Trends in Food Science & Technology 117 (2021) 163–181

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

# Biological action mechanisms of fucoxanthin extracted from algae for application in food and cosmetic industries

C. Lourenço-Lopes [a,b], M. Fraga-Corral [a,b], C. Jimenez-Lopez [a,b], M. Carpena [a], A.G. Pereira [a,b], P. Garcia-Oliveira [a,b], M.A. Prieto [a,**], J. Simal-Gandara [a,*]

[a] Nutrition and Bromatology Group, Analytical and Food Chemistry Department. Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E-32004, Ourense, Spain

[b] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253, Bragança, Portugal

ARTICLE INFO

*Keywords:*
Fucoxanthin
Carotenoid
Extractions
Algae
Bioactivities

ABSTRACT

*Background:* Fucoxanthin is a pigment present in diverse marine organisms such as micro and macro-algae. The most relevant source of fucoxanthin has been described to be the group of the brown macroalgae, also known as *Phaeophyceae*. The presence of the fucoxanthin, a xanthophyll, found as an accessory pigment in the chloroplasts of the brown algae is responsible for providing them their characteristic color. The market size of this carotenoid, expected to reach US$ 120 million by 2022, reflects its industrial importance, especially remarkable as a food or cosmetic ingredient.

*Scope and approach:* Therefore, it is critical to recognize the main sources of fucoxanthin as well as the most efficient extraction and purification methods that allow obtaining the best production ratio for such a valuable molecule. Throughout this review very different preventive properties of the fucoxanthin have been included, such as antioxidant, anticancer, antiangiogenic, anti-inflammatory, cytoprotective, antiobesity, neuroprotective and its skin protective effects. The stability, bioavailability and toxicity of the fucoxanthin have also been reviewed through diverse biological, *in vitro* and *in vivo* assays.

*Key findings and conclusions:* Thus, the main aim of this work is to provide a wide and global vision of the fucoxanthin in terms of productive species, efficient recovery techniques and multiple industrial applications.

## 1. Algae as a source of fucoxanthin

Approximated 70% of the surface of the Earth is covered by marine water. Over the last decades, marine organisms have been revealed as a promising source of functional compounds, beneficial for our organism and human health, such as vitamins, and essential minerals, dietary fibers, omega-3 polyunsaturated fatty acids (PUFAs), besides a large number of peptides, enzymes, and antioxidant substances. Thus, the biodiversity contained in the oceans is contemplated as a provider of unknown molecules with potential bioactivities to discover (Heo et al., 2008). Marine resources, such as algae, microalgae or seaweeds have been traditionally used as nutrients or remedies. In fact, scientific evidence supports the use of different marine organisms, from distant coastal areas since 14000 AD with medicinal and nutritional purposes (Dillehay et al., 2008). Algae have been part of the Eastern diet since immemorial times, due to its high nutritional content (Dawczynski et al., 2007), which have prompted their utilization as a food ingredient all over the world (Kummar et al., 2008; Soo-Jin You-Jin et al., 2008). In the eastern countries, the most used algae for this purpose are *Undaria*, *Porphyra* and *Laminaria* genera, colloquially known as wakame, nori, and kombu, respectively. Their inclusion in the diet has been generalized and its consumption has triggered the controlled production of species of interest which are also considered prolific and sustainable organisms. Thus, algae aquaculture is undergoing an important evolution that allows manufacturing huge amounts of algae, exceeding exponentially those obtained by manual collection. In addition to this ancestral use in food, algae contain other compounds, such as hydrocolloids, which have been widely applied in pharmaceutical and/or cosmetic industries (Kummar et al., 2008). Hence, algae are focusing the attention of different research and industry areas in order to magnify their use as a possible source of compounds with bioactive potential, such as fatty acids, carotenoids, polysaccharides, phytosterols, phenolics, etc., since these are molecules associated with many beneficial

* Corresponding author.
** Corresponding author.
*E-mail addresses:* mprieto@uvigo.es (M.A. Prieto), jsimal@uvigo.es (J. Simal-Gandara).

https://doi.org/10.1016/j.tifs.2021.03.012
Received 28 May 2020; Received in revised form 14 September 2020; Accepted 14 March 2021
Available online 20 March 2021

**Abbreviations**

*Generic*

| | |
|---|---|
| PAR | photosynthetically active solar radiation |
| DW | dry weight |
| WAT | white adipose tissue |
| BAT | brown adipose tissue |
| UV | ultraviolet |
| $EC_{50}$ | half maximal effective concentration |
| $IC_{50}$ | half maximal inhibitory concentration |
| $LD_{50}$ | median lethal dose |
| ROS | reactive oxygen species |
| CNS | central nervous system |
| UVB | ultraviolet B-rays |
| JAK-STAT | Janus kinases signal transducer and activator of transcription proteins pathway |
| PI3K/Akt | intracellular signal transduction pathway |
| MAPKs | mitogen-activated protein kinase pathway |
| I/R | ischemia/reperfusion injury |
| ARE | antioxidant response elements |
| TBI | traumatic brain injury |
| SHRSP | stroke-prone spontaneously hypertensive rats |
| NF-kB | nuclear factor kappa-light-chain-enhancer of activated B cells pathway |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| mTOR | mammalian target of rapamycin |
| MAPK | mitogen-activated protein kinase |
| Wnt | wingless and int-1 signaling pathways |
| Hippo | Salvador-Warts-Hippo pathway |
| Notch | Notch signaling pathway |
| FoxO | Forkhead box protein O1 |
| PUFAs | omega-3 polyunsaturated fatty acids |
| $NAD(P)^+$ | nicotinamide adenine dinucleotide phosphate |
| EtOH | ethanol |
| DE | diethyl ether |
| $H_2O_2$ | hydrogen peroxide |
| Nrf2 | nuclear factor erythroid 2-related factor 2 |
| NQO1 | quinine oxidoreductase |
| DEN | diethylnitrosamine |
| HO-1 | heme oxygenase-1 |
| AMPK | activated protein kinase |
| LDL | low-density lipoprotein |
| Bcl-xL | anti-apoptotic protein |
| ADP | adenosine diphosphate |
| TRAIL | tumor necrosis factor-related apoptosis-inducing ligand |
| NO | nitric oxide |
| iNOS | inducible nitric oxide synthase |
| COX-2 | cyclooxygenase 2 |
| TNF-α | tumor necrosis factor-α |
| RNA | ribonucleic acid |
| DNA | deoxyribonucleic acid |
| PAI-1 | plasminogen activator inhibitor-1 |
| UPC1 | mitochondrial uncoupling protein 1 |
| DHA | docosahexaenoic acid |
| Adrb3 | β3-adrenergic receptor |
| GLUT4 | glucose transporter 4 |
| MCP-1 | monocyte chemoattractant protein-1 |
| HRAR | recombinant aldose reductase |
| RLAR | rat lens aldose reductase |
| PTP1B | protein tyrosine phosphatase 1B |
| AGE | advanced glycation end-product |
| NAC | N- acetylcysteine |
| TAG | long-chain triacylglycerols |
| C | chitosan |
| G | glycolipid |
| ST | sodium tripolyphosphate |

*Techniques*

| | |
|---|---|
| P-TLC | preparative thin layer chromatography |
| NMR | nuclear Magnetic Resonance |
| HPLC | high-performance liquid chromatography |
| LC | liquid chromatography |
| DAD | photodiode-array detector |
| MS | mass spectrometry |
| DPPH | 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical assay |
| FRAP | ferric reducing antioxidant power assay |
| ABTS | 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay |

*Cellular lines*

| | |
|---|---|
| CaCo-2 | human colon epithelial cancer cell line |
| Hep G2 | human liver carcinoma cell line |
| Vero | kidney fibroblast cells from monkey |
| BNL CL.2 | murine hepatic cells from mouse |
| 3 T3-L1 | adipocytes from mouse |
| SH-SY5Y | human neuroblastoma cell line |
| L02 | human hepatic cell line |
| B16-F10 | melanoma mouse cell line |
| BNL CL.2 | embryonic liver mouse cell line |
| Neuro2a | neuroblastoma murine cell line |
| HL-60 | human leukemia cell line |
| DLD-1 | colorectal adenocarcinoma cell line |
| HT-29 | colorectal adenocarcinoma cell line |
| HCT116 | human colorectal carcinoma cell line |
| PC-3 | human prostate cancer cell lines |
| DU145 | human prostate cancer cell line |
| LNCaP | human prostate adenocarcinoma cell line |
| MCF-7 | breast cancer cell line |
| MDA-MB-231 | epithelial, human breast cancer cell line |
| HUC-Fm | human male umbilical cord fibroblasts |
| EJ-1 | urinary bladder cancer cell line |
| GOTO | human neuroblastoma |
| SK-Hep-1 | immortal human cell line |
| H1299 | lung cancer cell line |
| WiDr | human colon carcinoma |
| U87 | primary glioblastoma cell line |
| U251 | human glioblastoma astrocytoma cell line |
| SGC-7901 | human gastric cancer cell line |
| BGC-823 | human gastric carcinoma cell line |
| NSCLC-N6 | non-small-cell lung carcinoma cell line |
| K562 | myelogenous leukemia cell line |
| TK6 | human lymphoblastoid cell line |
| HeLa | human cervical adenocarcinoma cell line |
| SiHa | human cervical cancer cell line |

*Units*

| | |
|---|---|
| h | hour |
| min | minute |
| mL | milliliter |
| g | gram |
| ºC | Celsius degrees |
| nm | nanometer |
| mg | milligram |
| w | weight |
| v | volume |
| μg | microgram |
| cm | centimeter |
| M | molar |
| μM | micromolar |
| kg | kilogram |

| *Extraction techniques* | | | |
|---|---|---|---|
| ME | Maceration extraction | MAE | Microwave-assisted extraction |
| EAE | Enzyme-assisted extraction | PLE | Pressurized liquid extraction |
| | | SFE | Supercritical fluid extraction |

health effects, such as anticoagulant, antitumor properties (Saet et al., 2008) or antioxidants (Kummar et al., 2008). Therefore, the development of new functional ingredients for food, cosmetic and pharmaceutical formulations (Perez-Gregorio & Simal-Gandara, 2017) from compounds present in algae are gaining importance. The studies carried out with brown algae are the ones that reported the greatest amount of bioactive components, surpassing red and green ones (Prabhasankar et al., 2009). An example of such interesting compounds are phlorotannins, phylopheophylin, and fucoxanthin, among other metabolites (Maeda et al., 2006).

Fucoxanthin is a compound belonging to the group of carotenoids, considered a secondary metabolite and one of the most abundant and characteristic pigments of brown algae. Some examples of edible algae that contain fucoxanthin belong to the genera *Undaria*, *Sargassum*, *Laminaria, Eisenia*, *Alaria*, *Cystoseira, or Hijikia* (Maoka et al., 2007; Willstatter & Page, 1914). Nevertheless, it has been also found in large quantities in other matrices such as the diatom *Phaeodactylum tricornutum* (Li et al., 2018).

Fucoxanthin is a pigment present in chloroplasts of eukaryotic algae Chromalveolata (phylum Heterokontophyta, Ochrophyta class) involved in the photosynthesis process which is considered to be more productive than that occurring in the most efficient land plants (Gao & McKinley, 1994). Besides, it is a very abundant molecule, it accounts for approximately 10% of the total carotenoids in nature (Maeda et al., 2006). Currently, the market size of this pigment maintains an average annual growth rate of 2.47% which in American dollars represented a rise from 92 million $ in 2014 to 99 million $ in 2017. Scientific analysts have estimated a future expansion of the fucoxanthin market size that may reach 120 million $ by 2022 (Market Reports World, 2017).

The biological and therapeutic activities related to this carotenoid have been repeatedly underlined throughout scientific works that have reported its antioxidant, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, anti-angiogenic capacities and also photoprotective (Chuyen & Eun, 2017; D'Orazio et al., 2012; Gao & McKinley, 1994; Heo et al., 2008; Heo & Jeon, 2009; Kotake-Nara et al., 2015; Kumar et al., 2013; Maoka et al., 2007; Mikami & Hosokawa, 2013; Sugawara et al., 2009). However, to date, the most studied and researched bioactivity remains to be the antioxidant which is regarded for its beneficial effects on health for potential new applications in the food sector and the pharmaceutical industry (Kim & Pangestuti, 2011; Muradian et al., 2015). Even though the possible applications for this pigment are very promising, they are limited because the commercialization of fucoxanthin is almost non-existent (Kanazawa et al., 2008). Indeed, the production of fucoxanthin has to face several challenges since its chemical synthesis represents a complex process that is not efficient and the extraction method from marine organisms has been not standardized (Kanda et al., 2014; Yamamoto et al., 2011). As discussed below, there are very different extractive techniques that have been used for pigment extraction, such as the traditional heat-assisted extraction (maceration)(Shannon & Abu-Ghannam, 2017). For a product to be profitable, it is necessary to obtain it by using simple, fast and based on low-cost technologies (Raguraman et al., 2018), thus future and innovative studies regarding extraction methods can be of great help in the progress towards its commercialization (López et al., 2018).

Thus, even though carotenoids may be artificially synthesized, following laboratory protocols, their extraction from algae presents many more advantages. In the specific case of fucoxanthin, the accessibility to the natural producers, mostly algae, convert this pigment in an economic and ecological alternative that provides a natural ingredient avoiding the safety issues prompted using chemical compounds. However, the extraction yield of fucoxanthin has been found to be very variable depending on the selected species and the recovery technique. Therefore, the main aim of this work is to underline the best algae in terms of fucoxanthin production and the most promising extraction and purification methods, while offering a complete panorama of the described bioactivities of fucoxanthin which includes bioavailability, administration via, doses and stability of the molecule.

### 1.1. Principal matrices

The first time that fucoxanthin was isolated was in 1914 in Germany from brown seaweeds (Phaeophyceae) *Dictyota*, *Fucus*, and *Laminaria* (Willstatter & Page, 1914). However, since that moment the presence of this carotenoid has been detected in many other macroalgae genera such as *Alaria, Ascophyllum, Carpophyllum, Cladosiphon*, *Cystophora, Cystoseira Dictyota, Ecklonia, Ectocarpus, Eisenia, Fucus, Himanthalia, Hizikia, Ishige, Kjellmaniella, Laminaria, Myagropsis, Padina, Pelvetia, Petalonia, Saccharina, Sargassum, Schytosiphon, Sporochnus, Turbinaria,* and *Undaria* (Muradian et al., 2015). From these wide varieties of genera, currently, the alga species mostly used for the extraction of the fucoxanthin is *Undaria pinnatifida* (wakame) due to its high content can reach 10% in the lipid fraction (Billakanti et al., 2013). Nevertheless, unicellular microalgae have been found to synthesize fucoxanthin, besides, they are considered very efficient producer organisms since they can be easily and economically cultured to yield high concentrations of different biomolecules, such as pigments among others. The production of fucoxanthin has been studied mostly in diatoms (Bacillariophyceae) including *Phaeodactylum tricornutum, Chaetoceros sp., Cylindrotheca closterium,* and *Odontella aurita* (Peng et al., 2011).

Fucoxanthin is a pigment mostly associated with brown algae even though its presence has been reported as a minor carotenoid in other algae groups such as Chlorophytes. Fucoxanthin is a not typical carotenoid from Chlorophytes however two different biosynthetic pathways for the metabolism of fucoxanthin in brown algae have been suggested to happen based on the conversion of β-carotene in fucoxanthin with diadinoxanthin and neoxanthin as precursors. Along these pathways, some carotenogenic genes have been identified, which are shared by both brown and green algae. However, the presence of fucoxanthin in green algae may be due to the biotransformation of different carotenoids, and this process is still not completely clear (Mikami & Hosokawa, 2013). The presence of fucoxanthin in green algae has been reported in a study that used supercritical fluid extraction, with ethanol as co-solvent, as a tool to obtain extracts from freshwater green macroalgae which naturally occur in inland waters in Poland (Table 2). Extracts from *Cladophora glomerata,* and *Chara fragilis*, two of the studied species, among others were analyzed trough HPLC-DAD-MS allowing the tentative assignment of diverse carotenoids including fucoxanthin, especially in *C. glomerata* extracts (Fabrowska et al., 2016).

The presence of fucoxanthin has been reported several times in the Rhodophyceae family but on several occasions, it was attributed to the presence of biological contaminants, like diatoms and Chrysophyceae mixed with the red algae. In a few cases, natural red algae compared with cultured ones showed that fucoxanthin was not produced by the algae themselves (Palermo et al., 1991). These analyses permit to eliminate the fucoxanthin contributions from biological contaminates, particularly microalgae and brown algae, by comparing naturally occurring red algae with cultured ones assuring that fucoxanthin and other carotenoids were in fact produced by the studied red algae analyzed (Bjørnland & Aguilar-Martinez, 1976). In additional

microscopic studies the examination of three different red algae, *Corallina officinalis*, *Corallina elongata* and *Jania sp.* did not show the presence of diatoms or other contaminants that could be responsible for the fucoxanthin yields detected (Table 2). Despite these results, the origin of fucoxanthin in red algae is still not certain, because its production by a microscopic symbiotic organism cannot be ruled out, so more studies with Rhodophyceae are necessary (Palermo et al., 1991).

Brown algae, also known as Phaeophyceae, are responsible for the biggest fucoxanthin production, with this pigment being their major carotenoid. Fucoxanthin is synthesized through the xanthophyll-cycle pathway that converts zeaxanthin in fucoxanthin via antheraxanthin, violaxanthin, and diadionoxanthin; and usually, algae that possess this cycle are able to produce fucoxanthin (Terasaki et al., 2009). The concentration of this compound has been shown to be very variable when testing not only different species but also when the same species have been grown under different environmental conditions and seasonal changes (Fariman et al., 2016).

The duration of sunshine, PAR (photosynthetically active solar radiation), and seawater temperature were studied to determine the effects of these parameters. Results displayed that higher concentrations of fucoxanthin were detected between January and March (wintertime) where the sunlight hours were less, and the water temperature reaches the lowest values (Terasaki et al., 2009). Another work also evaluated how seasonal variations, and additionally geographical distributions, affected to the fucoxanthin content in two brown algae, *Sargassum horneri* and *Cystoseira hakodatensis*. The algae were collected in different locations but were lately cultivated in the same place. Regarding the geographical distribution, identical species collected from distant areas 700 km from each other displayed different fucoxanthin concentrations. Thus, the authors suggested that genetic variation from different origins may be responsible for the fucoxanthin amount variability. Regarding seasonal changes, fucoxanthin concentrations started to increase in October reaching a maximum in January in both species with a 15% increase in winter months (Nomura et al., 2013). Therefore, from these studies, it can be deduced that the low light in winter months and lower water temperatures lead to an increase in fucoxanthin production in brown algae.

On a similar note, the *Sargassum fusiforme*'s biological composition was tested while changing environmental factors that affect growth and biochemical composition, like temperature and salinity. The best fucoxanthin content was obtained at 15 °C and 10 psi at a maximum of 2.62 mg/g (Table 2) (Li et al., 2019).

