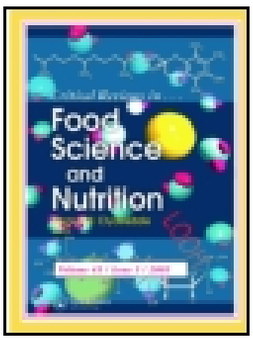

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Marine Carotenoids: Bioactivities and Potential Benefits to Human Health

Van Hoang Chuyen & Jong-Bang Eun

To cite this article: Van Hoang Chuyen & Jong-Bang Eun (2015): Marine Carotenoids: Bioactivities and Potential Benefits to Human Health, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1063477

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1063477

Accepted author version posted online: 13 Nov 2015.

Marine Carotenoids: Bioactivities and Potential Benefits to Human Health

Hoang Van Chuyen [1, 2] and Jong-Bang Eun[1,*]

[1] Department of Food Science and Technology and Functional Food Research Center, Chonnam National University, Buk-gu, Gwangju 500-757, Korea

[2] Department of Food and Agricultural Products Processing and Preservation, Faculty of Agriculture and Forestry, Tay Nguyen University, Daklak Province, Vietnam

[*]Corresponding author: Jong-Bang Eun, Department of Food Science and Technololgy, College of Agriculture & 17 Life Science, Chonnam National University, Buk-gu, Gwangju 500-757, Korea Tell: +82-62-530-2145 19 FAX:+82-62-530-2149 E-mail: jbeun@jnu.ac.kr; jongbang@hotmail.com

Abstract

Among natural pigments, carotenoids play important roles in physiological functions. The characteristics of carotenoids and their effects on human health have been reported for a long time, but most studies have focused on carotenoids from vegetables, fruits, and other parts of higher plants. Few reports are available on carotenoids from marine sources, such as seaweeds, microalgae, and marine animals, which have attracted attention in recent decades. Hundreds of carotenoids have been identified and isolated from marine organisms and their beneficial physiological functions, such as anti-cancer, anti-obesity, anti-diabetic, anti-inflammatory, and cardioprotective activities have been reported. The purpose of this review is to discuss the literature on the beneficial bioactivities of some of the most abundant marine carotenoids,

including fucoxanthin, astaxanthin, cantaxanthin, peridinin, fucoxanthinol, and halocynthiaxanthin.

Keywords

Biological activity, fucoxanthin, astaxanthin, cantaxanthin

Introduction

Carotenoids are yellow to red isoprenoid polyene pigments and one of the most abundant pigment groups in nature (Gross, 1991). More than 700 naturally occurring carotenoids exist, and this number continues to rise with additional discoveries (Hosokawa et al., 2009). Carotenoids are divided into two major classes depending on their structure. The first class is carotenes (which are pure hydrocarbons containing no oxygen) including α, β, ε-carotene, and lycopene (Figure 1). The second class is xanthophylls (which are oxygenated derivatives of carotenes and contain one or more oxygenated group), such as cryptoxanthin, lutein, and zeaxanthin (Figure 2). Carotenoids are the principal color compounds in many plants, algae, and bacteria. Unlike plants, animals and humans cannot synthesize carotenoids and must obtain them through the diet and convert them to functional metabolites. Carotenoids are indispensable components in living cells of almost all organisms because they play important roles in various physiological functions (Ghidalia, 1985). The best-known function of carotenoids is the provitamin A activity of β-carotene and β-cryptoxanthin. Lutein and zeaxanthin absorb damaging blue and near-ultraviolet light to protect the macula lutea from light-associated damage (Krinsky & Johnson, 2005). Carotenoids have also exhibited a number of biological activities, including antioxidant, anti-cancer, anti-obesity, anti-inflammatory, and cardioprotective activities (Mein et al., 2008; Molnar et al., 2005).

