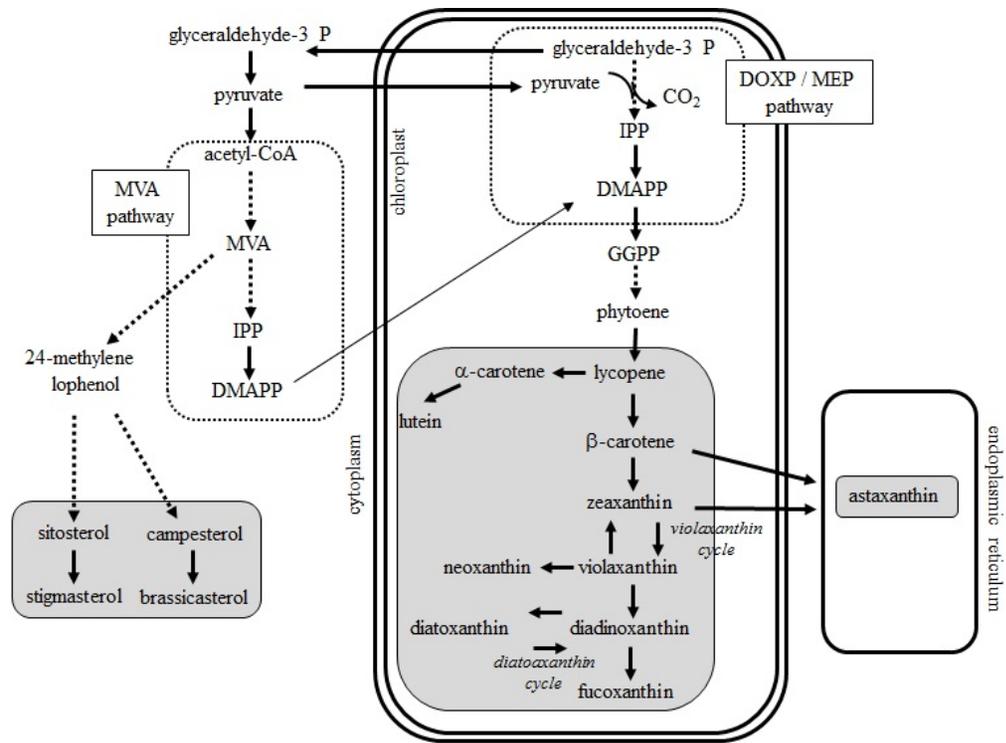


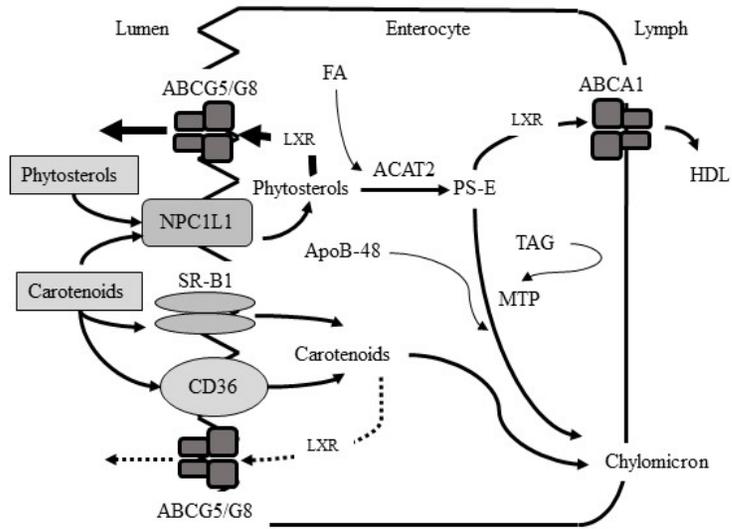


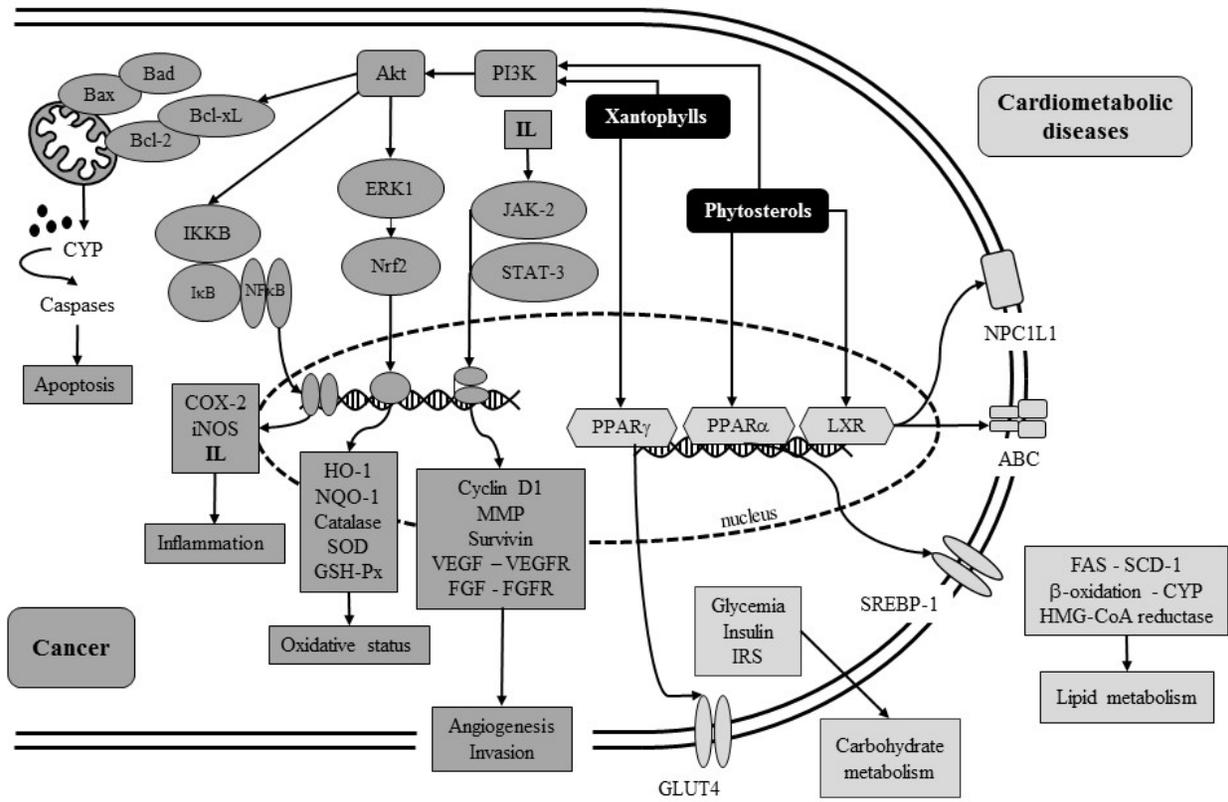
Journal



Journal







1 **Highlights**

2

3 • Microalgae produce high added value molecules involved in human health and disease  
4 prevention

5 • Microalgal xanthophylls and phytosterols regulate cell signaling pathways involved in  
6 chronic disease

7 • Astaxanthin and fucoxanthin exhibit anti-oxidant, hypolipidemic, anti-inflammatory  
8 and anti-tumoral activities

9 • Microalgal sterols regulate cholesterol and lipid metabolisms and possess anti-cancer  
10 activities

Journal Pre-proof