Even though, *Undaria pinnatifida* is another brown alga well-known to possess high contents in fucoxanthin, with values that reach up to 4.96 mg/g (Table 2) (Fung et al., 2013), some additional studies were able to extract even higher concentrations (Rajauria et al., 2017b). evaluated *Himanthalia elongata* as a potential source for dietary fucoxanthin and used low polarity solvents like n-hexane, diethyl ether, and chloroform to perform the extractions. The crude extract was then purified with preparative thin layer chromatography (P-TLC) and the identification, quantification and structure elucidation were elucidated trough LC-DAD-MS and NMR ($^{1}$H and $^{13}$C). The purification step led to a yielded of 18.6 mg/g of fucoxanthin (Table 2) with a purity of 97% (Rajauria et al., 2017b).

Another significant source of fucoxanthin is the group of the microalgae, some species, like the Bacillariophycean *Odontella auritais* is capable of producing more than 18 mg/g DW of this compound (Xia et al., 2013). Another Bacillariophycean, *Chaetoceros calcitrans* is a very important marine species because they are the base of the marine food chain. Fucoxanthin is the major light-harvesting complex in these microalgae, contributing to more than 10% of the estimated total production of carotenoids in nature. The detected amounts of fucoxanthin in this species are around 5 mg/g of DW (Table 2) (Foo et al., 2015, 2017). The main advantage of using microalgae as a source of fucoxanthin is that they can be cultured and grown in close photobioreactors providing an available, stable and sustainable supply of biomass all year round (Foo et al., 2015). Besides, as microalgae can grow in photobioreactors, its growing conditions can be optimized for maximizing the extraction yield of a specific compound. In (McClure et al., 2018) they studied the effect of different culture conditions, light intensity, carbon dioxide addition and medium composition, on the production of fucoxanthin by *Phaeodactylum tricornutum*. They found that on day 7 of the cultivation, the fucoxanthin production was around 42.8 mg/g of biomass at low light conditions (100 μmol photons $m^{-2}s^{-1}$) and an increase in light intensity resulted in a significant decrease in fucoxanthin production to only 9.9 mg/g. The effect of carbon dioxide addition did not affect significantly the cell growth rate or the fucoxanthin production but the supplementation of the medium with nitrate increased the fucoxanthin levels on day 7 up to 57.4 mg/g (Table 2) (McClure et al., 2018).

Table 2 compiles an extended list of matrices in which fucoxanthin has been detected at different concentrations, as well as the respective extraction and detection methods used.

### 1.2. Fucoxanthin metabolites

Fucoxanthin represents one of the major accessory pigments found in the chloroplasts of the brown algae, together with chlorophyll *a* and *c*, and β-carotene. The chemical structure of the fucoxanthin is a carotene backbone oxygenated under the shape of different functional groups such as epoxy, hydroxyl, carbonyl and carboxyl moieties, besides it includes an allenic bond which reinforces the uniqueness of this molecule (Fig. 1A) (Peng et al., 2011). This singular pigment that provides the characteristic brown or olive-green color to the *Phaeophyceae* absorbs light in the blue-green to yellow-green part of the visible spectrum, mostly in the range of 450–540 nm. It behaves as a primary light-harvesting carotenoid that transfers energy to chlorophyll-protein complexes. Fucoxanthin exhibits high energy transfer efficiency that is thought to be related to the unique structure of this carotenoid (Kajikawa et al., 2012). Some characteristics and physical properties of fucoxanthin are resumed in Table 1 (see Fig. 2).

The presence of different fucoxanthin metabolites has been evident, and mostly, detected in plasma, liver, or adipose tissue depending on the species selected for the *in vivo* assay, the absence/presence of induced pathologies on these experimental models, the duration of the assay and the concentration of the fucoxanthin administrated. The wide variability observed along the published studies hinders their full comparison. However, general results can be extracted from the bioavailability and metabolism of these works. *In vivo* studies performed in mice and rats have demonstrated that fucoxanthin, after its oral administration, suffers different chemical modifications, catalyzed by the specific enzymes of each target organ. The gastrointestinal hydrolysis of fucoxanthin seems to take place for 2 h, so in that period, it would be likely to detect it in the plasma or the liver in mice (Asai et al., 2004; Matsumoto et al., 2010). A study performed with the cell line named CaCO-2, used as an intestinal epithelium barrier model to evaluate intestinal absorption rates, showed that fucoxanthin become fucoxanthinol (Fig. 1B) (Sugawara et al., 2002, 2009). Based on different animal models, this biotransformation has been suggested to be mediated by lipases or esterases that trigger its hydrolysis and it has been proven to occur after the administration of fucoxanthin in different species such as hens, mice or rats (Asai et al., 2004; Sangeetha et al., 2010; Strand et al., 1998; Sugawara et al., 2002). Once deacetylated, fucoxanthin becomes into a non-polar molecule of greater bioavailability ready to be incorporated into chylomicrons that permit to fucoxanthinol to reach different systemic organs, mainly the liver, lungs, kidney, heart, and spleen (Kotake-Nara et al., 2015; Sugawara et al., 2002, 2009). Whereas, in other organs, fucoxanthin, already transformed in fucoxanthinol, seems to be rapidly metabolized by the action of a short-chain reductase or dehydrogenase to be converted into another metabolite known as amarouciaxanthin A (Fig. 1C). Studies based on the use of hepatic cells (HepG2) demonstrated that fucoxanthinol becomes into

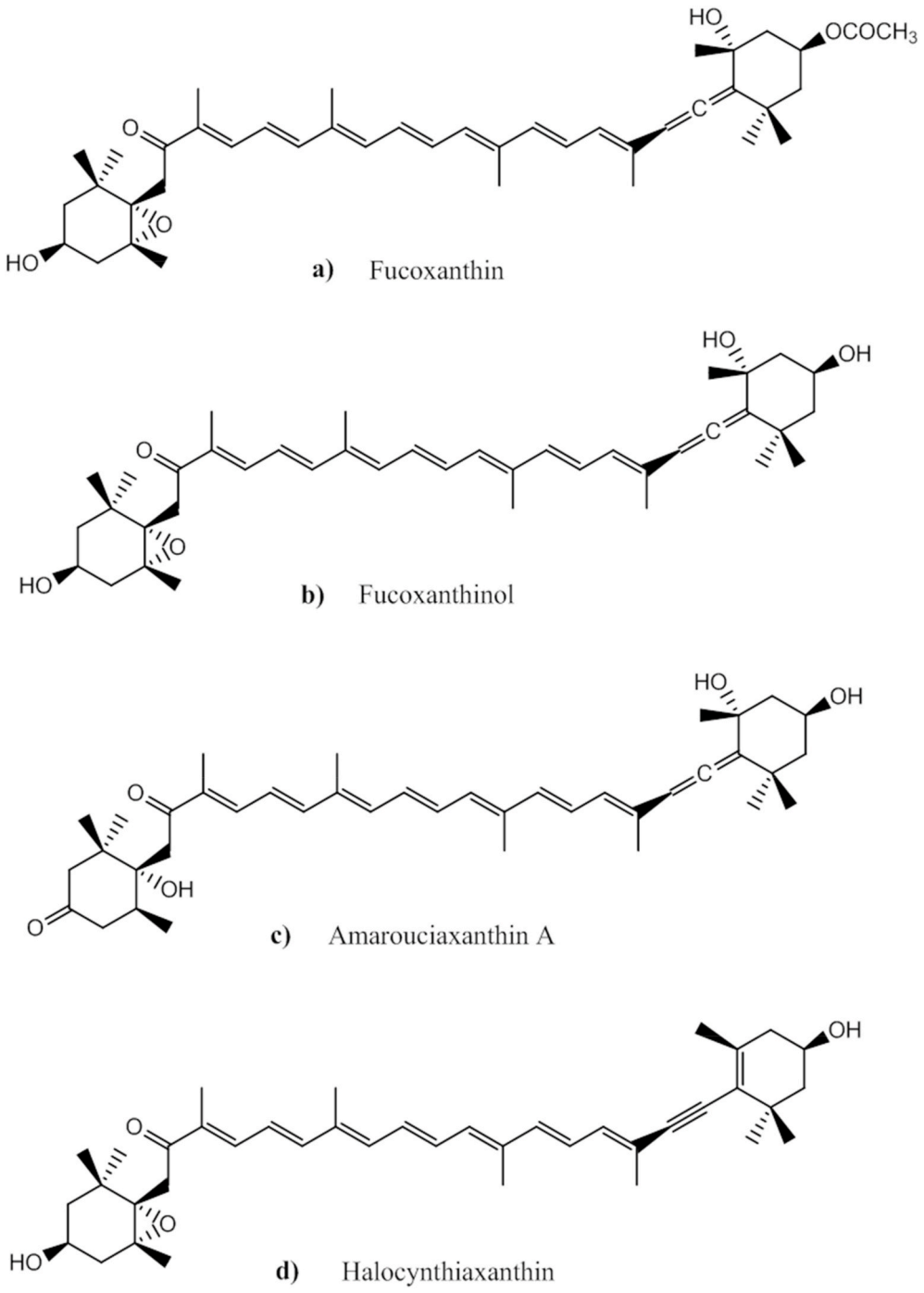


**Fig. 1.** Chemical representation of a) fucoxanthin, b) fucoxanthinol, c) amaroucianxanthin A and d) halocynthiaxanthin.

amarouciaxanthin A (Fig. 1C) by a dehydrogenation/isomerization. This step is probably catalyzed by a NAD(P)$^+$-dependent dehydrogenase and it was predominantly shown in liver microsomes (Asai et al., 2004). As in the case of the fucoxanthinol, amarouciaxanthin A also reaches blood circulation and additional targets, primarily the adipose tissue (Hashimoto et al., 2009; Peng et al., 2011; Yonekura et al., 2010). In fact, when animals were fed for periods longer than a week, fucoxanthin and its metabolites were detected in blood and other organs or tissues apart from those considered as principals (Hashimoto et al., 2009; Yonekura et al., 2010). In the case of hens fed with *Fucus serratus* fucoxanthinol reach the egg yolk being the major carotenoid identified (Strand et al., 1998).

Apart from tested in animal models, fucoxanthin was also administrated to humans in different experiments where its metabolization was evaluated. One of the experiments consisted of the administration of a concentration of 6.1 mg of fucoxanthin (extracted from wakame) for a week while the other one provided a unique dose of 31 mg of fucoxanthin (obtained from Kombu). Fucoxanthinol was detected on blood tests in both experiments, in fact in the latter assay displayed higher relative amounts of fucoxanthinol in humans than in animals whereas amarouciaxanthin A was absent (Asai et al., 2008; Hashimoto et al., 2012). But an interesting observation was provided by the second assay where human data was compared against the results obtained for mice. They concluded that the administrated dose for humans was seven times lower while the fucoxanthinol concentration in plasma was just a third or a half of that in mice. Data obtained from other experiments

**Table 1**
Fucoxanthin characteristics.

| | |
|---|---|
| ***Molecular formula:*** | $C_{42}H_{58}O_6$ |
| ***IUPAC Name:*** | [(1S,3R)-3-hydroxy-4-[(3E,5E,7E,9E,11E,13E,15E)-18-[(1S,4S,6R)-4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl]-3,7,12,16-tetramethyl-17-oxooctadeca-1,3,5,7,9,11,13,15-octaenylidene]-3,5,5-trimethylcyclohexyl] acetate |
| ***Molecular weight:*** | 658.9 g/mol |
| ***Density:*** | 1.1 ± 0.1 g/cm3 |
| ***Boiling Point:*** | 764.1 ± 60.0 °C at 760 mmHg |
| ***Flash Point:*** | 223.0 ± 26.4 °C |

yielded similar results, showing that a dietary supplement of 0.024 mg of fucoxanthin per kilo and per day was enough to reduce abdominal WAT in humans (Abidov et al., 2010) while for achieving the same results in obese mice a dose higher than 100 mg of fucoxanthin per kilo and per day was required administrated (Airanthi et al., 2011; Maeda et al., 2009). The lower concentration of fucoxanthin or fucoxanthinol detected or the high doses needed for having effects on mice may be due to the faster metabolism of small and short-lived animals as well as to the differential fucoxanthin-like molecules absorption rates of each species (Mordenti, 1986). Instead, the lack of arouciaxanthin A in human plasma may be owed to the lack of information about the metabolic pathway that fucoxanthin follows in different organs which may hinder the quantification of the final metabolic compounds. As far as scientific works get published additional metabolites of fucoxanthin are disclosed. This is the case of a study in which besides fucoxanthinol, halocynthiaxanthin have been isolated from *U. pinnatifida* (Fig. 1D) (Sachindra et al., 2007). Many factors remain to be studied in the metabolism of the fucoxanthin, thus the research on the metabolic pathway of fucoxanthin and the study of these compounds is really interesting since it has been proved that, at least, fucoxanthinol can produce even greater functional effects than fucoxanthin (Sun et al., 2019).

## 2. Extraction methods

The development of fucoxanthin extraction techniques with high-efficiency rates is one of the main goals of the industry involved in the production of natural pigments.

The most common technique for obtaining fucoxanthin is through maceration extraction (ME, Table 2). Maceration consists of a solid/liquid extraction in which different variables are evaluated: biomass-solvent ratio, type, and percentage of solvent, or time and temperature of incubation. Usually, these parameters need to be optimized under different combinations for which is useful a response surface methodology (RSM) (Pinela et al., 2019). Temperatures and times of extraction tested for fucoxanthin were very variable (from 4 °C up to 65 °C, during 15 min–96 h) and solvents too (methanol, acetone, and ethanol, being the most commons) (Table 2). Among the scientific literature reviewed the best yield result obtained with ME for macroalgae has been accomplished with *Himanthalia elongata* using equal volumes of n-hexane, diethyl ether, and chloroform (18.6 mg/g DW) (Rajauria and Abu-Ghannam, 2017). Whereas the maximum yield in microalgae was obtained with *Phaeodactylum tricornutum* and ethanol (57.4 mg/g DW) however, in this experiment the main key was the modification of the growing parameters (McClure et al., 2018) (Table 2). Other innovative techniques imply the use of expensive instruments or reagents such as for enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), or supercritical fluid extraction (SFE). EAE permits to hydrolyze cellulose walls to facilitate the exit of the pigment from the cell A study using *Fucus vesiculosus* showed that the best conditions were enzyme-to-water ratio 0.52%, seaweed-to-water ratio 5.37% and enzyme incubation time 3 h

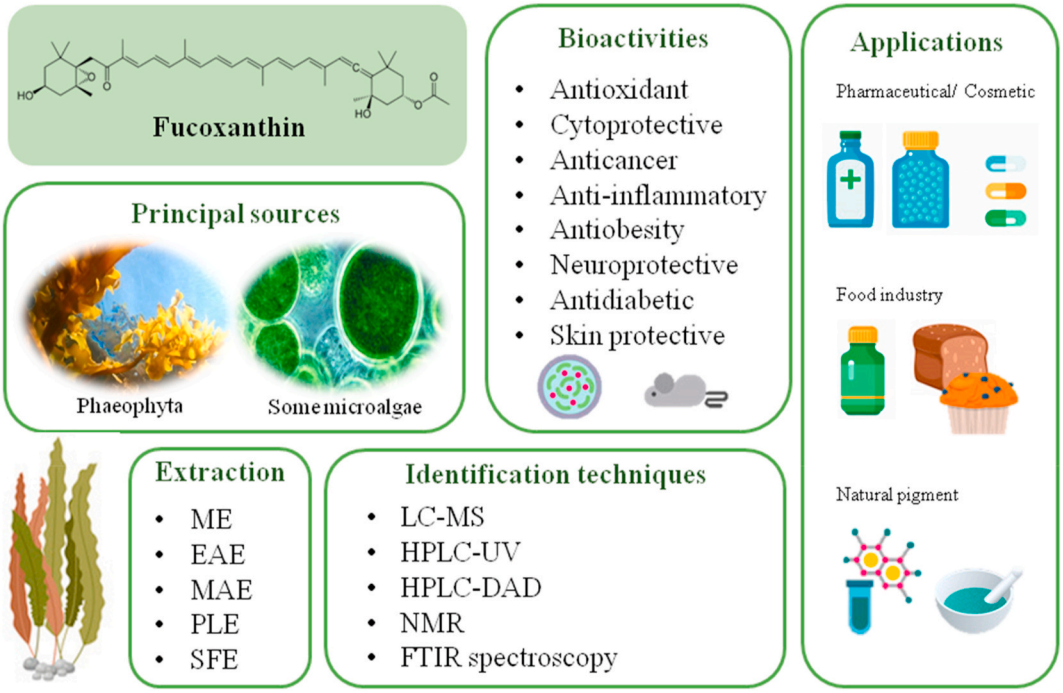


**Fig. 2.** The main sources of fucoxanthin are brown algae (Phaeophytes) and some microalgae. Several techniques have been employed to extract this compound, such as maceration extraction (ME), enzyme assisted extraction (EAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE) or supercritical fluid extraction (SFE). Following the extraction, the identification step has been carried out using different chromatographic and spectroscopic techniques. Fucoxanthin has gained attention in the last decades, due to the wide variety of attributed beneficial activities, such as antioxidant, anti-inflammatory, anticancer or neuroprotective effects. These bioactivities are interesting for its diverse applications in the industry, including the development of innovative pharmaceutical, cosmetic and food products. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**
Algae species described as source of fucoxanthin, solvent used for specific extraction techniques, detection methods employed for determining its presence and quantification values (fucoxanthin concentration are expressed in mg/g of dry weight, DW, or where not detected, nd, was indicated).