Although many studies have been carried out on plant sources of carotenoids, marine carotenoids and their bio-functions have only been investigated in recent decades. Hundreds of carotenoids have been found in marine organisms, and they contribute considerably to total natural carotenoid production. Marine carotenoids are mainly synthesized by phytoplankton in the well-lit ocean surface and are also present in marine plants and animals (Matsuno, 2001). Because

carotenoids are responsible for the color of many marine organisms, they play an important role determining the quality of seafood, such as shrimp, lobsters, crabs, salmon, and tuna.

Among marine carotenoids, fucoxanthin (the dominant carotenoid in brown seaweed) is the most abundant and contributes about 10% to the total production of natural carotenoids (Matsuno, 2001). Production of astaxanthin and canthaxanthin from algae and perinidin from dinoflagellates is also noteworthy and continues to increase. Other carotenoids have been identified at significant concentrations in marine sources, including violaxanthin, halocynthiaxanthin, echinenone, and tunaxanthin.

In addition, a variety of the biological functions of these carotenoids have been reported, such as anti-cancer, anti-obesity, anti-diabetic activities, and positive effects against inflammation and cardiovascular diseases (Hosokawa et al., 2009). These results suggest that marine carotenoids are a natural carotenoid resource that could provide physiological benefits for human health. Marine carotenoid-rich sources have been used in aquaculture feed but also to produce commercial well-being products, such as fucoTHIN® and Xanthigen™. Because of the high potential of marine carotenoids for human health, this review was conducted to discuss the literature on the biological activities of major carotenoids from marine organisms, including fucoxanthin, astaxanthin, canthaxanthin, peridinin, fucoxanthinol, and halocynthiaxanthin (Figure 3).

Bioactivities of Marine Carotenoids

Anti-cancer Activity

Several *in vitro* and *in vivo* studies as well as clinical trials have investigated the anti-cancer activity of marine carotenoids. The results demonstrate that carotenoids inhibit the growth of many cancer cells, including breast, intestinal, hepatic, bladder, prostate, oral, and leukemic. In addition, the mechanisms of action include induction of apoptosis and suppression of cell proliferation.

Fucoxanthin inhibits viability of HL-60 (human leukemia) cells. Doses of 11.3 μM and 45.2 μM fucoxanthin isolated from seaweed *(Undaria pinnatifida)* decrease cell viability to 46.0% and 17.3% of the control, respectively. In addition, characteristics of apoptotic cells were clearly observed on the DNA ladder at a very low concentration (4.5 μM) (Hosokawa et al., 1999). Fucoxanthin isolated from *U. pinnatifida* also has anti-cancer effects on the Caco-2, DLD-1, and HT-29 human colorectal adenocarcinoma cell lines. Although fucoxanthin treatment reduces cell viability, the effects differed in different cells. Viability of Caco-2, DLD-1, and HT-29 cells decreased to 14.8%, 29.4%, and 50.8% after treatment with 15.2 μM fucoxanthin for 72 h, respectively. The decrease in cell viability was related to apoptosis, as shown by the typical morphological properties of apoptotic cells and a significant increase in DNA fragmentation (Hosokawa et al., 2004). In that study, fucoxanthin also showed higher anti-cancer activity than that of β-carotene and astaxanthin. Fucoxanthin treatment (7.6 μM) resulted in increased DNA fragmentation, whereas the other two carotenoids did not induce DNA fragmentation at this concentration. Kim et al., (2010) carried out a study to discover the mechanisms of fucoxanthin-induced apoptosis in human leukemia (HL-60) cells. As results, fucoxanthin generated reactive oxygen species (ROS) during cytotoxicity and apoptosis, cleavage of caspases -3 and -7 and poly-ADP-ribose polymerase (PARP), and decreased Bcl-xL levels (Kim et al., 2010).