| ALGAE SPECIE | SOLVENT | EXTRACTION METHOD | DETECTION METHOD | FUCOXANTHIN (mg/g DW) | REFERENCE |
|---|---|---|---|---|---|
| **MACROALGAE** | | | | | |
| **Green** | | | | | |
| *Chara fragilis* | EtOH | SFE | HPLC-DAD–MS | nd | Fabrowska et al. (2016) |
| *Cladophora glomerata* | EtOH | SFE | HPLC-DAD–MS | nd | Fabrowska et al. (2016) |
| **Red** | | | | | |
| *Corallina officinalis* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.04 | Palermo et al. (1991) |
| *Corallina elongata* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.02 | Palermo et al. (1991) |
| *Euchema cottoni* | EtOH | ME | HPTLC | 0.94 | Agatonovic-Kustrin et al. (2018) |
| *Jania sp.* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.08 | Palermo et al. (1991) |
| **Brown** | | | | | |
| *Alaria crassifolia* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.1 | Terasaki et al. (2009) |
| *Alaria esculenta* | AcO 62.2% | ME (30°, 36.5 min) | HPLC-DAD | 0.87 | Shannon and Abu-Ghannam (2017) |
| *Analipus japonicas* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.40 | Terasaki et al. (2009) |
| *Ascophylum nodossum* | EtOH | VAE | HPLC-PDA | 0.022 | (Nunes et al., 2019) |
| *Cladosiphon okamuranus* | MeOH | ME (RT, 1h) | HPLC-DAD | 0.27 | (Mise, Ueda, & Yasumoto, 2011) |
| *Corallina elongate* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.02 | Palermo et al. (1991) |
| *Corallina officinalis* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.04 | Palermo et al. (1991) |
| *Cystoseira hakodatensis* | Ch, MeOH | ME | HPLC-DAD | 2.01 | (Susanto, Fahmi, Abe, Hosokawa, & Miyashita, 2016) |
| *Cystoseira Hakodatensis* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 2.01 | (Susanto et al., 2016) |
| *Cystoseira hakodatensis* | MeOH | ME (RT, 12h) | HPLC-PDA | 2.40 | Terasaki et al. (2009) |
| *Cystoseira hakodatensis* | Ch/MeOH (1:2) | ME (RT, 1h) | HPLC-DAD | 3.47 | Nomura et al. (2013) |
| *Desmarestia viridis* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.10 | Terasaki et al. (2009) |
| *Dictyopteris australis* | AcO | ME (4 °C, 12h) | Spec | 0.23 | (Verma, Kumar, Mishra, & Sahoo, 2017) |
| *Dictyota dichotoma* | EtOH | ME | HPTLC | 0.44 | () |
| *Dictyota dichotoma* | EtOH | VAE | HPLC-PDA | 0.60 | (Nunes et al., 2019) |
| *Dictyota dicotoma* | AcO | ME (4 °C, 12h) | Spec | 0.18 | (Verma et al., 2017) |
| *Dictyota dicotoma* | MeOH | ME (RT, 24h) | HPLC-PDA | 6.42 | (S. J. Heo et al., 2010) |
| *Ecklonia kurome* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 1.68 | (Susanto et al., 2016) |
| *Eisenia bicyclis* | EtOH | PLE | HPLC-PDA | 0.42 | Shang et al. (2011) |
| *Euchema cottoni* | EtOH | ME | HPTLC | 0.94 | Agatonovic-Kustrin et al. (2018) |
| *Fucus distichus* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.90 | Terasaki et al. (2009) |
| *Fucus distichus* | AcO | ME | Spec | 0.16 | (Seely, Duncan, & Vidaver, 1972) |
| *Fucus serratus* | EtOH | SFE | HPLC-DAD | 2.18 | (Heffernan et al., 2016b) |
| *Fucus serratus* | Hx/AcO (70:30) | ME | HPLC-DAD | 3.57 | (Heffernan et al., 2016b) |
| *Fucus vesiculosus* | EtOH | VAE | HPLC-PDA | 0.02 | (Nunes et al., 2019) |
| *Fucus vesiculosus* | AcO | VAE | HPLC-DAD | 0.70 | Shannon and Abu-Ghannam (2017) |
| *Fucus vesiculosus* | W | EAE | HPLC-UV, LC-MS | 0.66 | Shannon and Abu-Ghannam (2018) |
| *Himanthalia elongata* | n-Hx, DE, Ch | ME | LC-MS, HPLC, $^{1}$H NMR | 18.60 | (Rajauria et al., 2017) |
| *Hizikia fusiformis* | MeOH | ME | HPLC-DAD | 0.02 | Kanazawa et al. (2008) |
| *Ishige okamurae* | MeOH | ME | HPLC-DAD | nd | (Kanda et al., 2014) |
| *Iyengaria stellate* | AcO | ME (4 °C, 12h) | Spec | 0.18 | (Verma et al., 2017) |
| *Jania sp.* | DCM | ME (RT) | HPLC, $^{1}$H NMR, $^{13}$C NMR | 0.08 | Palermo et al. (1991) |
| *Kjellmaniella crassifolia* | MeOH | ME (RT, 15 min) | HPLC-DAD | 0.15 | Foo et al. (2017) |
| *Laminalia japonica* | MeOH | ME | HPLC-DAD | 0.19 | Kanazawa et al. (2008) |
| *Laminaria digitata* | AcO 62.2% | ME (30°, 36.5 min) | HPLC-DAD | 0.65 | Shannon and Abu-Ghannam (2017) |
| *Laminaria japonica* | Hp, AcO, W | MAE | LC-MS, HPLC, $^{1}$H NMR | 0.04 | Xiao et al. (2012) |
| *Laminaria religiosa* | MeOH | ME (RT, 96h) | HPLC-DAD, $^{1}$H NMR, $^{13}$C NMR | 0.24 | (Mori et al., 2004) |
| *Laminaria saccharina* | AcO | ME | Spec | 0.24 | (Seely et al., 1972) |
| *Leathesia difformis* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.30 | Terasaki et al. (2009) |
| *Lobophora variegata* | AcO | ME (4 °C, 12h) | Spec | 0.23 | (Verma et al., 2017) |
| *Melanosiphon intestinalis* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.90 | Terasaki et al. (2009) |
| *Myagropsis myagroides* | MeOH | ME (RT, 24h) | HPLC-PDA | 9.01 | (S. J. Heo et al., 2010) |
| *Padina australis* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 1.29 | (Susanto et al., 2016) |
| *Padina gymnospora* | AcO | ME (4 °C, 12h) | Spec | 0.44 | (Verma et al., 2017) |
| *Padina minor* | EtOH | ME | HPTLC | 0.50 | Agatonovic-Kustrin et al. (2018) |
| *Padina pavonica* | EtOH | ME | HPTLC | 0.43 | Agatonovic-Kustrin et al. (2018) |
| *Padina tetrastromatica* | AcO | ME (4 °C, 12h) | Spec | 0.41 | (Verma et al., 2017) |
| *Padina tetrastromatica* | EtOH | UAE | HPLC-DAD | 0.75 | (Raguraman et al., 2018b) |
| *Petalonia binghamiae* | MeOH | ME (RT, 48h) | HPLC-DAD, $^{1}$H NMR, $^{13}$C NMR | 0.59 | (Mori et al., 2004) |
| *Saccharina japonica* | MeOH | ME (RT, 15 min) | HPLC-DAD | 0.07 | Foo et al. (2017) |
| *Saccharina japonica* | n-Hx | SAE (40 °C, 16h) | HPLC-DAD | 0.45 | |
| *Saccharina sculpera* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.70 | Terasaki et al. (2009) |

(*continued on next page*)

**Table 2** (*continued*)

| ALGAE SPECIE | SOLVENT | EXTRACTION METHOD | DETECTION METHOD | FUCOXANTHIN (mg/g DW) | REFERENCE |
|---|---|---|---|---|---|
| *Sargassum binderi* | MeOH | ME (RT, 12h x2) | HPLC-DAD | 0.73 | Jaswir, Noviendri, Salleh, & Miyashita (2012) |
| *Sargassum confusum* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.60 | Terasaki et al. (2009) |
| *Sargassum crassifolium* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 1.64 | (Susanto et al., 2016) |
| *Sargassum duplicatum* | MeOH | ME (RT, 12h x2) | HPLC-DAD | 1.01 | (Jaswir et al., 2012) |
| *Sargassum fulvellum* | MeOH | ME | HPLC-DAD | 0.01* | Kanazawa et al. (2008) |
| *Sargassum fusiforme* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.10 | Terasaki et al. (2009) |
| *Sargassum fusiforme* | AcO/EtOH (1:40) | ME (65 °C, 80 min) | Spec | 2.62 | (J. Li et al., 2019) |
| *Sargassum fusiforme* | Hp, AcO, W | MAE | LC-MS, HPLC, $^{1}$H NMR | 0.02 | Xiao et al. (2012) |
| *Sargassum horneri* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 2.12 | (Susanto et al., 2016) |
| *Sargassum horneri* | MeOH | ME (RT, 12h) | HPLC-PDA | 3.70 | Terasaki et al. (2009) |
| *Sargassum horneri* | Ch/MeOH (1:2) | ME (RT, 1h) | HPLC-DAD | 4.49 | Nomura et al. (2013) |
| *Sargassum horneri* | $CO_2$, EtOH | SFE (45°, 250 bar) | HPLC-DAD | 0.77 | Sivagnanam et al. (2015) |
| *Sargassum japonica* | $CO_2$, EtOH | SFE (45°, 250 bar) | HPLC-DAD | 0.41 | Sivagnanam et al. (2015) |
| *Sargassum linearifolium* | AcO | ME (4 °C, 12h) | Spec | 0.38 | (Verma et al., 2017) |
| *Sargassum muticum* | AcO | ME | Spec | 0.29 | (Seely et al., 1972) |
| *Sargassum plagiophyllum* | AcO/MeOH (7:3) | ME (ice, 15 min) | HPLC | 0.71 | (Jaswir et al., 2013) |
| *Sargassum polycystum* | EtOH | ME | HPTLC | 0.41 | Agatonovic-Kustrin et al., 2018 |
| *Sargassum siliquastrum* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 1.99 | (Susanto et al., 2016) |
| *Sargassum thunbergii* | MeOH | ME (RT, 12h) | HPLC-PDA | 1.80 | Terasaki et al. (2009) |
| *Sargassum vulgare* | EtOH | VAE | HPLC-PDA | 0.40 | (Nunes et al., 2019) |
| *Scytosiphon lomentaria* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.50 | Terasaki et al. (2009) |
| *Scytosiphon lomentaria* | MeOH | ME (RT, 96h) | HPLC-DAD, $^{1}$H NMR, $^{13}$C NMR | 0.56 | (Mori et al., 2004) |
| *Silvetia babingtonii* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.70 | Terasaki et al. (2009) |
| *Spatoglossum asperum* | AcO | ME (4 °C, 12h) | Spec | 0.58 | (Verma et al., 2017) |
| *Sphaerotrichia divaricata* | MeOH | ME (RT, 12h) | HPLC-PDA | 0.20 | Terasaki et al. (2009) |
| *Stoechospermum marginatum* | AcO | ME (4 °C, 12h) | Spec | 0.37 | (Verma et al., 2017) |
| *Turbinaria ornata* | Ch/MeOH (1:2) | ME (RT, 2h) | HPLC-DAD | 1.27 | (Susanto et al., 2016) |
| *Turbinaria spp.* | AcO | ME (4 °C, 12h) | Spec | 0.43 | (Verma et al., 2017) |
| *Turbinaria turbinata* | AcO/MeOH (7:3) | ME (ice, 15 min) | HPLC | 0.59 | (Jaswir et al., 2013) |
| *Undaria pinnatifida* | MeOH | ME (RT, 96h) | HPLC-DAD, $^{1}$H NMR, $^{13}$C NMR | 2.67 | (Mori et al., 2004) |
| *Undaria pinnatifida* | Hp, AcO, W | MAE | LC-MS, HPLC, $^{1}$H NMR | 0.90 | Xiao et al. (2012) |
| *Undaria pinnatifida* | MeOH | ME (RT, 1h) | HPLC-DAD | 2.08 | Fung et al. (2013) |
| *Undaria pinnatifida* | MeOH | ME (RT, 1h) | HPLC-DAD | 4.96* | Fung et al. (2013) |
| *Undaria pinnatifida* | EtOH | ME (RT, 1h) | HPLC-DAD | 0.07* | Billakanti et al. (2013) |
| *Undaria pinnatifida* | EtOH | ME (RT, 1h) | HPLC-DAD | 0.70 | Billakanti et al. (2013) |
| *Undaria pinnatifida* | $CO_2$, EtOH | SFE (50°, 200 bar) | HPLC-UV | >0.01 | (Roh et al., 2008) |
| *Undaria pinnatifida* | $CO_2$, EtOH | SFE (60°, 400 bar) | HPLC-UV | 0.99 | Kanda et al. (2014) |
| *Undaria pinnatifida* | EtOH | PLE (12h, 78°) | HPLC-UV | 0.05 | Kanda et al. (2014) |
| *Zonaria tournefortii* | EtOH | VAE | HPLC-PDA | 0.80 | (Nunes et al., 2019) |
| **MICROALGAE** | | | | | |
| *Amphora sp.* | AcO | ME (ice) | HPLC-PDA | 1.21** | (Ishika et al., 2017) |
| *Chaetoceros calcitrans* | DE | UAE | HPLC-DAD | 4.49 | Foo et al. (2015) |
| *Chaetoceros calcitrans* | DCM | UAE | HPLC-DAD | 5.25 | Foo et al. (2015) |
| *Chaetoceros calcitrans* | MeOH | ME (RT, 15 min) | HPLC-DAD | 5.13 | Foo et al. (2017) |
| *Chaetoceros gracilis* | EtOH | ME (RT, 1h) | HPLC-DAD | 2.24 | (S. M. Kim, Kang, Kwon, Chung, & Pan, 2012) |
| *1.1.1. Chaetoceros muelleri* | AcO | ME (ice) | HPLC-PDA | 2.92** | (Ishika et al., 2017) |
| *Chrysotila carterae* | AcO | ME (ice) | HPLC-PDA | 1.04** | (Ishika et al., 2017) |
| *Cylindrotheca closterium* | AcO | MAE | HPLC | 4.24 | (Pasquet et al., 2011) |
| *Isochrysis aff. galbana* | EtOH | ME (RT, 1h) | HPLC-DAD | 18.23 | (S. M. Kim, Kang, et al., 2012) |
| *Isochrysis galbana* | EtOH | ME (RT, 1h) | HPLC-DAD | 6.04 | (S. M. Kim, Kang, et al., 2012) |
| *Isochrysis galbana* | MeOH | ME (RT, 15 min) | HPLC-DAD | 2.19 | Foo et al. (2017) |
| *Mallomonas sp.* | Ch, MeOH, EtOH, AcON | ME (RT, 20 min) | HPLC-DAD | 26.60 | (Petrushkina et al., 2017) |
| *Navicula- sp.* | AcO | ME (ice) | HPLC-PDA | 1.49** | (Ishika et al., 2017) |
| *Nitzschia sp.* | EtOH | ME (RT, 1h) | HPLC-DAD | 4.92 | (S. M. Kim, Kang, et al., 2012) |
| *Odontella aurita* | EtOH | ME (RT, 1h) | LC-DAD-MS | 18.47 | Xia et al. (2013) |
| *Odontella sinensis* | MeOH | ME (RT, 15 min) | HPLC-DAD | 1.18 | Foo et al. (2017) |
| *Phaeodactylum tricornutum* | EtOH | ME (45 °C, 2h) | HPLC | 3.51 | (F. L. Li et al., 2018) |
| *Phaeodactylum tricornutum* | EtOH | ME (RT, 1h) | HPLC-DAD | 8.55 | (S. M. Kim, Kang, et al., 2012) |
| *Phaeodactylum tricornutum* | EtOH | ME | HPLC-PDA | 57.40 | McClure et al. (2018) |
| *Phaeodactylum tricornutum* | EtOH | ME (RT, 30 min) | HPLC-UV, LC-MS, $^{1}$H NMR, $^{13}$C NMR | 15.71 | (S. M. Kim, Jung, et al., 2012) |
| *Pheodactylum tricornutum* | AcO | ME (ice) | HPLC-PDA | 1.87** | (Ishika et al., 2017) |

(*continued on next page*)

**Table 2** (*continued*)

| ALGAE SPECIE | SOLVENT | EXTRACTION METHOD | DETECTION METHOD | FUCOXANTHIN (mg/g DW) | REFERENCE |
|---|---|---|---|---|---|
| *Phaeodactylum tricornutum* | MeOH | ME (RT, 15 min) | HPLC-DAD | 0.07 | Foo et al. (2017) |
| *Skeletonema costatum* | MeOH | ME (RT, 15 min) | HPLC-DAD | 0.36 | Foo et al. (2017) |
| *Tisochrysis lutea* | AcO | ME (ice) | HPLC-PDA | 2.05** | (Ishika et al., 2017) |

**Solvent:** Ethanol (EtOH); Methanol (MeOH); Acetone (AcO); Chloroform (Ch); Hexane (Hx); Dichloromethane (DCM); Heptane (Hp); Water (W); n-Hexane (n-Hx); Diethyl ether (DE); Acetonitrile (AcN).

**Extraction method:** Supercritical fluid extraction (SFE); Supercritical $CO_2$ Extraction; Ultrasonic assisted extraction (UAE); Maceration extraction (ME); Pressurized liquid extraction (PLE); Microwaves assisted extraction (MAE); Vortex-assisted solid-liquid micro-extraction (VAE), Enzyme-assisted extraction (EAE), Soxhlet assisted extraction (SAE).

**Detection method:** High Performance Liquid Chromatography (HPLC); HPLC with diode array detector (HPLC-DAD); HPLC with ultraviolet detector (HPLC-UV); HPLC-DAD coupled to Mass Spectrometry (HPLC-DAD–MS); Liquid Chromatography Mass Spectrometry (LC-MS); LC with DAD and MS (LC-DAD-MS); High Performance Thin Layer Chromatography (HPTLC); Proton nuclear magnetic resonance ($^1$H NMR); Carbon-13 nuclear magnetic resonance ($^{13}$C NMR); Spectrophotometry (Spec).

**Fucoxanthin content:** not determined: fucoxanthin was found but not quantified (nd); DW: Dry Weight; Fresh Wight (* FW); Ash Free Dry Wight (** AFDW).

(Shannon & Abu-Ghannam, 2018).MAE applied for recovering fucoxanthin has been revealed a great approach in a study carried out using different solvents in *Laminaria japonica*, *Undaria pinnatifida*, and *Sargassum fusiforme*. The optimized parameters (solvent:sample ratio of 10:1 mL/g, 50 °C,10 min) provided high extraction efficiencies: 5, 109, and 2 mg/100 g, respectively (Xiao et al., 2012). The application of PLE for *Undaria pinnatifida* using Soxhlet with ethanol (78 °C, 12h) yielded of 50 μg/g DW of fucoxanthin (Kanda et al., 2014) while when applied to *Eisenia bicyclis* (110 °C, 90% ethanol, 5 min) using an accelerated solvent extractor the efficiency increased nearly 10 times with a yield of 0.42 mg of fucoxanthin/g DW (Shang et al., 2011). For SFE applied for fucoxanthin extraction the mostly used solvent is $CO_2$ with ethanol as entrainer to improve the method efficiency. ASFE-based work compared the application of $CO_2$ and $CO_2$+ethanol for *Undaria pinnatifida*. The optimized method (70 °C,400 bar, 3 h) using just $CO_2$ achieved a fucoxanthin yield of 60.12 μg/g. Whereas the method based in the co-elution of ethanol as entrainer (60 °C, 400 bar, 3 h) reached an extraction efficiency 16 times higher (994.53 μg of fucoxanthin/g DW) (Kanda et al., 2014). SFE using just (45 °C, 250 bar, 2 h) was also tested in *Saccharina japonica* and *Sargassum horneri* and allowed the recovery or rich-fucoxanthin oil fractions with respective 0.41 ± 0.05 and 0.77 ± 0.07 mg/g DW (Sivagnanam et al., 2015). Thus, temperature, pressure, and co-solvent need to be set up to obtain the best results with SFE.