Fucoxanthin (5 and 10 µM) was investigated for its effect on viability of six cancer cell types during a 72 h incubation, including MRC-5 (human normal embryonic lung fibroblast), HUC-Fm (human male umbilical cord fibroblasts), B16 (mouse melanoma), Caco-2 (human colorectal adenocarcinoma), HCT116 (human colorectal carcinoma), and PC-3 (human prostate cancer). The results showed significant decreases in viability of the five cancer cell lines (except MRC-5) with the highest reductions in the HCT116 and PC-3 cancer cells. Moreover, the effects of fucoxanthin on these cell types were even higher compared to lycopene at the same concentrations (Kotake-Nara et al., 2005). The potential chemopreventive effect of fucoxanthin from brown seaweed (*Laminaria japonica)* on urinary bladder cancer cells (EJ-1) via inhibiting growth and apoptosis was determined. Fucoxanthin in the diet significantly inhibited EJ-1 cell proliferation in a time- and dose- dependent manner. Specifically, treatment with 20 µM fucoxanthin for 72 h resulted in > 93% apoptotic cells, which was characterized by morphological changes, the DNA ladder, increased hypodiploid-cell percentage, and activation of caspase-3 (Zhang et al., 2008). The effects of different carotenoids including fucoxanthin from brown algae on viabilities of three prostate cancer cell lines (PC-3, DU 145, and LNCaP) have been evaluated. Fucoxanthin and neoxanthin showed the highest rates of inhibiting proliferation, whereas phytoene, canthaxanthin, β-cryptoxanthin, and zeaxanthin had no effect. The viabilities of cells cultured in 20 µM fucoxanthin for 72 h were 14.9% for PC-3, 5.0% for DU 145, and 4.8% for LNCaP cells compared to those cultured in control medium (Kotake-Nara et al., 2001). In another study, the anti-cancer effects of fucoxanthin and neoxanthin were assessed on PC-3 cells in an apoptosis assay. The ratio of apoptotic cells was > 30% after a 48 h treatment with 20 µM fucoxanthin and the apoptosis inducing effect of this carotenoid was the result of activated

caspase-3. In addition, those authors also reported that fucoxanthinol, a fucoxanthin metabolite, induces apoptosis and has a greater inhibitory effect on PC-3 cells than that of fucoxanthin. The 50% inhibitory concentration (IC_{50}) of fucoxanthinol on proliferation of PC-3 cells was 2.0 μM compared to 3.0 μM for fucoxanthin (Kotake-Nara et al., 2005).

Halocynthiaxanthin, another fucoxanthin metabolite extracted from the sea squirt, shows higher anti-cancer activity than that of fucoxanthin. This carotenoid completely inhibits growth of human neuroblastoma (GOTO) cells, whereas fucoxanthin reduced cell growth by 88.8% at the same concentration (5 μg/ml) after 2 days (Nishino et al., 1992). The effects of halocynthiaxanthin and fucoxanthinol isolated from *Halocynthia roretzi* (sea pineapple) on induction of apoptosis and viability of human leukemia (HL-60), breast (MCF-7), and colon (Caco-2) cancer cells were compared. The 12.5 μM halocynthiaxanthin and fucoxanthinol treatments resulted in HL-60 cell viabilities of 12.1% and 5.7%, respectively, after 48 h of incubation. In addition, morphological changes (chromosomal condensation and DNA degradation) demonstrated that these two carotenoids induced apoptosis in all three cell lines. Relative DNA fragmentation in HL-60 cells treated with halocynthiaxanthin and fucoxanthinol increased 5- and 7-fold of that in control cells, respectively. Treatment with these compounds also raised relative DNA fragmentation of breast and colon cancer cells from 3- to 7-fold. In a comparison with fucoxanthin, these metabolites exhibited markedly higher inhibitory effects and apoptosis-inducing activity than those of fucoxanthin in all tested cells (Konishi et al., 2006). Halocynthiaxanthin also shows sensitizing effects on tolerance of colon cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a promising candidate cancer therapeutic. Individual treatments of halocynthiaxanthin or TRAIL only induced apoptosis

slightly in colorectal cancer (DLD-1) cells, whereas their combination significantly increased apoptosis synergistically, with clear nuclear condensation and PARP cleavage (Yoshida et al., 2007).

Fucoxanthin showed chemopreventive effects in an *in vivo* study on mouse colon carcinogenesis based on a decrease in the number of putative pre-neoplastic aberrant crypt foci (ACF), an end-point marker lesion of colon carcinogenesis. The results showed that adding 0.01% fucoxanthin from *Hijikia fusiforme* (dark brown, bushy algae called *Hijiki* in Japan) to drinking water caused a 25% reduction of ACF in colon cancer-induced mice after 7 weeks of treatment (Kim et al., 1998).

Consequently, *in vitro* and *in vivo* studies on fucoxanthin and its metabolites demonstrate that these carotenoids possess significant anti-cancer effects on a variety of cancer cells via apoptosis. However, their influences varied considerably among cancer cell types, and the metabolites (halocynthiaxanthin and fucoxanthinol) showed higher anti-cancer activity than fucoxanthin. A summary of treatments with fucoxanthin and its metabolites and their effects on various cancer cells is presented in Table 1.

Canthaxanthin, a carotenoid found in algae and crustaceans, also has considerable anti-cancer activity. In a study on the growth of murine tumor cells, up to 100 µM canthaxanthin significantly reduced the growth of JB/MS, B16F10 (melanoma tumor), and PYB6 (fibrosarcoma tumor) cells (Huang et al., 1992). An apoptosis-inducing effect of canthaxanthin was indicated in WiDr (human colon adenocarcinoma) and SK-MEL-2 (human melanoma) cells by an increase in the number of *in situ* nick end labeled-positive nuclei (Palozza et al., 1998).

Canthaxanthin increased the number of apoptotic cells in both cell lines in dose- and time-dependent manners. Treatment with 10 μM canthaxanthin for 48 h resulted in 18% and 20% apoptosis in WiDr and SK-MEL-2 cells, respectively. Moreover, the growth of WiDr cells was more inhibited than that of SK-MEL-2 cells, but no difference was observed in the number of apoptotic cells between the cell types. This result suggests that further studies should identify other pathways that may be involved in the inhibitory effect of canthaxanthin, such as stimulation of tumor necrosis factor-α (TNF-α) and other cytokines (Abdel-Fatth et al., 1993) or downregulation of the epidermal growth factor receptor (Muto et al., 1995).

The chemopreventive effect of canthaxanthin on chemically induced mammary cancer in mice has been reported. The frequency of this cancer decreased 65% in models fed a canthaxanthin-supplemented diet for 3 weeks prior to inducing cancer with dimethylbenzanthracene (Grubbs et al., 1991). The modulatory effect of canthaxanthin on colon carcinogenesis was investigated in another *in vivo* study on male F344 rats. After a 34 week treatment with 500 ppm canthaxanthin, the incidence and multiplicity of neoplasms in the large intestine of rats were significantly smaller than those in the control group. Additionally, the development of aberrant crypt foci induced by azoxymethane was considerably inhibited by canthaxanthin supplementation (Tanaka et al., 1995). Canthaxanthin also markedly reduced the size of skin papillomas in mice induced by 200 mg/kg/day 9, 10-dimethyl-1, 2-benzanthracene for 14 days (Katsumura et al., 1996). Oral administration of canthaxanthin starting 15 days before tumor inoculation delayed the appearance of macroscopic ascites and prolonged survival of BALB/c mice bearing thymoma cells in a dose-dependent manner. In addition, the effect of this carotenoid on females was greater than that in males because it was more easily absorbed in tumor cells by females than by

males (Palozza et al., 1997). Several studies have suggested that cancer chemopreventive action is highly correlated with the ability of anti-cancer agents to upregulate gap junctional intercellular communication. Even at a low dose (1 μM), canthaxanthin increases the level of connexin43, a gene encoding a major gap junction protein in C3H10T1/2 (mouse embryo) cells (Hanusch et al., 1995). In a clinical trial, 235 women from the Bronx, New York City (USA) were treated with a carotenoid-supplemented diet to investigate the effects of some carotenoids on cervical intraepithelial neoplasia (CIN) and cervical cancer, based on decreases in plasma levels of these compounds (Palan et al., 1996). Mean plasma canthaxanthin levels were 1.91 ± 1.8 μg/dl in patients with cervical cancer and 2.48 ± 2.4, 1.83 ± 1.5, and 1.71 ± 1.1 μg/dl in patients with CIN grades 1, 2, and 3 disease, respectively, compared to that of a control group (3.33 ± 2.5 μg/dl). These results suggest that dietary canthaxanthin may be a risk factor for CIN and cervical cancer, and that the antioxidant properties of this carotenoid may be responsible for preventing cancer. Therefore, it is necessary to carry out further studies to clarify the anti-cancer effect of this carotenoid.