As explained above, even though, the development of an optimized extraction procedure is essential to achieve a high-throughput, the culture conditions of algae are crucial (McClure et al., 2018). Additionally, the selection of an adequate quantification technique will provide more accurate results. The most utilized ones are the liquid chromatography mass spectrometry (LC-MS) for a mass-to-charge ratio ($m/z$)-based identification, high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) or diode-array detector (DAD) for the identification based on a maximum absorbance peak at 446 nm or the nuclear magnetic resonance (NMR) for determining its structure (Xia et al., 2013).

## 3. Biological activities

In the last decades, numerous studies have reported the wide range of biological activities of fucoxanthin, such as radical scavenging, anti-inflammatory, anti-tumor, anti-obesity, neuroprotective, and many others as reported in Table 3 (Chuyen & Eun, 2017).

### 3.1. Antioxidant activity

The excellent antioxidant properties of fucoxanthin against oxidative stress have been confirmed throughout numerous studies. The unique chemical structure of fucoxanthin possesses an allenic bond and an acetyl functional group which are considered to be responsible for its antioxidant properties (Guvatova et al., 2020; Liu et al., 2011; Miyashita et al., 2020). Fucoxanthin has been shown to scavenge different free radicals, like DPPH, ABTS, hydrogen peroxide, hydroxyl radical, superoxide anion and singlet oxygen, and also the 12-doxyl-stearic acid and nitrobenzene with linoleic acid (Liu et al., 2011; Ristivojević et al., 2021). For DPPH, its radical scavenging activity may be quantified in terms of effective or inhibitory concentrations (EC or IC, respectively). The concentration required to obtain a 50% antioxidant effect ($EC_{50}$) is often used to express the antioxidant capacity and allows the comparison among different compounds. Whereas the concentration of the sample required inhibiting 50% of DPPH radicals usually permits the comparison against other assays (Barros et al., 2011; Lopes et al., 2018). Fucoxanthin exhibited an $EC_{50}$ for DPPH scavenging of 165 μM while its two derivative compounds, fucoxanthinol and halocynthiaxanthin, presented an $EC_{50}$ of 154, and 826 μM, respectively (Sachindra et al., 2007). A recent study estimated that fucoxanthin extracted from the diatom *Phaeodactylum tricornutum,* presented an $IC_{50}$ of 201 μg/mL in DPPH assay. FRAP assay revealed that fucoxanthin is equal to 65 mmol $Fe^{2+}$ per gram (Neumann et al., 2019). The antioxidant activity of fucoxanthin has been assessed in numerous studies *in vitro* using animal and human cell cultures. For animal cell lines the effect of fucoxanthin was tested in different tissue cells from diverse species, such as the kidney fibroblast cells from monkey (Vero), the murine hepatic cells from mouse (BNL CL.2) or the adipocytes from mouse (3 T3-L1). In kidney cells Vero, fucoxanthin isolated from *Sargassum siliquastrum* inhibited the oxidative damage caused by $H_2O_2$, reduced intracellular levels of reactive oxygen species (ROS), DNA fragmentation and apoptotic processes (Heo et al., 2008). Similar results were obtained when fucoxanthin (1–20 μM) was tested in hepatocyte cells, BNL CL.2, previously treated with ferric nitrilotriacetate, a compound described to cause apoptosis. In this assay, fucoxanthin reduced the production of intracellular ROS, DNA damage and increased the levels of glutathione, with an important role in the cellular defense against oxidants and the maintenance of the redox cell homeostasis highly related with the prevention of apoptotic processes (Liu et al., 2011). Following this trend, fucoxanthin was demonstrated to inhibit lipid accumulation and ROS formation in 3 T3-L1 mouse adipocytes (Jang et al., 2018). Fucoxanthin also exhibited antioxidant effects on human cell lines with similar results than those observed for animal models. On fibroblasts irradiated with UVB, fucoxanthin protected cells from oxidative damage, reducing the levels of intracellular ROS and DNA damage in a dose-dependent manner (Jin Heo & Jeon, 2009; Wijesinghe & Jeon, 2011). The reduction on the production of intracellular ROS promoted by fucoxanthin isolated from *Laminaria japonica* (50 μg/mL) was also demonstrated in retinal pigment epithelium cells exposed to damage by excessive visible-light radiation, reaching almost normal levels and no cytotoxic effects (Liu et al., 2016). The same reduction on the oxidative damage, caused by $H_2O_2$, was produced when fucoxanthin (5 μM) was applied to

**Table 3**
Summary of fucoxanthin bioactivities and effects.

| ACTIVITIES | MODEL | REFERENCES |
|---|---|---|
| ***Antioxidant*** | DPPH, ABTS, hydrogen peroxide, hydroxyl radical, superoxide anion and singlet oxygen. | (Liu et al., 2011; Wang, Park, et al., 2018a) |
| | DPPH. | Sachindra et al. (2007) |
| | FRAP assay. | Neumann et al. (2019) |
| | Monkey kidney fibroblast cells (Vero). | Heo et al. (2008) |
| | Inhibit lipid accumulation and ROS formation *in vitro* 3 T3-L1 mouse adipocytes. | Jang et al. (2018) |
| | SH-SY5Y human neuroblastoma cells, primary cerebellar granule neurons and human hepatic L02 cells. | (X. Wang, Park, et al., 2018a; Yu et al., 2017) |
| | Protected against oxidative stress by reducing the levels of lipid peroxidation and increasing the activity of catalase and glutathione transferase. | Sangeetha, Bhaskar, & Baskaran (2009) |
| ***Cytoprotective*** | Human fibroblasts | (S. J. Heo & Jeon, 2009b) |
| | Human hepatic LO2 cell | Wang, Park, et al. (2018b) |
| | Pre-treated *in vitro* Hep G2 human liver cancer cells | Jang et al. (2018) |
| | Protected photoreceptor cells (rod cells and cone cells) on an *in vivo* rabbit model | Liu et al. (2016) |
| ***Anticancer*** | Inhibit growth and induce apoptosis on B16-F10 melanoma, BNL CL.2 embryonic liver and Neuro2a neuroblastoma murine cell lines | Chuyen and Eun (2017) |
| | GOTO, human colon carcinoma WiDr, HepG2, Burkitt's and Hodgkin's lymphoma and T-cell leukemia cells | Satomi (2017) |
| | Caco-2, HT-29, DLD-1 | Hosokawa et al. (2004) |
| | Human cervical cancer SiHa cells | (Y. Jin et al., 2018) |
| ***Anti-inflammatory*** | Inhibiting inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein expression | (S. Heo et al., 2010) |
| | Inhibition of mRNA expressions of interleukin-6, plasminogen activator inhibitor-1 (PAI-1), COX-2 and iNOS in Raw 264.7 macrophages co-cultures, and in 3 T3-L1 adipocyte cells | (L. Wang, Park, et al., 2018a) |
| ***Antiobesity*** | Obese mice and rats | Okada et al. (2008) |
| ***Neuroprotective*** | Inhibition in the expression of N-myc, a proto-oncogene protein, as well as proliferation of GOT0 cells | Okuzumi et al. (1990) |
| | Activation of Nrf2 and its target genes | Hu et al. (2018) |
| | Nrf2-ARE and Nrf2-autophagy pathways in *in vivo* and *in vitro* models of Traumatic Brain Injury | Zhang et al. (2017) |
| ***Antidiabetic*** | Adrb3, GLUT4, MCP-1 | (L. Wang, Park, et al., 2018a) |
| | Supercharged mice, Adrb3, GLUT4, MCP-1 | Maeda et al. (2009) |
| | HRAR, RLAR, PTP1B, AGE | Jung et al. (2012) |
| | Diabetic mice | Maeda et al. (2007) |
| ***Skin protective*** | *In vivo* and *in vitro* studies | (Mio Matsui et al., 2016) |
| | Inhibitory effect on tyrosinase activity in guinea pigs | Shimoda et al. (2010) |
| | Human fibroblast cells | (S. J. Heo & Jeon, 2009a) |
| | | Ikeda et al. (2003). |

**Table 3** (*continued*)

| ACTIVITIES | MODEL | REFERENCES |
|---|---|---|
| ***Cardiovascular protection*** | Stroke-prone spontaneously hypertensive rats (SHRSP) | |
| | *Drosophila melanogaster* and *Caenorhabditis elegans* | Lashmanova et al. (2015) |
| | Transcriptome analysis | Moskalev et al. (2018) |
| | Normal human cells LECh4 (81) | Guvatova et al. (2020) |
| | Rat glioma cells model | Afzal et al. (2019) |
| ***Hepatoprotection*** | Mice fed a high-fat diet | Woo et al. (2010) Park et al. (2011) |
| ***Antiplasmodial*** | *Plasmodium falciparum* | Afolayan et al. (2008) |

SH-SY5Y human neuroblastoma cells, primary cerebellar granule neurons and human hepatic L02 cells (Wang, Park, et al., 2018a; Yu et al., 2017). The antioxidant activity of fucoxanthin demonstrated in so different cell models has been related to the enhancement of the expression levels of the nuclear factor erythroid 2-related factor 2 (Nrf2). The Nrf2 is a transcription factor related to the antioxidant mechanism, fundamental to protect the cell against oxidative damage. In the absence of cellular stress, Nrf2 is usually located in the cytoplasm and transcriptionally inactive. However, oxidative stress promotes its translocation to the nucleus which triggers the expression of stress-induced genes, such as those for the quinine oxidoreductase 1 (NQO1) or the heme oxygenase-1 (HO-1), two cytoprotective enzymes which defend the cell against stress factors. Dietary ingredients with antioxidant capacity have been reported as potential cell protectors from oxidative damage by modulating the Nrf2 pathway (Tang et al., 2014, pp. 13079–13087; X.; Wang, Park, et al., 2018a). In fact, one of these dietary compounds is fucoxanthin since it has been described to produce its antioxidant effects by altering this Nrf2 pathway (X. Wang, Park, et al., 2018a).

Finally, fucoxanthin has been found to present antioxidant effects *in vivo* very similar to those observed *in vitro*. The action of fucoxanthin was tested in animals with pathologies induced, such as the obese/diabetes KK-$A^y$ mice model. The treatment with 0.2% of fucoxanthin decreased lipid peroxidation levels of liver and abdominal adipose tissue and reduced the oxidative damage associated with diabetes and obesity (Rokkaku et al., 2013). When evaluated in rats with retinol deficiency, the results showed the same effects for fucoxanthin (0.83 μM): it protected against oxidative stress by reducing the levels of lipid peroxidation and increasing the activity of two antioxidant enzymes, catalase and glutathione transferase (Baskaran et al., 2009). Another pathology induced to rats administrating them diethylnitrosamine (DEN), the hepatocarcinogenesis, was treated with fucoxanthin. As demonstrated before, lower intracellular ROS levels and lipid peroxidation were detected for those rats feed with fucoxanthin. Besides lower liver stress markers were found and significant cellular antioxidant defenses were increased for fucoxanthin-fed rats (Jin et al., 2019).

### 3.2. Cytoprotective effect

Several studies have reported the protective effects of fucoxanthin in *in vitro* assays using different cell lines, in many cases related to its antioxidant properties, as previously exposed. The pre-treatment with fucoxanthin before exposing cells to different types of damage and stress has been demonstrated to restore the cell survival comparing to control cells. In different animal models, fucoxanthin displayed different protective routes against the damage that were caused by $H_2O_2$ or UVB prolonged radiation. In monkey kidney fibroblasts exposed to $H_2O_2$, the pre-treatment with fucoxanthin significantly increased in a dose-dependent manner the cell survival (Soo-Jin You-Jin et al., 2008). In the case of rat glioma cells treated with UVB, fucoxanthin supplementation before and after the UVB exposition increased significantly cell viability and reduced the DNA damage caused by the radiation and even

promoted differentiation to functional cells (Afzal et al., 2019). Similar experiments were developed using the same cell types and same damage reagents showing results according to those observed in animal models. On human fibroblasts, the treatment with fucoxanthin before the exposure to UVB caused an increment dose-dependent in cell survival, comparing to untreated cells, the maximum cell survival rate reached 81.47% with a concentration of 100 μM of the carotenoid (Heo & Jeon, 2009). As previously mentioned, the fucoxanthin pre-treatment (5 μM) of human L02 hepatic cells increased their viability after the exposition to $H_2O_2$. The authors suggested that the increment of Nrf2 expression levels leads to the activation of both cytoprotective enzymes, NQO1 and HO-1, which help to palliate oxidative damage (Wang, Park, et al., 2018a). Arachidonic acid and iron were used to stimulate oxidative damage and apoptosis in hepatic cells (Hep G2) pre-treated with fucoxanthin extracted from *Laminaria japonica*. The results showed that the fucoxanthin pre-treatment (30 μM/mL) allowed reducing cell death, mitochondria dysfunction and oxidative damage and triggered autophagy through the activation of AMP-activated protein kinase (AMPK) which promotes the removal of damaged cellular components representing a fundamental pathway for cell homeostasis (Jang et al., 2018). Concordant effects to those described above were observed in human umbilical vein endothelial cells exposed to oxidized LDL cell damage, confirming that protective effects of fucoxanthin are related to the AMPK pathway (Ou et al., 2019).

Several studies have reported the cytoprotective effects of fucoxanthin *in vivo* animal models. Topical application of a solution with fucoxanthin in hairless mice submitted to UVB-induced skin damage demonstrated to prevent wrinkle formation, epidermal hypertrophy and other sings of photo-aging and oxidative damage (Urikura et al., 2011). Mice were exposed to excessive UV radiation and the results showed that topic treatment with fucoxanthin prevented UV-induced sunburns and also cured four days sunburns (Matsui et al., 2016). Similarly, on an *in vivo* rabbit model submitted to intense light to induce retinal damage, it was found that fucoxanthin protected photoreceptor cells (rod cells and cone cells) (Liu et al., 2016). In the previously cited study, conducted on rats using DEN to stimulate hepatocarcinogenesis, it was demonstrated that fucoxanthin reverted significantly the cytotoxic effects of DEN when compared against rats without fucoxanthin administration (Jin et al., 2019). Finally, as observed in in vitro models, the oral administration of fucoxanthin (10 mg/kg) increased the levels of Nrf2 expression, activating the antioxidant defenses, and reduced the corneal damage caused by UVB radiation *in vivo* rats (Chen et al., 2019).

### 3.3. Anticancer activity

Fucoxanthin is considered an anticancer carotenoid and its activity has been demonstrated against a wide range of cancer cell lines, both human and animal. Several studies have reported that fucoxanthin exerts different anticancer mechanisms of action, such as inhibition of cell proliferation, cell cycle arrest, an increase of intracellular ROS, induction of apoptosis and anti-angiogenic effects (Bae et al., 2020; Chuyen & Eun, 2017; Cianciosi et al., 2018; Kumar et al., 2013; Ou et al., 2019; Satomi, 2017).

Fucoxanthin has been found to inhibit growth and induce apoptosis on mouse cell models such as melanoma (B16-F10), embryonic liver (BNL CL.2) and neuroblastoma murine cell lines (Neuro2a) (Kim et al., 2013; Kumar et al., 2013; Satomi, 2017). However, most of the *in vitro* assays have been performed using human cell lines. Fucoxanthin has been evaluated over a large number of cell lines such as human leukemia cells HL-60, human colorectal adenocarcinoma cell lines Caco-2, DLD-1 and HT-29, human colorectal carcinoma HCT116, human prostate cancer cell lines PC-3, DU145 and LNCaP, breast cancer MCF-7 and MDA-MB-231, human male umbilical cord fibroblasts HUC-Fm, urinary bladder cancer EJ-1, human neuroblastoma GOTO, human liver cancer Hep G2, hepatoma SK-Hep-1 and lung cancer H1299 cell line. Several studies have demonstrated the ability of fucoxanthin to induce cell-cycle arrest in numerous cancer lines, such as GOTO, human colon carcinoma WiDr, HepG2, Burkitt's and Hodgkin's lymphoma and T-cell leukemia cells. The results showed that fucoxanthin affected the expression of cellular molecules and cellular signal transduction pathways, which lead to cell cycle arrest (Chuyen & Eun, 2017; Kumar et al., 2013; Satomi, 2017). Recent studies have reported that fucoxanthin promoted apoptosis and reduced cell proliferation in bladder cancer T24 and EJ-1 cell lines, human glioma cancer U87 and U251 cell lines, human gastric adenocarcinoma MGC-803, human liver cancer Hep G2, and breast cancer MCF-7 and MDA-MB cell lines (Jin et al., 2018; Neumann et al., 2019; Yu et al., 2017). Fucoxanthin has been demonstrated to be more effective in promoting apoptosis than β-carotene and astaxanthin on the colorectal adenocarcinoma cell lines Caco-2, HT-29, and DLD-1 (Hosokawa et al., 2004). Fucoxanthin induced apoptosis on human gastric adenocarcinoma SGC-7901 or BGC-823 cells by JAK/STAT pathway, which is related to apoptosis, among other processes. Besides, SGC-7901 presented cell cycle arrest at phase S and BGC-823 at the G2/M phase (Yu et al., 2017). In human non-small cells, bronchopulmonary carcinoma NSCLC-N6 were observed apoptotic cells characteristic, whereas in human lung epithelial cells no cytotoxic effects were observed, indicating that fucoxanthin action is a target to carcinogenic cells. Fucoxanthin reduced cell proliferation of two human leukemia cell lines, erythromyeloblastoid leukemia cell line K562, and the human lymphoblastoid cell line TK6, and presents cytotoxic effects against K562 cells. These results showed that cytotoxic effects were not related to apoptosis, but deeper research is necessary to fully understand the molecular pathways involved in fucoxanthin cytotoxicity against this human leukemia cell lines (Almeida et al., 2018). In this sense, leukemia cells (HL-60) were treated with fucoxanthin that induced apoptosis through the production of ROS, reduced the levels of the anti-apoptotic Bcl-xL protein, triggered the cleavage of caspases and the consecutive inactivation of the poly-ADP-ribose polymerase. Similar results were observed in prostate cancer (PC-3) cells and cervical adenocarcinoma cells (HeLa), in which the fucoxanthin induced caspase-dependent apoptosis, modulated by changes in BAX/Bcl-2 proteins related to the apoptotic process (Chuyen & Eun, 2017; Kumar et al., 2013; Satomi, 2017). In human cervical cancer SiHa cells, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) administration with fucoxanthin promoted apoptosis through the suppression of PI3K/Akt and NF-κB pathways. These two pathways are strongly related to cell proliferation and therefore, to cancer (Jin et al., 2018). In the human glioma U251 cell line, the results showed that fucoxanthin extracted from microalgae *Nitzschia sp* induced apoptosis through the production of ROS and dysfunction of MAPKs and PI3K/AKT pathways (Wu et al., 2019).

But not only the fucoxanthin presents anticancer effects, its two metabolites fucoxanthinol, and halocynthiaxanthin have been reported to have anti-cancer activity, in many cases showing stronger capacity than fucoxanthin. In fact, when fucoxanthin and fucoxanthinol effects were tested on PC-3 cells, the $IC_{50}$ for fucoxanthinol was 2.0 μM while for fucoxanthin it was 3.0 μM (Kotake-Nara et al., 2015). Another study reported halocynthiaxanthin at 5 μg/mL, completely inhibited GOTO human neuroblastoma cell growth, while fucoxanthin, at the same concentration, caused an 88% reduction on cell growth. While on leukemia (HL-60), breast cancer (MCF-7) and colorectal adenocarcinoma (Caco-2) cells, both metabolite induced apoptosis in a stronger manner than fucoxanthin (Chuyen & Eun, 2017; Kumar et al., 2013). In another experiment, fucoxanthin and fucoxanthinol were displayed to induce apoptosis in both breast cancer MCF-7 and MDA-MB-231 cell lines. Nevertheless, only fucoxanthinol reduced the expression of some NF-κB pathway members in the MDA-MB-231 cell line (Chuyen & Eun, 2017; Kumar et al., 2013; Satomi, 2017). A recent study conducted on human adenocarcinoma colorectal cells demonstrated that fucoxanthinol induced anoikis, a type of apoptosis that occurs in anchorage-dependent cells when they detach from the extracellular matrix or neighbor cells (Terasaki, Iida, et al., 2019a).

Regarding *in vivo* studies, fucoxanthin administered in different ways has been demonstrated to inhibit many types of cancers. In mice, oral administration of fucoxanthin reduced duodenal and liver carcinogenesis growth and also the number of aberrant crypt foci in 1,2-dimethylhydrazine-submitted colon and azoxymethane-treated mice (Afrin et al., 2020; Kim et al., 2013; Kumar et al., 2013; Satomi, 2017). Similarly, mice submitted to two carcinogens using azoxymethane and dextran sodium sulphate were treated with fucoxanthin showing a reduced polyp formation and induced anoikis. The same author used BALB/c nu/nu mice to inject them colorectal cancer cells and treated them with fucoxanthin administrated via oral. Results showed that fucoxanthin reduced the proliferation of the subcutaneous tumors, without producing cytotoxic effects (Terasaki, Matsumoto, et al., 2019b). Some other studies reported that the administration of fucoxanthin suppressed tumor growth of primary effusion lymphoma, sarcomas, and osteosarcoma in mice (Chuyen & Eun, 2017; Kumar et al., 2013; Satomi, 2017). Intraperitoneal injection of fucoxanthin has been reported to inhibit tumor growth of melanoma (B16F10) and cervical adenocarcinoma (HeLa) cell lines implanted in *in vivo* mice models (Kim et al., 2013; Kumar et al., 2013; Satomi, 2017). Ultimately, the topic application of fucoxanthin, in combination with 12-O-tetradecanoylphorbol 13-acetate, inhibited skin tumor in mice (Chuyen & Eun, 2017; Kumar et al., 2013; Satomi, 2017).

Consequently, both *in vitro* and *in vivo* assays using fucoxanthin and its metabolites demonstrate that these compounds possess important anti-proliferative effects which may be focused on cancer cells. For instance, all these results point to fucoxanthin as a promising compound to control and treat cancer.

### 3.4. Anti-inflammatory activity

The anti-inflammatory potential of fucoxanthin isolated from a brown algae, *Myagropsis myagroides*, was analyzed through the inhibition of the production of nitric oxide (NO), one of the determinants of inflammation (Heo et al., 2010). This alga was treated using different solvents from which the extract obtained using chloroform was the one that showed a greater inhibitory effect of NO, being fucoxanthin the active substance detected. Fucoxanthin was capable of inhibiting inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein expressions, in addition to reducing the expressions of other pro-inflammatory factors such as mRNA, tumor necrosis factor-α (TNF-α) and interleukins-1b and 6.

Similarly, another research group discovered that both fucoxanthin and its primary metabolite, fucoxanthinol, were able to inhibit NO production, reducing inflammation caused by obesity. They also concluded that there was a significant inhibition of mRNA expressions of interleukin-6, plasminogen activator inhibitor-1 (PAI-1), COX-2 and iNOS in Raw 264.7 macrophages co-cultures, as well as in 3 T3-L1 adipocyte cells by both compounds (Wang, Park, et al., 2018a). Therefore, the possibilities of achieving very favorable and beneficial results with these compounds opens the door to possible and future therapeutic applications, since it could contribute to the fight against many inflammatory diseases or that involve inflammation.

A recent study also reports the potential protective effect of fucoxanthin on several inflammation-related diseases, due to fucoxanthin's strong antioxidant capacity and gut microbiota regulation. Some of the important molecules to this regulation include Nrf2, cAMP response element binding protein, extracellular signal-regulated kinase, adenosine monophosphate (AMP)-dependent protein kinase, Akt serine/threonine kinase/phosphatidylinositol-3-kinase, and peroxisome proliferator-activatedreceptorγcoactivator-1α (M. Liu et al., 2020).

### 3.5. Antiobesity activity

Obesity is becoming a worldwide health problem with an increasing number of affected people. This pathology appears when food ingested, and its proportional kilocalories (kcal), far exceeds the energy used to perform daily tasks and activities, so there is an excess of caloric intake that it takes accumulated in the body. Several conditions have been analyzed as possible triggers of this disease, such as metabolic or dietary factors. It has been also studied the capacity of some active compounds present in food since some of them can cause changes in metabolism by contributing to the imbalance of energy in tissues. Therefore, research on possible remedies against this condition is of great interest. One of the most promising strategies is the development of diet-induced adaptive thermogenesis through the positive regulation of mitochondrial uncoupling protein 1 (UCP1). In this sense, fucoxanthin is a key compound, since it is a food component that can favor such thermogenic adaptation by regulating UCP1 in both beige, white (WAT) and brown adipose tissues (BAT), and managing to increase daily energy expenditure (Miyashita et al., 2020; Miyashita & Hosokawa, 2017). In a study carried out in mice and rats, in which the weight of the WAT was analyzed, it was shown that the mice fed with fucoxanthin displayed a significantly lower weight than the control groups, fed with a diet not based on the contribution of this molecule (Maeda et al., 2008). Among reducing WAT, fucoxanthin has a systemic effect, reducing total body weight. Rodents fed with fucoxanthin displayed UCP1 expression in abdominal WAT that involved the oxidation of fatty acids and the consequent thermogenesis in WAT mitochondria. On the other hand, the consumption of fucoxanthin was able to lower blood glucose levels and plasma insulin. Moreover, and as a novel fact, adding fucoxanthin to the diet significantly increases hepatic docosahexaenoic acid (DHA) levels in rodents, the most important polyunsaturated fatty acid due to its functionality (Okada et al., 2008). Another work claimed that fucoxanthin obtained from *Undaria pinnatifida*, and its derivative fucoxanthinol, produced an inhibitory effect on the differentiation of preadipocytes into adipocytes. This effect may be mediated by the down-regulation of adipogenic transcription factors, such as peroxisome proliferator-activated receptor-c, and that achieves a decrease in adipose tissue content in the body (Maeda et al., 2006; Okada et al., 2008). Likewise, it has been suggested that such suppression of adipocyte differentiation can be generated due to the unique allenic bond present in the structure of fucoxanthin and its derivative. Other carotenoids with different types of substituents in their structures have been tested, and fail to produce the same effect (Kim & Pangestuti, 2011; Okada et al., 2008).

Therefore, all these capabilities and attributes associated with the intake of fucoxanthin support the importance of this bioactive carotenoid in the prevention of metabolic disorders, such as obesity. Nevertheless, deeper evaluations about the mechanism of action of this group are necessary for its further therapeutic application (Miyashita et al., 2020; Miyashita & Hosokawa, 2017).

### 3.6. Neuroprotective effect

In 20 years, neurodegenerative diseases are expected to be placed as the second leading cause of death in the world, even overcoming those caused by cancer (Ansari et al., 2010; Bjarkam et al., 2001). This is one of the reasons why scientists and researchers have given great importance to developing neuroprotective strategies and therapies in order to minimize the impact of these predictions. Neuroprotection can be defined as the mechanisms and procedures used to protect neuronal cells belonging to the central nervous system (CNS) against possible damage, apoptosis, loss of function or accelerated degeneration (Tucci & Bagetta, 2008). The substances capable of exerting beneficial effects are named neuroprotective agents and may have natural or synthetic origin. However, and as it happens in other fields, the administration of synthetic compounds is usually associated with side effects. In the case of the neuroprotector agents, the described side effects are drowsiness, fatigue, dry mouth, imbalance, anxiety or nervousness, among others (Narang et al., 2008; Pangestuti & Kim, 2010). In the last years, in order to prevent the apparition of these side effects, researchers have opted for

searching and studying the neuroprotective abilities of natural compounds. Algae are an emerging source of natural compounds with bioactive capabilities, thus their biomolecules are been evaluated in order to try to disclose compounds capable of generating neuroprotection (Pangestuti & Kim, 2011).

Fucoxanthin extracted and isolated from *Hizikia fusiformis*, a brown alga widely consumed in Korea, Japan, and China, has been demonstrated to inhibit the expression of N-myc, a proto-oncogene protein, as well as the proliferation of GOTO cells, a cell line responsible for human neuroblastoma. An amount of fucoxanthin equivalent to 1–10 g/mL is enough to inhibit the growth rate of such a cell line (GOTO) at 38%, decreasing the rate of progression of this neurodegenerative disease, so even though results using fucoxanthin are positive; it will take time to combat it (Okuzumi et al., 1990). Another study showed that fucoxanthin obtained from wakame also achieved a decrease in cellular damage of cortical neurons when hypoxia and oxygen reperfusion conditions were induced. Since an excessive amount of ROS usually occurs during these processes, the mechanism of action of fucoxanthin, as previously exposed, is associated with a radical scavenging activity, producing an antioxidant effect. This is especially interesting for the protection of neurites since they are very important components of neurons, necessary for neuronal development during the embryonic state and also in the adult human brain (Khodosevich & Monyer, 2010).

As explained before fucoxanthin has been also demonstrated to be implied on the activation of the Nrf2 pathway and its related genes, such as those for the cytoprotective enzymes NQO1 and HO-1, showing that it may protect the brain against ischemia/reperfusion (I/R) injury. In this study, oxidative stress was significantly alleviated upon fucoxanthin treatment, indicating that fucoxanthin might induce Nrf2/HO-1 activation and thereby leading to attenuation of oxidative stress from cerebral I/R injury. This study indicated that treatment with fucoxanthin effectively reduced middle cerebral artery occlusion-induced cerebral I/R injury and the apoptosis of oxygen-glucose deprivation and reoxygenation -treated neurons, and these effects were partly mediated by the activation of Nrf2/HO-1 signaling. Therefore, this assay concluded that fucoxanthin could be used as a potential supplementary ingredient against cerebral I/R injury in the future (Hu et al., 2018).

The neuroprotective effects of fucoxanthin through the Nrf2-pathway were further evaluated by different approaches. As in the case of the study were the possible role of Nrf2-antioxidant response elements (ARE) and Nrf2-autophagy pathways in the neuroprotective effects of fucoxanthin were examined in *in vivo* and *in vitro* models of Traumatic Brain Injury (TBI). This batch of experiments found that: fucoxanthin provided neuroprotection after TBI; specifically, it improved neurobehavioral performance, alleviated brain edema and decreased lesion volume. Besides the treatment using fucoxanthin decreased TBI-induced apoptosis and oxidative stress; attenuated apoptosis and oxidative stress through activation of the Nrf2-ARE and Nrf2-autophagy pathways (Zhang et al., 2017).

The global analysis of the results presented above allows confirming the neuroprotective capacity that some natural ingredients possess. The use of natural compounds is usually considered to produce less or non-unwanted side effects, which makes them very interesting compounds for including them in the formulation of new foods or drugs (Munekata et al., 2019, pp. 53–96). Natural products may represent novel and functional ingredients for the treatment and/or prophylaxis of neurodegenerative diseases. However, it is necessary to continue with the studies, to reach the successive clinical phases, more *in vivo* studies and, subsequently, in humans (Pangestuti & Kim, 2011).

### 3.7. Antidiabetic effect

Fucoxanthin has been confirmed as a substance capable of exerting an antidiabetic effect, for example, by regulating the level of blood glucose, as well as that of insulin, possibly exerting a promotional effect of the β3-adrenergic receptor (Adrb3) and glucose transporter 4 (GLUT4), as well as an inhibition of the expression of monocyte chemoattractant protein-1 (MCP-1) (Wang, Park, et al., 2018b). In fact, a fucoxanthin-rich lipids fraction obtained from wakame was described to possess anti-diabetic properties and demonstrated in mice with obesity induced by the hyperlipidemic diet (Bae et al., 2020). According to the results, plasma glucose and insulin levels increased significantly in mice fed with a high-fat diet, while they decreased in mice given fucoxanthin as a dietary supplement. The mechanism of action was associated with an increase in Adrb3 and GLUT4 receptors, and a decrease in MCP-1 expression (Maeda et al., 2009). Interestingly, the hypoglycemic effect of fucoxanthin is only observed in mice models with a dietary pathology induced, such as diabetes (Maeda et al., 2007), since overfed but healthy mice, it means not suffering from diabetes or obesity, the effect of fucoxanthin on reducing the glycemia was not observed (Maeda et al., 2009; Park et al., 2011; Woo et al., 2009). However, a decrease in postprandial glucose concentration, plasma insulin level and insulin resistance was observed in the overfed and obese mice (Maeda et al., 2006; Park et al., 2011). It was determined that the antidiabetic effect of this marine carotenoid may be mainly due to two possible biological mechanisms: the effect of fucoxanthin and its accumulated metabolites in visceral WAT, which regulate the release of cell mediators, and/or the regulation of the expression of GLUT4 and its appearance in the cell membrane (Miyashita et al., 2011, 2013)

Another work that analyzed the antidiabetic capacity of fucoxanthin, also extracted from wakame, showed different mechanisms of action. Results displayed that this compound was able to inhibit the activities of certain human enzymes, related to the metabolic diabetes disease, such as recombinant aldose reductase (HRAR), rat lens aldose reductase (RLAR), protein tyrosine phosphatase 1B (PTP1B), and advanced glycation end-product (AGE) formation (Jung et al., 2012).

### 3.8. Skin protective effect

Nowadays, the harmful effect of UV radiation is well-known and wide world recognized. Mild or moderate exposure to this radiation is enough to create damage to the skin, leading to photosensitivity, and even burns. Among the already cited properties, fucoxanthin has been also demonstrated to have a positive effect in preventing these damages caused by UV. In vitro and *in vivo* studies carried out with fucoxanthin obtained from wakame supported its skin protector feature. When fucoxanthin was tested in in vitro assay it was observed that it decreased the production of intracellular ROS, triggered after exposure of dermal fibroblasts to UV radiation, as much as other compounds with strong antioxidant capacity, such as N- acetylcysteine (NAC). *In vivo* results, showed a stronger efficiency of fucoxanthin since it had skin protective effect against burns and filaggrin disorder (filament aggregating protein) induced by radiation, while NAC or clobetasol (a drug from the group of corticosteroids) were unable to protect the dermal layers. The data obtained suggest that the dermo-protective capacity of fucoxanthin against burns induced by UV radiation can be explained through the promotion of filaggrin, which generates a protective dermal barrier, instead of its ROS-sensing effect (Matsui et al., 2016).

Fucoxanthin extracted from different sources, such as *Laminaria japan*, was also reported to exert an inhibitory effect on tyrosinase activity in *in vivo* assays performed on guinea pigs. Animals previously exposed to UV radiation and treated with fucoxanthin displayed a reduction of the harmful effects of irradiation. It was demonstrated that an oral administration of fucoxanthin produced a suppression of transcription of the melanogenesis factor, due to the inhibition of dermal mRNA expression related to this disease (Shimoda et al., 2010). The photoprotective capacity of the fucoxanthin has been also related to the remediation of DNA damage and its potent antioxidant activity, in human fibroblast cells (Heo & Jeon, 2009). Therefore, it can be concluded that oral administration of fucoxanthin, either as a food supplement or as a drug, can reduce or even prevent the negative effects induced by exposure to UV radiation, such as the appearance of

melanomas.

### 3.9. Geroprotective activity

The rate of aging and longevity is directly correlated with the organism's ability to effectively respond to exterior or physiological stress. Thus, an approach to increase lifespan is through the pharmacological activation of stress-defense mechanisms. Fucoxanthin has been evaluated as a potential geroprotective agent. A recent study analyzed the effects of fucoxanthin in the lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans*. The use of fucoxanthin decreased flies fecundity, increased the spontaneous locomotor activity and improve the resistance to oxidative stress which translated into an enhanced median and maximum lifespan of both species (Lashmanova et al., 2015). With the aim of developing potential drugs to prolong human life and prevent the development of age-related diseases, further studies were made to determine signaling pathways affected by this carotenoid both at molecular and systemic level. At the organism level, fucoxanthin improved nighttime sleep, fertility and intestinal barrier function. To determine the differentially expressed genes involved a transcriptome analysis was performed. Up to 17 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were involved. Those were related to longevity, such as mTOR, MAPK, Wnt, Hippo and Notch, translation, oxidative phosphorylation, autophagy, apoptosis, glycolysis, neurogenesis, immune response, sleep, and response to DNA damage. Thus, fucoxanthin was demonstrated to alter the expression of longevity associated genes which may confer it geroprotective effects (Moskalev et al., 2018).

Another study, evaluated fucoxanthin's antioxidant activity in embryonic lung fibroblasts under oxidative stress conditions using a new generation sequence tool, RNA-seq. Transcriptomic changes were studied during the replicative senescence of fibroblasts in the presence of fucoxanthin. Results showed an increment in the expression level of genes related to the Nrf2/ARE pathway. Besides, when analyzing the enriched KEGG pathways, it was evident that fucoxanthin was able to alter cellular processes like lipid metabolism, ribosome biogenesis and cell cycle regulation and the previously mentioned Wnt, JAK-STAT, and FoxO signaling pathways. Thus, fucoxanthin may represent an alternative molecule to treat or, at least prevent, age-related diseases (Guvatova et al., 2020).

Lastly, the anti-stress and differentiation-inducing potential of fucoxanthin were tested through a model with rat glioma cells. At low and nontoxic concentrations, fucoxanthin was able to protect cells against heat-induced protein misfolding, aggregation of proteins, UV-induced DNA damage and heavy metals. Long term treatments of glioma cells using fucoxanthin induced physiological differentiation into astrocytes, which suggests the great potential of this carotenoid for preventing and protecting against age-related pathologies (Afzal et al., 2019).