The literature on anticancer activity of canthaxanthin suggests that it may be a candidate for cancer treatment due to its inhibitory effects on various types of *in vitro* cancer cell lines as well as in *in vivo* and clinical cancer models. The treatments and effects of canthaxanthin on cancer cells and models are summarized in Table 2.

Because canthaxanthin and astaxanthin are present abundantly together in algae, several studies have investigated the biological functions of these carotenoids simultaneously. Tanaka and coauthors carried out a series of studies on the chemopreventive effects of both canthaxanthin

and astaxanthin against various cancer types. In a study on oral carcinogenesis of F344 male rats induced by 4-nitroquinoline I-oxide (4-NQO), the incidence of preneoplastic lesions and neoplasms in rats treated with 100 ppm astaxanthin or canthaxanthin were significantly smaller than those of the control group (Tanaka et al., 1995). In particular, treatment with either astaxanthin or canthaxanthin during 4-NQO treatment completely prevented the development of oral neoplasms. The number of silver-stained nucleolar organizer region proteins (AgNORs), an index of cell proliferation, and the BrdUrd-labeling index also indicated a decrease in cell proliferation activity in the non-lesional squamous epithelium of rats fed a diet supplemented with astaxanthin or canthaxanthin (Tanaka et al., 1995). They also investigated the effects of these two xanthophylls on mouse urinary bladder carcinogenesis. Administration of water containing 50 ppm astaxanthin or canthaxanthin for 20 weeks resulted in a lower incidence of preneoplastic lesions and neoplasms in the bladder of treated mice compared to those in untreated mice. The number of AgNORs in the transitional epithelium also decreased in mice treated with astaxanthin or canthaxanthin. In addition, although both astaxanthin and canthaxanthin showed anti-proliferative effects on the mouse urinary bladder, the magnitude of the astaxanthin effect was greater than that of canthaxanthin (Tanaka et al., 1994). In another study by these authors, diets supplemented with 500 ppm astaxanthin or canthaxanthin individually for 34 weeks during the post-initiation phase of colon carcinogenesis significantly reduced the incidence and multiplicity of neoplasms in the large intestine of rats. These treatments also inhibited development of aberrant crypt foci and reduced cell proliferation (Tanaka et al., 1995). A study was conducted on mammary tumors in female BALB/c mice to compare anti-cancer effects of astaxanthin, canthaxanthin and β-carotene. Decreases in

mammary tumor volume were observed in the groups fed the carotenoid diet prior to inoculation with tumor cells, and astaxanthin showed a greater inhibitory effect on growth of this tumor than that of the other carotenoids (Chew et al., 1999). The inhibitory activity of astaxanthin, canthaxanthin, and six other plant carotenoids on AH109A hepatoma cell invasion was also studied. All of these carotenoids inhibited AH109A cell invasion in a dose-dependent manner, and the inhibition reached a plateau at ≥ 5 μM. Among these carotenoids, canthaxanthin had a greater effect than the others, including β-carotene, lycopene, astaxanthin, β-cryptoxanthin, α-carotene, zeaxanthin, and lutein (Kozuki et al., 2000).