### 3.10. Other biological activities

Some studies show that terpenes (a chemical group to which xanthophylls belong), specifically diterpenes, from *Dictyota dichotoma* seaweed, among others, can reduce the risk of hypertension and heart failure. Diterpenes constitute a pharmacological group of compounds that are widely used in the treatment of cardiovascular diseases. Fucoxanthin is a molecule that binds to nitrogen through strong bonds, so it is also able to reduce nitric oxide levels, thereby decreasing its vasodilator efficacy (Agatonovic-Kustrin et al., 2018). This data has been supported by an additional work where fucoxanthin extracted from wakame was able to reduce the development of hypertension, as well as its associated problems, in stroke-prone spontaneously hypertensive rats (Ikeda et al., 2003; Miyashita et al., 2020).

Similarly, the effects of fucoxanthin on other disorders have been studied, demonstrating that it is capable of inhibiting differentiation of the RAW264.7 macrophage cell line, a cell line that can derive in osteoclast cells if stimulated with the activating ligand of the NF-kB receptor, reducing osteoclastogenesis (Das et al., 2010).

Concerning lipid metabolism, fucoxanthin has been also demonstrated in *in vivo* assays to reduce the triglyceride concentration in plasma which increases the content of lipids in faces. Additionally, the hepatic lipid content was reduced in the group fed with fucoxanthin which was associated with a parallel reduction in the enzymatic activity of the hepatic tissue. (Woo et al., 2010). Other authors corroborate these results by the associating the same reasons (a possible reduction in hepatic synthesis of fatty acids, due to the decrease in the activity of related enzymes). This study also demonstrated that the effect of the fucoxanthin is not dose-dependent since the same results were observed when using doses of 0.05% and 0.2%, thus the lower administration dose was enough to produce these benefits. Besides, and as a complementary activity, fucoxanthin has been described to possess the ability to significantly activate glycolytic enzymes, such as hepatic glucokinase, regulating hepatic levels of glucokinase/glucose-6-phosphatase and glycogen in mice fed with excess fat (Park et al., 2011).

Moreover, fucoxanthin also has been demonstrated to have anthelmintic properties. Malaria is a tropical and subtropical disease caused by the protozoan *Plasmodium falciparum*, a parasite that is transmitted by mosquito. An extract obtained from the brown seaweed *Sargassum heterophyllum*, rich in fucoxanthin, sargahydroquinoic acid, sargaquinoic acid and sargaquinal, was tested against *Plasmodium falciparum*. The extract produced an effective response against the parasite, so then the individual components were purified and newly tested. The fraction containing fucoxanthin was the one that showed the greatest anthelmintic potential. Authors associated such antimalarial activity with the strong antioxidant capacity of the algal molecules, although they also encouraged new tests to deepen the mechanism of action (Afolayan et al., 2008).

Another study tested the effects of fucoxanthin in men infertility, since the oxidative stress has been recognized as one of the triggering causes of this pathology that can affect 5 of 10 men. A rich fucoxanthin extract obtained from *Sargassum glaucescens* was evaluated in a lipopolysaccharide-induced inflammation model in macrophages and in a Cisplatin-induced model that replicates reproductive damages in hamsters. The administration of fucoxanthin for 5 days reduced the level of malondialdehyde and reactive oxygen species in macrophages and rats' testis. Besides it showed protective effects on mitochondrial membrane potential, related to its antioxidant and anti-inflammatory capacity. Furthermore, the alpha-glucosidase activity and testosterone levels were improved and the sperm count also increased while the sperm abnormality decreased. These findings suggest that fucoxanthin extracts can be used as an alternative treatment for testicular damage (Wang et al., 2020).

## 4. Bioavailability,Toxicity, and stability

Fucoxanthin absorption is affected by the amount and type of lipids and fiber consumed in the daily diet, as well as by the food matrix in which this carotenoid is included (Bohn, 2008). When fucoxanthin (Fig. 1A) is ingested it undergoes hydrolyzing in the gastrointestinal tract resulting in fucoxanthinol (Fig. 1 B) which is further transformed into amarouciaxanthin A (Fig. 1C), mostly in the hepatic tissue (Asai et al., 2004). The esterification step, in addition to allowing the transport and distribution of fucoxanthin, transforms it into a non-polar molecule of greater bioavailability, thus reducing its possible toxic effects, protecting the organism (Sugawara et al., 2002, 2009).

As explained in section 1.2, differences among species have been observed by few authors who have underlined that for obtaining similar results mice required the administration of higher doses of fucoxanthin than humans (Abidov et al., 2010; Hashimoto et al., 2012; Maeda et al., 2009). The explanation of the differences between the effective dose in humans and mice may be due to different reasons. The lower concentration of fucoxanthin or fucoxanthinol detected or needed for having

effects on mice may be due to the faster and different pharmacokinetic profile that small and short-lived animals possess which may trigger quicker detoxification pathways (Mordenti, 1986). It has been also stated that different absorption rates may be involved in the sensitivity to the fucoxanthin or its metabolites since the vegetable or food matrix in which the pigment is wrapped directly influences the absorption rate of this compound. A study on the solubility of fucoxanthin in different micelles demonstrated the variability of the process. The results displayed that the greatest solubility was achieved with the long-chain triacylglycerols (TAG) micelles, followed by medium-chain TAG and, finally, those of indigestible oil (orange/mineral oil) (Salvia-Trujillo et al., 2015). Fucoxanthin naturally found in alga tissues is mainly embedded in a matrix of glycoglycerolipids. A greater presence of these compounds is associated with greater absorption of fucoxanthin, since the ingestion of glycoglycerolipids favors the formation of lyso-type compounds that increase and facilitate the absorption and, therefore, its bioavailability (Kotake-Nara et al., 2015; Miyashita et al., 2013).

As previously mentioned, algae have been part of the Asian diet for generations, algae that include as beneficial molecules as fucoxanthin in their composition, so, over time, safety and toxicity tests have been carried out in animals. For example, in a study conducted by (Beppu et al., 2009) it was found that the administration to mice of a single dose, whether it was 1000 or 2000 mg/kg of fucoxanthin, did not produce abnormalities, much less mortality. Therefore, they conducted a new trial in which mice were also given a repeated treatment of 500 or 1000 mg/kg for 30 days, concluding that there were no adverse effects after this treatment. Similarly (Kadekaru et al., 2008), also reported no toxicity in their study carried out by applying purified fucoxanthin in rats. Moreover, metabolic derivatives of fucoxanthin such as fucoxanthinol (Fig. 1), also showed no significant adverse effects or mutagenicity in *in vivo* trials, and it was found that fucoxanthinol reaches human plasma after the consumption of brown algae, specifically, by *U. pinnatifida* (Asai et al., 2004; Miyashita & Hosokawa, 2017).

Fucoxanthin, like most carotenoids from marine environments, has low toxicity. A study conducted with oily extract rich in fucoxanthin extracted from the *Chaetoceros sp*. microalgae used to analyze genotoxicity in mice through the bacterial mutation and the micronucleus tests concluded that no signs of toxicity were observed. In another study conducted in mice, the results showed the safety of fucoxanthin, as no adverse effects were observed, even though fucoxanthin induced a hepatic stimulation that resulted in the production of an omega-3 fatty acid, DHA, in large quantities (Okada et al., 2008). In addition, a study in rats showed that the mean lethal dose ($LD_{50}$) in a single administration of fucoxanthin amounted to more than 2 g/kg of body weight, while no mortality or abnormalities were observed in a 13-week treatment using 200 mg of fucoxanthin/kg/day (Muradian et al., 2015). On the other hand, in a study in humans, a daily supplement of 10 mg of fucoxanthin was administered. When the treated group was compared against the control one, with a placebo supplement administered, no adverse side effects were reported. These promising results regarding the absorption, bioavailability, distribution, non-toxicity, biocompatibility, and bioactivity of fucoxanthin support the conduct of future and more in-depth clinical trials in humans (Holt, 2008). However, for the further development of clinical trials, the stability of the fucoxanthin has to be considered. The degradation of the putative fucoxanthin may modify the absorbance degree and the initial properties of the molecule. The most used presentations of fucoxanthin (free, emulsified or encapsulated) are briefly analyzed for the development of food or cosmetic products. When using free fucoxanthin obtained from *Sargassum binderi* at different pH conditions it was observed that at both very acidic (pHs of 1 and 3) and in very alkaline environments (pHs of 9, 11 and 13) fucoxanthin rich extracts suffered strong degradation, while a pH range between 5 and 7 kept their stability (Yip et al., 2014). Another determinant of carotenoid stability are temperature and light (López et al., 2019; Ye & Eitenmiller, 2005). Regarding the temperature, no significant differences were found after storing extracts rich in fucoxanthin at 4 °C or 25 °C ($p > 0.05$) for one month, at 50 °C, a significant decrease was detected after 3 weeks of storage. In terms of light exposition, extracts preserved in the dark were more stable than samples exposed to light (Yip et al., 2014). Another study conducted on the stability of fucoxanthin after its emulsification in an oil/water system showed no stability efficiency for fucoxanthin, and all its trans-, 13-cis and 13′-cis isomers, except for the 9′-cis isomer. As in the case of the free molecule, low values of pH reduce the stability of this isomer while it was even capable of considerably increase in concentration under a neutral pH of 7.5. Similarly, in the emulsion, the increase of temperature greatly promoted the degradation of the molecules (Zhao et al., 2019). The third alternative for preserving fucoxanthin is through the encapsulation, a process that allows the isolation of substances that are not very stable or difficult to apply to formulations by enclosing them in a capsule. This system may provide an exact dosage in a comfortable administration format which converts it in a method that facilitates the adherence to the treatment by the consumer (Taofiq et al., 2018). The stability and bioavailability of fucoxanthin when encapsulated, using different nanogels based on mixing variable amounts of chitosan (C) and glycolipid (G) or sodium tripolyphosphate (ST) has been evaluated. Fucoxanthin remained more stable when introduced into C:G nanogels than when embedded in C nanogels. The presence of G stabilizes this carotenoid and extends it up to nearly two days. In terms of bioavailability, C:G nanogels offered the highest rate with 68% (C nanogels 51%, and G nanogels, 35%) (Ravi & Baskaran, 2015).

Therefore, the ideal conditions to incorporate fucoxanthin into formulations, whether pharmaceutical, or as a food ingredient would be encapsulated and embedded in solutions with pH 5 to 7 kept stored both in a refrigerator at 4 °C and room temperature of 25 °C, or up to 50 °C but for periods shorter than 3 weeks in order to keep the features of the ingredient.

## 5. Future trends

Algae-extracted fucoxanthin represents a natural molecule with a wide range of noteworthy features and bioactivities with many different possible applications. For instance, it is important to find a viable way to exploit these properties in food, cosmetics, and even in the pharmaceutical industries. The growing scientific evidence from *in vitro* and *in vivo* studies have shown that fucoxanthin has great potential in the prevention of illnesses, and also, in the care of human health. As a carotenoid, fucoxanthin is a potent antioxidant that protects free radical damage cells. A diet rich in fucoxanthin may help to reduce the accumulation of body fat and modulate the levels of glucose and insulin in the blood. The administration of fucoxanthin has been shown to be safe, without secondary effects and even providing other health benefits, including cardiovascular protection, inflammatory activity, anti-cancer activity and neuroprotective effect. In addition, it has been shown to be an effective chelator of heavy and toxic metals. All of these biological and chemical properties prove that fucoxanthin is a very interesting component to be included in the formulation of functional foods or drugs. Nevertheless, despite the great advances in the characterization of its biological activities, the biosynthetic route of fucoxanthin in the brown algae is still not fully understood. Therefore, further studies deepening this area should be performed to maximize the application of fucoxanthin.

The potential applications of this pigment are very promising; however, they are limited because the commercialization of fucoxanthin is almost non-existent. Its production must face several challenges since its chemical synthesis represents a complex process that is not efficient and the extraction method from marine organisms has been not standardized. The main characteristic of a profitable product is to be obtained by using simple, fast and based on low-cost technologies. This work provides an overview of different extraction conditions that may help in the standardization of the process, especially important at an industrial level. The optimization of the extraction technique permits

maximizing the yield and reducing its production costs related to solvent and energy consumption and helping to guide the choice for an efficient and sustainable way to produce extracts rich in fucoxanthin. Thus, future and innovative studies regarding efficient, quick and fast extraction methods can speed up the progress towards its commercialization.

Besides, even though carotenoids can be artificially synthesized, following laboratory protocols, their extraction from algae presents many advantages. In the specific case of fucoxanthin, the accessibility to the natural producers, mostly algae, convert this pigment in an economic and ecological alternative that provides a natural ingredient avoiding safety issues prompted using chemical compounds. However, the extraction yield of fucoxanthin has been found to be very variable depending on the selected species and the recovery technique as demonstrated by the data provided from the analyzed scientific literature. Therefore, the main aim of this work has been to underline the best algae species in terms of fucoxanthin production and the most promising extraction and purification methods, while offering a complete panorama of the described bioactivities of fucoxanthin which includes bioavailability, administration via, doses and stability of the molecule under different conditions.

## Acknowledgments

The research leading to these results was funded by FEDER under the program Interreg V Spain-Portugal (POPTEC, ref. 0377-Iberphenol-6-E); by MICINN supporting the Ramón&Cajal grant for M.A. Prieto (RYC-2017-22891); by Xunta de Galicia and University of Vigo supporting the post-doctoral grant of M.Fraga-Corral (ED481B-2019/096), the pre-doctoral grants for A.G. Pereira (ED481A-2019/0228) and P. García-Oliveira (ED481A-2019/295); by Axudas Conecta Peme (Xunta de Galicia) supporting the IN852A 2018/58 NeuroFood Project and Alga-Mar (www.algamar.com) for supporting the pre-doctoral grant for C. Lourenço-Lopes; NANOEATERS Project (0181_NANOEATERS_01_E) for supporting the pre-doctoral work of C. Jimenez-Lopez; EcoChestnut Project (Erasmus+KA202) for supporting the work of M. Carpena; Ibero-American Program on Science and Technology (CYTED - AQUA-CIBUS, P317RT0003) and UP4HEALTH Project (H2020-BBI-JTI-2019) for financial support.

## References

Abidov, M., Ramazanov, Z., Seifulla, R., & Grachev, S. (2010). The effects of Xanthigen™ in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. *Diabetes, Obesity and Metabolism, 12*(1), 72–81. https://doi.org/10.1111/j.1463-1326.2009.01132.x

Afolayan, A. F., Bolton, J. J., Lategan, C. A., Smith, P. J., & Beukes, D. R. (2008). Fucoxanthin, tetraprenylated toluquinone and toluhydroquinone metabolites from *Sargassum heterophyllum* inhibit the *in vitro* growth of the malaria parasite *Plasmodium falciparum*. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences, 63*, 848–852. https://doi.org/10.1515/znc-2008-11-1211

Afrin, S., Giampieri, F., Gasparrini, M., Forbes-Hernández, T. Y., Cianciosi, D., Reboredo-Rodriguez, P., Zhang, J., Manna, P. P., Daglia, M., Atanasov, A. G., & Battino, M. (2020). Dietary phytochemicals in colorectal cancer prevention and treatment: A focus on the molecular mechanisms involved. *Biotechnology Advances, 38*, 107322. https://doi.org/10.1016/j.biotechadv.2018.11.011

Afzal, S., Garg, S., Ishida, Y., Terao, K., Kaul, S. C., & Wadhwa, R. (2019). Rat glioma cell-based functional characterization of anti-stress and protein deaggregation activities in the marine carotenoids, astaxanthin and fucoxanthin. *Marine Drugs, 17*(3), 189. https://doi.org/10.3390/md17030189

Agatonovic-Kustrin, S., Kustrin, E., Angove, M. J., & Morton, D. W. (2018). A screening method for cardiovascular active compounds in marine algae. *Journal of Chromatography A, 1550*, 57–62. https://doi.org/10.1016/j.chroma.2018.03.054

Airanthi, M. K. W. A., Sasaki, N., Iwasaki, S., Baba, N., Abe, M., Hosokawa, M., & Miyashita, K. (2011). Effect of brown seaweed lipids on fatty acid composition and lipid hydroperoxide levels of mouse liver. *Journal of Agricultural and Food Chemistry, 59*(8), 4156–4163. https://doi.org/10.1021/jf104643b

Almeida, T. P., Ferreira, J., Vettorazzi, A., Azqueta, A., Rocha, E., & Ramos, A. A. (2018). Cytotoxic activity of fucoxanthin, alone and in combination with the cancer drugs imatinib and doxorubicin, in CML cell lines. *Environmental Toxicology and Pharmacology, 59*, 24–33. https://doi.org/10.1016/j.etap.2018.02.006

Ansari, J. A., Siraj, A., & Inamdar, N. N. (2010). Pharmacotherapeutic approaches of Parkinson's disease. *International Journal of Pharmacology, 6*(5), 584–590. https://doi.org/10.3923/ijp.2010.584.590

Asai, A., Sugawara, T., Ono, H., & Nagao, A. (2004). Biotransformation of fucoxanthinol into amarouciaxanthin a in mice and HepG2 cells: formation and cytotoxicity of fucoxanthin metabolites. *Drug Metabolism and Disposition, 32*, 205–211. https://doi.org/10.1124/dmd.32.2.205

Asai, A., Yonekura, L., & Nagao, A. (2008). Low bioavailability of dietary epoxyxanthophylls in humans. *British Journal of Nutrition, 100*(2), 273–277. https://doi.org/10.1017/S0007114507895468

Bae, M., Kim, M. B., Park, Y. K., & Lee, J. Y. (2020). Health benefits of fucoxanthin in the prevention of chronic diseases. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1865*(11), 158618. https://doi.org/10.1016/j.bbalip.2020.158618

Barros, L., Cabrita, L., Boas, M. V., Carvalho, A. M., & Ferreira, I. C. F. R. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry, 27*(4), 1600–1608. https://doi.org/10.1016/j.foodchem.2011.02.024

Baskaran, V., Sangeetha, K. R., & Bhaskar, N. (2009). Comparative effects of β-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats. *Molecular and Cellular Biochemistry, 331*(1–2), 59–67. https://doi.org/10.1007/s11010-009-0145-y

Beppu, F., Niwano, Y., Tsukui, T., Hosokawa, M., & Miyashita, K. (2009). Single and repeated oral dose toxicity study of fucoxanthin (FX), a marine carotenoid, in mice. *Journal of Toxicological Sciences, 34*(5), 501–510. https://doi.org/10.2131/jts.34.501

Billakanti, J. M., Catchpole, O. J., Fenton, T. A., Mitchell, K. A., & Mackenzie, A. D. (2013). Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from Undaria pinnatifida using dimethyl ether and ethanol. *Process Biochemistry, 48*(12), 1999–2008. https://doi.org/10.1016/j.procbio.2013.09.015

Bjarkam, C. R., Sørensen, J. C., Sunde, N. A., Geneser, F. A., & Østergaard, K. (2001). New strategies for the treatment of Parkinson's disease hold considerable promise for the future management of neurodegenerative disorders. *Biogerontology, 2*(3), 193–207. https://doi.org/10.1023/A:1011565207964

Bjørnland, T., & Aguilar-Martinez, M. (1976). Carotenoids in red algae. *Phytochemistry, 15*(2), 291–296. https://doi.org/10.1016/S0031-9422(00)89006-8

Bohn, T. (2008). Bioavailability of non-provitamin A carotenoids. *Current Nutrition & Food Science, 4*, 240–258. https://doi.org/10.2174/157340108786263685

Chen, S. J., Lee, C. J., Lin, T. B., Peng, H. Y., Liu, H. J., Chen, Y. S., & Tseng, K. W. (2019). Protective effects of fucoxanthin on ultraviolet B-induced corneal denervation and inflammatory pain in a rat model. *Marine Drugs, 17*(3), 152. https://doi.org/10.3390/md17030152

Chuyen, H. Van, & Eun, J. B. (2017). Marine carotenoids: Bioactivities and potential benefits to human health. In *Critical reviews in food science and nutrition* (Vol. 57, pp. 2600–2610). Taylor & Francis. https://doi.org/10.1080/10408398.2015.1063477

Cianciosi, D., Varela-Lopez, A., Forbes-Hernandez, T. Y., Gasparrini, M., Afrin, S., Reboredo-Rodriguez, P., Zhang, J. J., Quiles, J. L., Nabavi, S. F., Battino, M., & Giampieri, F. (2018). Targeting molecular pathways in cancer stem cells by natural bioactive compounds. *Pharmacological Research, 135*, 150–165. https://doi.org/10.1016/j.phrs.2018.08.006

Das, S. K., Ren, R., Hashimoto, T., & Kanazawa, K. (2010). Fucoxanthin induces apoptosis in osteoclast-like cells differentiated from RAW264.7 cells. *Journal of Agricultural and Food Chemistry, 58*, 6090–6095. https://doi.org/10.1021/jf100303k

Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry, 103*, 891–899. https://doi.org/10.1016/j.foodchem.2006.09.041

Dillehay, T. D., Ramírez, C., Pino, M., Collins, M. B., Rossen, J., & Pino-Navarro, J. D. (2008). Monte verde: Seaweed, food, medicine, and the peopling of south America. *Science, 320*(5877), 784–786. https://doi.org/10.1126/science.1156533

D'Orazio, N., Gemello, E., Gammone, M. A., De Girolamo, M., Ficoneri, C., & Riccioni, G. (2012). Fucoxantin: A treasure from the sea. *Marine Drugs, 10*, 604–616. https://doi.org/10.3390/md10030604

Fabrowska, J., Ibañez, E., Łęska, B., & Herrero, M. (2016). Supercritical fluid extraction as a tool to valorize underexploited freshwater green algae. *Algal Research, 19*, 237–245. https://doi.org/10.1016/j.algal.2016.09.008

Fariman, G. A., Shastan, S. J., & Zahedi, M. M. (2016). Seasonal variation of total lipid, fatty acids, fucoxanthin content, and antioxidant properties of two tropical brown algae (*Nizamuddinia zanardinii* and *Cystoseira indica*) from Iran. *Journal of Applied Phycology, 28*(2), 1323–1331. https://doi.org/10.1007/s10811-015-0645-y

Foo, S. C., Yusoff, F. M., Ismail, M., Basri, M., Chan, K. W., Khong, N. M. H., & Yau, S. K. (2015). Production of fucoxanthin-rich fraction (FxRF) from a diatom, *Chaetoceros calcitrans* (paulsen) takano 1968. *Algal Research, 12*, 26–32. https://doi.org/10.1016/j.algal.2015.08.004

Foo, S. C., Yusoff, F. M., Ismail, M., Basri, M., Yau, S. K., Khong, N. M. H., Chan, K. W., & Ebrahimi, M. (2017). Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. *Journal of Biotechnology, 241*, 175–183. https://doi.org/10.1016/j.jbiotec.2016.11.026

Fung, A., Hamid, N., & Lu, J. (2013). Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. *Food Chemistry, 136*(2), 1055–1062.

Gao, K., & McKinley, K. R. (1994). Use of macroalgae for marine biomass production and CO2 remediation: A review. *Journal of Applied Phycology, 6*(1), 45–60. https://doi.org/10.1007/BF02185904

Guvatova, Z., Dalina, A., Marusich, E., Pudova, E., Snezhkina, A., Krasnov, G., Kudryavtseva, A., Leonov, S., & Moskalev, A. (2020). Protective effects of carotenoid fucoxanthin in fibroblasts cellular senescence. *Mechanism of Ageing and Development, 189*(May), 111260. https://doi.org/10.1016/j.mad.2020.111260

Hashimoto, T., Ozaki, Y., Mizuno, M., Yoshida, M., Nishitani, Y., Azuma, T., Komoto, A., Maoka, T., Tanino, Y., & Kanazawa, K. (2012). Pharmacokinetics of fucoxanthinol in human plasma after the oral administration of kombu extract. *British Journal of Nutrition, 107*(11), 1566–1569. https://doi.org/10.1017/S0007114511004879

Hashimoto, T., Ozaki, Y., Taminato, M., Das, S. K., Mizuno, M., Yoshimura, K., Maoka, T., & Kanazawa, K. (2009). The distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice. *British Journal of Nutrition, 102*, 242–248. https://doi.org/10.1017/S0007114508199007

Heo, S. J., & Jeon, Y. J. (2009). Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. *Journal of Photochemistry and Photobiology B: Biology, 95*(2), 101–107. https://doi.org/10.1016/j.jphotobiol.2008.11.011

Heo, S. J., Ko, S. C., Kang, S. M., Kang, H. S., Kim, J. P., Kim, S. H., Lee, K. W., Cho, M. G., & Jeon, Y. J. (2008). Cytoprotective effect of fucoxanthin isolated from brown algae Sargassum siliquastrum against H2O2-induced cell damage. *European Food Research and Technology, 228*(1), 145–151. https://doi.org/10.1007/s00217-008-0918-7

Heo, S. J., Yoon, W., Kim, K., Ahn, G., Kang, S., Kang, D., Oh, C., Jung, W., & Jeon, Y. (2010). Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264 . 7 macrophages. *Food and Chemical Toxicology, 48*(8–9), 2045–2051. https://doi.org/10.1016/j.fct.2010.05.003

Holt, S. (2008). Fucose complexes , fucoxanthin, fucoid , and fat storage. *Townsend Letter*, 87–92.

Hosokawa, M., Kudo, M., Maeda, H., Kohno, H., Tanaka, T., & Miyashita, K. (2004). Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARγ ligand, troglitazone, on colon cancer cells. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1675*(1–3), 113–119. https://doi.org/10.1016/j.bbagen.2004.08.012

Hu, L., Chen, W., Tian, F., Yuan, C., Wang, H., & Yue, H. (2018). Neuroprotective role of fucoxanthin against cerebral ischemic/reperfusion injury through activation of Nrf2/HO-1 signaling. *Biomedicine & Pharmacotherapy, 106*(1), 1484–1489. https://doi.org/10.1016/j.biopha.2018.07.088

Ikeda, K., Kitamura, A., Machida, H., Watanabe, M., Negishi, H., Hiraoka, J., & Nakano, T. (2003). Effect of *Undaria pinnatifida* (Wakame) on the development of cerebrovascular diseases in stroke-prone spontaneously hypertensive rats. *Clinical and Experimental Pharmacology and Physiology, 30*, 44–48. https://doi.org/10.1046/j.1440-1681.2003.03786.x

Jang, E. J., Kim, S. C., Lee, J. H., Lee, J. R., Kim, I. K., Baek, S. Y., & Kim, Y. W. (2018). Fucoxanthin, the constituent of *Laminaria japonica*, triggers AMPK-mediated cytoprotection and autophagy in hepatocytes under oxidative stress. *BMC Complementary and Alternative Medicine, 18*(1), 97. https://doi.org/10.1186/s12906-018-2164-2

Jaswir, I, Noviendri, D, Salleh, HM, & Miyashita, K (2012). Fucoxanthin Extractions of Brown Seaweeds and Analysis of Their Lipid Fraction in Methanol. *Food Science and Technology Research, 18*(2), 251–257.

Jin Heo, S., & Jeon, Y. J. (2009). Protective effect of fucoxanthin isolated from Sargassum siliquastrum on UV-B induced cell damage. *Journal of Photochemistry and Photobiology B: Biology, 95*(2), 101–107. https://doi.org/10.1016/j.jphotobiol.2008.11.011

Jin, Y., Qiu, S., Shao, N., & Zheng, J. (2018). Fucoxanthin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically promotes apoptosis of human cervical cancer cells by targeting PI3K/Akt/NF-kB signaling pathway. *Medical Science Monitor, 24*, 11–18. https://doi.org/10.12659/MSM.905360

Jin, X., Zhao, T. T., Shi, D., Ye, M. B., & Yi, Q. (2019). Protective role of fucoxanthin in diethylnitrosamine-induced hepatocarcinogenesis in experimental adult rats. *Drug Development Research, 80*(2), 209–217. https://doi.org/10.1002/ddr.21451

Jung, H. A., Islam, M. N., Lee, C. M., Jeong, H. O., Chung, H. Y., Woo, H. C., & Choi, J. S. (2012). Promising antidiabetic potential of fucoxanthin isolated from the edible brown algae *Eisenia bicyclis* and *Undaria pinnatifida*. *Fisheries Science, 76*, 1321–1329. https://doi.org/10.1007/s12562-012-0552-y

Kadekaru, T., Toyama, H., & Yasumoto, T. (2008). Safety evaluation of fucoxanthin purified from *Undaria pinnatifida*. *Nippon Shokuhin Kagaku Kogaku Kaishi, 55*(6), 304–308. https://doi.org/10.3136/nskkk.55.304

Kajikawa, T., Okumura, S., Iwashita, T., Kosumi, D., Hashimoto, H., & Katsumura, S. (2012). Stereocontrolled total synthesis of fucoxanthin and its polyene chain-modified derivative. *Organic Letters, 14*(3), 808–811. https://doi.org/10.1021/ol203344c

Kanazawa, K., Ozaki, Y., Hashimoto, T., Das, S. K., Matsushita, S., Hirano, M., Okada, T., Komoto, A., Mori, N., & Nakatsuka, M. (2008). Commercial-scale preparation of biofunctional fucoxanthin from waste parts of Brown sea algae laminalia japonica. *Food Science and Technology Research, 14*(6), 573–582. https://doi.org/10.3136/fstr.14.573

Kanda, H., Kamo, Y., Machmudah, S., Wahyudiono, & Goto, M. (2014). Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. *Marine Drugs, 12*(5), 2383–2396. https://doi.org/10.3390/md12052383

Khodosevich, K., & Monyer, H. (2010). Signaling involved in neurite outgrowth of postnatally born subventricular zone neurons in vitro. *BMC Neuroscience, 11*(18), 1–11. https://doi.org/10.1186/1471-2202-11-18

Kim, K. N., Ahn, G., Heo, S. J., Kang, S. M., Kang, M. C., Yang, H. M., Kim, D., Roh, S. W., Kim, S. K., Jeon, B. T., Park, P. J., Jung, W. K., & Jeon, Y. J. (2013). Inhibition of tumor growth in vitro and in vivo by fucoxanthin against melanoma B16F10 cells. *Environmental Toxicology and Pharmacology, 35*(1), 39–46. https://doi.org/10.1016/j.etap.2012.10.002

Kim, S. K., & Pangestuti, R. (2011). Biological activities and potential health benefits of fucoxanthin derived from marine brown algae. In *Advances in food and nutrition research* (1st ed., Vol. 64). Elsevier Inc. https://doi.org/10.1016/B978-0-12-387669-0.00009-0

Kotake-Nara, E., Yonekura, L., & Nagao, A. (2015). Lysoglyceroglycolipids improve the intestinal absorption of micellar fucoxanthin by Caco-2 cells. *Journal of Oleo Science*. https://doi.org/10.5650/jos.ess15180

Kumar, S. R., Hosokawa, M., & Miyashita, K. (2013). Fucoxanthin: A marine carotenoid exerting anti-cancer effects by affecting multiple mechanisms. In *Marine drugs* (Vol. 11, pp. 5130–5147). https://doi.org/10.3390/md11125130

Kummar, C. S., Ganesan, P., Suresh, P. V., & Bhaskar, N. (2008). Seaweeds as a source of nutritionally beneficial compounds - a review. *Journal of Food Science & Technology, 45*, 1–13.

Lashmanova, E., Proshkina, E., Zhikrivetskaya, S., Shevchenko, O., Marusich, E., Leonov, S., Melerzanov, A., Zhavoronkov, A., & Moskalev, A. (2015). Fucoxanthin increases lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans*. *Pharmacological Research, 100*, 228–241. https://doi.org/10.1016/j.phrs.2015.08.009

Li, J., Liu, Y., Liu, Y., Wang, Q., Gao, X., & Gong, Q. (2019). Effects of temperature and salinity on the growth and biochemical composition of the brown alga Sargassum fusiforme (Fucales, Phaeophyceae). *Journal of Applied Phycology, 31*(5), 3061–3068. https://doi.org/10.1007/s10811-019-01795-9

Liu, C. L., Liang, A. L., & Hu, M. L. (2011). Protective effects of fucoxanthin against ferric nitrilotriacetate-induced oxidative stress in murine hepatic BNL CL.2 cells. *Toxicology in Vitro, 25*(7), 1314–1319. https://doi.org/10.1016/j.tiv.2011.04.023

Liu, M., Li, W., Chen, Y., Wan, X., & Wang, J. (2020). Fucoxanthin: A promising compound for human inflammation-related diseases. *Life Sciences, 255*(March), 1178503. https://doi.org/10.1016/j.lfs.2020.117850

Liu, Y., Liu, M., Zhang, X., Chen, Q., Chen, H., Sun, L., & Liu, G. (2016). Protective effect of fucoxanthin isolated from *Laminaria japonica* against visible light-induced retinal damage both *in vitro* and *in vivo*. *Journal of Agricultural and Food Chemistry, 64*(2), 416–424. https://doi.org/10.1021/acs.jafc.5b05436

Li, F., Wang, L. J., Fan, Y., Parsons, R. L., Hu, G. R., & Zhang, P. Y. (2018). A rapid method for the determination of fucoxanthin in diatom. *Marine Drugs, 16*(1), 1–13. https://doi.org/10.3390/md16010033

Lopes, C. L., Pereira, E., Soković, M., Carvalho, A. M., Barata, A. M., Lopes, V., Rocha, F., Calhelha, R. C., Barros, L., & Ferreira, I. C. F. R. (2018). Phenolic composition and bioactivity of lavandula pedunculata (mill.) cav. Samples from different geographical origin. *Molecules, 23*(5), 1–19. https://doi.org/10.3390/molecules23051037

López, C. J., Caleja, C., Prieto, M. A. A., Barreiro, M. F., Barros, L., & Ferreira, I. C. F. R. (2018). Optimization and comparison of heat and ultrasound assisted extraction techniques to obtain anthocyanin compounds from Arbutus unedo L. Fruits. *Food Chemistry, 264*(April), 81–91. https://doi.org/10.1016/j.foodchem.2018.04.103

López, C. J., Caleja, C., Prieto, M. A., Sokovic, M., Calhelha, R. C., Barros, L., & Ferreira, I. C. F. R. (2019). Stability of a cyanidin-3-O-glucoside extract obtained from *Arbutus unedo* L. and incorporation into wafers for colouring purposes. *Food Chemistry, 275*, 426–438. June 2018.

Maeda, H., Hosokawa, M., Sashima, T., & Miyashita, K. (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-A y mice. *Journal of Agricultural and Food Chemistry, 55*(19), 7701–7706. https://doi.org/10.1021/jf071569n

Maeda, H., Hosokawa, M., Sashima, T., Murakami-Funayama, K., & Miyashita, K. (2009). Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Molecular Medicine Reports, 2*, 897–902. https://doi.org/10.3892/mmr-00000189

Maeda, H., Hosokawa, M., Sashima, T., Takahashi, N., Kawada, T., & Miyashita, K. (2006). Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *International Journal of Molecular Medicine, 18*, 147–152.

Maeda, H., Tsukui, T., Sashima, T., Hosokawa, M., & Miyashita, K. (2008). Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. *Asia Pacific Journal of Clinical Nutrition, 17*(SUPPL. 1), 196–199.

Maoka, T., Fujiwara, Y., Hashimoto, K., & Akimoto, N. (2007). Characterization of fucoxanthin and fucoxanthinol esters in the Chinese surf clam, mactra chinensis. *Journal of Agricultural and Food Chemistry, 55*, 1563–1567. https://doi.org/10.1021/jf063139n

Matsui, M., Tanaka, K., Higashiguchi, N., Okawa, H., Yamada, Y., Tanaka, K., Taira, S., Aoyama, T., Takanishi, M., Natsume, C., Takakura, Y., Fujita, N., Hashimoto, T., & Fujita, T. (2016). Protective and therapeutic effects of fucoxanthin against sunburn caused by UV irradiation. *Journal of Pharmacological Sciences, 132*(1), 55–64. https://doi.org/10.1016/j.jphs.2016.08.004

Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M., Miyashita, K., Yajima, T., & Hara, H. (2010). Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *European Journal of Nutrition, 49*(4), 243–249. https://doi.org/10.1007/s00394-009-0078-y

McClure, D. D., Luiz, A., Gerber, B., Barton, G. W., & Kavanagh, J. M. (2018). An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae *Phaeodactylum tricornutum*. *Algal Research, 29*, 41–48. https://doi.org/10.1016/j.algal.2017.11.015. November 2017.

Mikami, K., & Hosokawa, M. (2013). Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in brown seaweeds. *International Journal of Molecular Sciences, 14*, 13763–13781. https://doi.org/10.3390/ijms140713763

Miyashita, K., Beppu, F., Hosokawa, M., Liu, X., & Wang, S. (2020). Bioactive significance of fucoxanthin and its effective extraction. *Biocatalysis and Agricultural Biotechnology, 26*(March), 101639. https://doi.org/10.1016/j.bcab.2020.101639

Miyashita, K., & Hosokawa, M. (2017). Fucoxanthin in the management of obesity and its related disorders. *Journal of Functional Foods, 36*, 195–202. https://doi.org/10.1016/j.jff.2017.07.009

Miyashita, K., Mikami, N., & Hosokawa, M. (2013). Chemical and nutritional characteristics of brown seaweed lipids: A review. *Journal of Functional Foods, 5*(4), 1507–1517. https://doi.org/10.1016/j.jff.2013.09.019

Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M., & Hosokawa, M. (2011). The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. *Journal of the Science of Food and Agriculture, 91*(7), 1166–1174. https://doi.org/10.1002/jsfa.4353

Mordenti, J. (1986). Man versus beast: Pharmacokinetic scaling in mammals. *Journal of Pharmaceutical Sciences, 75*(11), 1028–1040. https://doi.org/10.1002/jps.2600751104

Moskalev, A., Shaposhnikov, M., Zemskaya, N., Belyi, A., Dobrovolskaya, E., Patova, A., Guvatova, Z., Lukyanova, E., Snezhkina, A., & Kudryavtseva, A. (2018). Transcriptome analysis reveals mechanisms of geroprotective effects of fucoxanthin in Drosophila. *BMC Genomics, 19*(Suppl 3). https://doi.org/10.1186/s12864-018-4471-x

Munekata, P. E. S., Pateiro, M., Barba, F. J., Dominguéz, R., Gagaoua, M., & Lorenzo, J. M. (2019). Development of new food and pharmaceutical products: Nutraceuticals and food additives. *Advances in food and nutrition research*. https://doi.org/10.1016/bs.afnr.2019.12.001

Muradian, K., Vaiserman, A., Min, K. J., & Fraifeld, V. E. (2015). Fucoxanthin and lipid metabolism: A minireview. *Nutrition, Metabolism, and Cardiovascular Diseases, 25*(10), 891–897. https://doi.org/10.1016/j.numecd.2015.05.010

Narang, S., Gibson, D., Wasan, A. D., Ross, E. L., Michna, E., Nedeljkovic, S. S., & Jamison, R. N. (2008). Efficacy of dronabinol as an adjuvant treatment for chronic pain patients on opioid therapy. *The Journal of Pain, 9*(3), 254–264. https://doi.org/10.1016/j.jpain.2007.10.018

Neumann, U., Derwenskus, F., Flister, V. F., Schmid-Staiger, U., Hirth, T., & Bischoff, S. C. (2019). Fucoxanthin, a carotenoid derived from *Phaeodactylum tricornutum* exerts antiproliferative and antioxidant activities *in vitro*. *Antioxidants, 8*(6), 183. https://doi.org/10.3390/antiox8060183

Nomura, M., Kamogawa, H., Susanto, E., Kawagoe, C., Yasui, H., Saga, N., Hosokawa, M., & Miyashita, K. (2013). Seasonal variations of total lipids, fatty acid composition, and fucoxanthin contents of Sargassum horneri (Turner) and Cystoseira hakodatensis (Yendo) from the northern seashore of Japan. *Journal of Applied Phycology, 25*(4), 1159–1169. https://doi.org/10.1007/s10811-012-9934-x

Okada, T., Nakai, M., Maeda, H., Hosokawa, M., Sashima, T., & Miyashita, K. (2008). Suppressive effect of neoxanthin on the differentiation of 3T3-L1 adipose cells. *Journal of Oleo Science, 57*, 345–351. https://doi.org/10.5650/jos.57.345

Okuzumi, J., Nishino, H., Murakoshi, M., Iwashima, A., Tanaka, Y., Yamane, T., Fujita, Y., & Takahashi, T. (1990). Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells. *Cancer Letters, 55*(1), 75–81. https://doi.org/10.1016/0304-3835(90)90068-9

Ou, H. C., Chou, W. C., Chu, P. M., Hsieh, P. L., Hung, C. H., & Tsai, K. L. (2019). Fucoxanthin protects against oxLDL-induced endothelial damage via activating the AMPK-Akt-CREB-PGC1α pathway. *Molecular Nutrition & Food Research, 63*(10), 1–10. https://doi.org/10.1002/mnfr.201801353

Palermo, J. A., Gros, E. G., & Seldes, A. M. (1991). Carotenoids from three red algae of the Corallinaceae. *Phytochemistry, 30*(9), 2983–2986. https://doi.org/10.1016/S0031-9422(00)98236-0

Pangestuti, R., & Kim, S. K. (2010). Neuroprotective properties of chitosan and its derivatives. *Marine Drugs, 8*, 2117–2128. https://doi.org/10.3390/md8072117

Pangestuti, R., & Kim, S. K. (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods, 3*(4), 255–266. https://doi.org/10.1016/j.jff.2011.07.001

Park, H. J., Lee, M. K., Park, Y. B., Shin, Y. C., & Choi, M. S. (2011). Beneficial effects of *Undaria pinnatifida* ethanol extract on diet-induced-insulin resistance in C57BL/6J mice. *Food and Chemical Toxicology, 49*, 727–733. https://doi.org/10.1016/j.fct.2010.11.032

Peng, J., Yuan, J. P., Wu, C. F., & Wang, J. H. (2011). Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. In *Marine drugs* (Vol. 9, pp. 1806–1828). https://doi.org/10.3390/md9101806

Perez-Gregorio, R., & Simal-Gandara, J. (2017). A critical review of bioactive food components, and of their functional mechanisms, biological effects and health outcomes. *Current Pharmaceutical Design, 23*(999). https://doi.org/10.2174/1381612823666170317122913

Pinela, J., Prieto, M. A., Pereira, E., Jabeur, I., Barreiro, M. F., Barros, L., & Ferreira, I. C. F. R. (2019). Optimization of heat- and ultrasound-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces for natural food colorants. *Food Chemistry, 275*, 309–321. https://doi.org/10.1016/j.foodchem.2018.09.118

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., Hosokawa, M., & Miyashita, K. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry, 115*(2), 501–508. https://doi.org/10.1016/j.foodchem.2008.12.047

Raguraman, V., MubarakAli, D., Narendrakumar, G., Thirugnanasambandam, R., Kirubagaran, R., & Thajuddin, N. (2018). Unraveling rapid extraction of fucoxanthin from Padina tetrastromatica: Purification, characterization and biomedical application. *Process Biochemistry, 73*(July), 211–219.

Rajauria, F., & Abu-Ghannam. (2017). Characterization of dietary fucoxanthin from Himanthalia elongata brown seaweed. *Food Research International, 99*, 995–1001. https://doi.org/10.1016/j.foodres.2016.09.023

Rajauria, G., Foley, B., & Abu-Ghannam, N. (2017). Characterization of dietary fucoxanthin from *Himanthalia elongata* brown seaweed. *Food Research International, 99*, 995–1001. https://doi.org/10.1016/j.foodres.2016.09.023

Ravi, H., & Baskaran, V. (2015). Biodegradable chitosan-glycolipid hybrid nanogels: A novel approach to encapsulate fucoxanthin for improved stability and bioavailability. *Food Hydrocolloids, 43*, 717–725. https://doi.org/10.1016/j.foodhyd.2014.08.004

Reports World, M. (2017). *Global fucoxanthin market report 2017 – market reports world*.

Ristivojević, P., Jovanović, V., Opsenica, D. M., Park, J., Rollinger, J. M., & Velicković, T.Ć. (2021). Rapid analytical approach for bioprofiling compounds with radical scavenging and antimicrobial activities from seaweeds. *Food Chemistry, 334*. https://doi.org/10.1016/j.foodchem.2020.127562. November 2019.

Rokkaku, T., Kimura, R., Ishikawa, C., Yasumoto, T., Senba, M., Kanaya, F., & Mori, N. (2013). *In vivo* antioxidant activity of fucoxanthin on obese/diabetes KK-A mice. *International Journal of Oncology, 43*(4), 1176–1186. https://doi.org/10.3892/ijo.2013.2019

Sachindra, N. M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M., & Miyashita, K. (2007). Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry, 55*, 8516–8522. https://doi.org/10.1021/jf071848a

Saet, B. L., Joo, Y. L., Song, D. G., Pan, C. H., Chu, W. N., Min, C. K., Eun, H. L., Sang, H. J., Kim, H. S., Yeong, S. K., & Byung, H. U. (2008). Cancer chemopreventive effects of Korean seaweed extracts. *Food Science and Biotechnology*.

Salvia-Trujillo, L., Sun, Q., Um, B. H., Park, Y., & McClements, D. J. (2015). *In vitro* and *in vivo* study of fucoxanthin bioavailability from nanoemulsion-based delivery systems: Impact of lipid carrier type. *Journal of Functional Foods, 17*, 293–304. https://doi.org/10.1016/j.jff.2015.05.035

Sangeetha, RK, Bhaskar, N, & Baskaran, V (2009). Comparative effects of β-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats. *Mol. Cell. Biochem., 331*, 59–67.

Sangeetha, R. K., Bhaskar, N., Divakar, S., & Baskaran, V. (2010). Bioavailability and metabolism of fucoxanthin in rats: Structural characterization of metabolites by LC-MS (APCI). *Molecular and Cellular Biochemistry, 333*, 299–310. https://doi.org/10.1007/s11010-009-0231-1

Satomi, Y. (2017). Antitumor and cancer-preventative function of fucoxanthin: A marine carotenoid. *Anticancer Research, 37*(4), 1557–1562. https://doi.org/10.21873/anticanres.11484

Shang, Y. F., Kim, S. M., Lee, W. J., & Um, B. H. (2011). Pressurized liquid method for fucoxanthin extraction from Eisenia bicyclis (Kjellman) Setchell. *Journal of Bioscience and Bioengineering, 111*(2), 237–241. https://doi.org/10.1016/j.jbiosc.2010.10.008

Shannon, E., & Abu-Ghannam, N. (2017). Optimisation of fucoxanthin extraction from Irish seaweeds by response surface methodology. *Journal of Applied Phycology, 29*(2), 1027–1036. https://doi.org/10.1007/s10811-016-0983-4

Shannon, E., & Abu-Ghannam, N. (2018). Enzymatic extraction of fucoxanthin from brown seaweeds. *International Journal of Food Science and Technology, 53*(9), 2195–2204. https://doi.org/10.1111/ijfs.13808

Shimoda, H., Tanaka, J., Shan, S. J., & Maoka, T. (2010). Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. *Journal of Pharmacy and Pharmacology, 62*, 1137–1145. https://doi.org/10.1111/j.2042-7158.2010.01139.x

Sivagnanam, S. P., Yin, S., Choi, J. H., Park, Y. B., Woo, H. C., & Chun, B. S. (2015). Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO2 extraction. *Marine Drugs, 13*(6), 3422–3442. https://doi.org/10.3390/md13063422

Soo-Jin You-Jin, H., Seok-Chun, K., Sung-Myung, K., Hahk-Soo, K., Jong-Pyung, K., Soo-Hyun, K., Ki-Wan, L., Man-Gi, C., & Jeon. (2008). Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H2O2-induced cell damage. *European Food Research and Technology, 228*(1), 145–151. https://doi.org/10.1007/s00217-008-0918-7

Strand, A., Herstad, O., & Liaaen-Jensen, S. (1998). Fucoxanthin metabolites in egg yolks of laying hens. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 119*, 963–974. https://doi.org/10.1016/S1095-6433(98)00011-7

Sugawara, T., Baskaran, V., Tsuzuki, W., & Nagao, A. (2002). Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by caco-2 human intestinal cells and mice. *Journal of Nutrition, 132*, 946–951. https://doi.org/10.1093/jn/132.5.946

Sugawara, T., Yamashita, K., Asai, A., Nagao, A., Shiraishi, T., Imai, I., & Hirata, T. (2009). Esterification of xanthophylls by human intestinal Caco-2 cells. *Archives of Biochemistry and Biophysics, 48*, 205–212. https://doi.org/10.1016/j.abb.2008.10.007

Sun, P., Wong, C. C., Li, Y., He, Y., Mao, X., Wu, T., Ren, Y., & Chen, F. (2019). A novel strategy for isolation and purification of fucoxanthinol and fucoxanthin from the diatom Nitzschia laevis. *Food Chemistry, 277*(April 2018), 566–572. https://doi.org/10.1016/j.foodchem.2018.10.133

Tang, W., Jiang, Y. F., Ponnusamy, M., & Diallo, M. (2014). *Role of Nrf2 in chronic liver disease. In world journal of gastroenterology* (Vol. 20). Baishideng Publishing Group Inc. https://doi.org/10.3748/wjg.v20.i36.13079, 36.

Taofiq, O., Heleno, S. A., Calhelha, R. C., Fernandes, I. P., Alves, M. J., Barros, L., González-Paramás, A. M., Ferreira, I. C. F. R., & Barreiro, M. F. (2018). Mushroom-based cosmeceutical ingredients: Microencapsulation and in vitro release profile. *Industrial Crops and Products, 124*, 44–52. https://doi.org/10.1016/j.indcrop.2018.07.057

Terasaki, M., Hirose, A., Narayan, B., Baba, Y., Kawagoe, C., Yasui, H., Saga, N., Hosokawa, M., & Miyashita, K. (2009). Evaluation of recoverable functional lipid components of several brown seaweeds (phaeophyta) from Japan with special reference to fucoxanthin and fucosterol contents1. *Journal of Phycology, 45*(4), 974–980. https://doi.org/10.1111/j.1529-8817.2009.00706.x

Terasaki, M., Iida, T., Kikuchi, F., Tamura, K., Endo, T., Kuramitsu, Y., Tanaka, T., Maeda, H., Miyashita, K., & Mutoh, M. (2019a). Fucoxanthin potentiates anoikis in colon mucosa and prevents carcinogenesis in AOM/DSS model mice. *Journal of*

*Nutritional Biochemistry, 64*, 198–205. https://doi.org/10.1016/j.jnutbio.2018.10.007

Terasaki, M., Matsumoto, N., Hashimoto, R., Endo, T., Maeda, H., Hamada, J., Osada, K., Miyashita, K., & Mutoh, M. (2019b). Fucoxanthin administration delays occurrence of tumors in xenograft mice by colonospheres, with an anti-tumor predictor of glycine. *Journal of Clinical Biochemistry & Nutrition, 64*(1), 52–58. https://doi.org/10.3164/jcbn.18-45

Tucci, P., & Bagetta, G. (2008). How to study neuroprotection? *Cell Death & Differentiation, 15*(6), 1084–1085. https://doi.org/10.1038/cdd.2008.32

Urikura, I., Sugawara, T., & Hirata, T. (2011). Protective effect of fucoxanthin against UVB-induced skin photoaging in hairless mice. *Bioscience, Biotechnology and Biochemistry, 75*(4), 757–760. https://doi.org/10.1271/bbb.110040

Wang, X., Cui, Y. J., Qi, J., Zhu, M. M., Zhang, T. L., Cheng, M., Liu, S. M., & Wang, G. C. (2018a). Fucoxanthin exerts cytoprotective effects against hydrogen peroxide-induced oxidative damage in L02 cells. *BioMed Research International, 1–25*. https://doi.org/10.1155/2018/1085073

Wang, L., Park, Y. J., Jeon, Y. J., & Ryu, B. M. (2018b). Bioactivities of the edible brown seaweed, Undaria pinnatifida: A review. *Aquaculture, 495*(May), 873–880.

Wang, P. T., Sudirman, S., Hsieh, M. C., Hu, J. Y., & Kong, Z. L. (2020). Oral supplementation of fucoxanthin-rich brown algae extract ameliorates cisplatin-induced testicular damage in hamsters. *Biomedicine & Pharmacotherapy, 125*, 109992. https://doi.org/10.1016/j.biopha.2020.109992. November 2019.

Wijesinghe, W. A. J. P., & Jeon, Y. J. (2011). Biological activities and potential cosmeceutical applications of bioactive components from brown seaweeds: A review. *Phytochemistry Reviews, 10*(3), 431–443. https://doi.org/10.1007/s11101-011-9214-4

Willstatter, R., & Page, H. (1914). The pigments of the brown algae. *Justus Liebigs Annalen der Chemie, 404*(404), 237–271.

Woo, M. N., Jeon, S. M., Kim, H. J., Lee, M. K., Shin, S. K., Shin, Y. C., Park, Y. B., & Choi, M. S. (2010). Fucoxanthin supplementation improves plasma and hepatic lipid metabolism and blood glucose concentration in high-fat fed C57BL/6N mice. *Chemico-Biological Interactions, 186*, 316–322. https://doi.org/10.1016/j.cbi.2010.05.006

Woo, M. N., Jeon, S. M., Shin, Y. C., Lee, M. K., Kang, M. A., & Choi, M. S. (2009). Anti-obese property of fucoxanthin is partly mediated by altering lipid-regulating enzymes and uncoupling proteins of visceral adipose tissue in mice. *Molecular Nutrition & Food Research, 53*(12), 1603–1611. https://doi.org/10.1002/mnfr.200900079

Wu, H. L., Fu, X. Y., Cao, W. Q., Xiang, W. Z., Hou, Y. J., Ma, J. K., Wang, Y., & Fan, C. D. (2019). Induction of apoptosis in human glioma cells by fucoxanthin via triggering of ROS-mediated oxidative damage and regulation of MAPKs and PI3K-AKT pathways. *Journal of Agricultural and Food Chemistry, 67*(8), 2212–2219. https://doi.org/10.1021/acs.jafc.8b07126

Xiao, X., Si, X., Yuan, Z., Xu, X., & Li, G. (2012). Isolation of fucoxanthin from edible brown algae by microwave-assisted extraction coupled with high-speed countercurrent chromatography. *Journal of Separation Science, 35*(17), 2313–2317. https://doi.org/10.1002/jssc.201200231

Xia, S., Wang, K., Wan, L., Li, A., Hu, Q., & Zhang, C. (2013). Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom odontella aurita. *Marine Drugs, 11*(7), 2667–2681. https://doi.org/10.3390/md11072667

Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T., & Mori, N. (2011). Fucoxanthin and its deacetylated product, fucoxanthinol, induce apoptosis of primary effusion lymphomas. *Cancer Letters, 300*, 225–234. https://doi.org/10.1016/j.canlet.2010.10.016

Ye, L., & Eitenmiller, R. R. (2005). Fat-soluble vitamins. In *Handbook of food science, Technology, and engineering - 4 volume set*. https://doi.org/10.1016/S0022-3182(81)80091-X

Yip, W. H., Lim, S. J., Mustapha, W. A. W., Maskat, M. Y., & Said, M. (2014). Characterisation and stability of pigments extracted from Sargassum binderi obtained from Semporna, Sabah. *Sains Malaysiana, 43*(9), 1345–1354.

Yonekura, L., Kobayashi, M., Terasaki, M., & Nagao, A. (2010). Keto-carotenoids are the major metabolites of dietary lutein and fucoxanthin in mouse tissues. *Journal of Nutrition, 140*(10), 1824–1831. https://doi.org/10.3945/jn.110.126466

Yu, J., Lin, J. J., Yu, R., He, S., Wang, Q. W., Cui, W., & Zhang, J. R. (2017). Fucoxanthin prevents H2O2-induced neuronal apoptosis via concurrently activating the PI3-K/Akt cascade and inhibiting the ERK pathway. *Food & Nutrition Research, 61*(1). https://doi.org/10.1080/16546628.2017.1304678

Zhang, L., Wang, H., Fan, Y., Gao, Y., Li, X., Hu, Z., Ding, K., Wang, Y., & Wang, X. (2017). Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways. *Scientific Reports, 7*(January), 1–15. https://doi.org/10.1038/srep46763

Zhao, D., Yu, D., Kim, M., Gu, M. Y., Kim, S. M., Pan, C. H., Kim, G. H., & Chung, D. (2019). Effects of temperature, light, and pH on the stability of fucoxanthin in an oil-in-water emulsion. *Food Chemistry, 291*(April), 87–93. https://doi.org/10.1016/j.foodchem.2019.04.002