The effects of algal extracts containing at least 14% astaxanthin and synthetic astaxanthin were investigated on UVA-irradiated human skin fibroblasts (1BR-3), human melanocytes (HEMAc), and human intestinal (CaCo-2) cancer cells (Lyons and O'Brien, 2002). Single cell gel electrophoresis and the comet assay indicated that the astaxanthin and synthetic astaxanthin treatments significantly prevented DNA damage induced by UVA in all three cell lines. Superoxide dismutase (SOD) activity of 1BR-3 cells was significantly higher and glutathione (GSH) content was markedly reduced after exposure to UVA. Nevertheless, cells pre-treated with algal astaxanthin and synthetic astaxanthin (10 μM 18 h prior to exposure to UVA) had unchanged SOD activity and GSH content compared to cells before UVA exposure. Similarly, the GSH content of intestinal cells treated with 10 μM astaxanthin during exposure to UVA was unchanged, whereas untreated cells had a considerably lower level of GSH after exposure to UVA. Although both algal and synthetic astaxanthin exhibited significant modulatory effects on the cells, the effects of astaxanthin from the algal extract were lower than those of the synthetic compound in all cell types. This difference may be related with the significantly lower

absorption of algal astaxanthin in cells compared to the synthetic compound (Lyons and O'Brien, 2002). Astaxanthin inhibits growth of human prostate cancer cells induced by androgens in a dose-dependent manner (Sharoni et al., 2002). Several *in vivo* studies have investigated the anti-cancer effect of astaxanthin. In a study on transplantable methylcholanthrene-induced fibrosarcoma (Meth-A tumor) cells of mice, reduced weight and size of tumors were observed following astaxanthin treatment (Jyonouchi et al., 2000). Tumor weight and size in mice fed a astaxanthin diet (40 µg/kg body wt/day) for 1 and 3 weeks before tumor inoculation decreased significantly, whereas feeding during inoculation reduce tumor size or weight. Correspondingly, cytotoxic T lymphocyte activity and interferon-γ (IFN-γ) production (two modes of anticancer activity) increased markedly by feeding astaxanthin for 1 and 3 weeks before tumor inoculation, but no effect was observed when astaxanthin was fed during tumor inoculation, except IFN-γ production by spleen cells (Jyonouchi et al., 2000). Daily oral administration (1 mg/kg/day) of astaxanthin for 14 days also significantly lowered hepatic metastasis induced by restraint stress in mice by inhibiting stress-induced lipid peroxidation (Kurihara et al., 2002).

Perinidin, a carotenoid found in dinoflagellates, has strong anti-cancer activity. The apoptosis-inducing ability of perinidin isolated from the dinoflagellate *Heterocapsa triquetra* was investigated in DLD-1 cells. This ability was clearly demonstrated based on the morphological changes (condensed chromatin fragments) caused by an increase in caspase-8 (2.4-fold) and caspase-9 (1.8-fold) activities after a 20 µM perinidin treatment for 48 h. Furthermore, these results showed a dose-dependent reduction in the viability of perinidin-treated DLD-1 cells. DLD-1 cell viability was about 40% compared to that of control cells after a 72 h treatment with 20 µM perinidin (Sugawara et al., 2007). In another investigation, perinidin was a potential

sensitizer of colorectal cancer (DLD-1) cell tolerance to TRAIL. The combination of TRAIL and perinidin isolated from clams caused a significant increase in the proportion of apoptotic cells. Moreover, the effect of perinidin was greater than that of halocynthiaxanthin at the same concentration (Yoshida et al., 2007).

Anti-obesity and Anti-diabetic Effects

Most reports on the anti-obesity and anti-diabetic effects of marine carotenoids have focused on fucoxanthin and astaxanthin due to their strong activities.

Maeda and coauthors carried out a series of studies related to the effects of fucoxanthin and its metabolites on obesity and diabetes *in vitro* and *in vivo*. In a study on anti-obesity activity of a seaweed (*U. pinnatifida*) extract, they reported that fucoxanthin is the active component in the lipid fraction of this seaweed (Maeda et al., 2005). A diet containing 2% *Undaria* lipids significantly reduced white adipose tissue (WAT) weight in rats and mice after 4 weeks compared to that in control fed animals. To clarify the contributions of bioactive compounds in the diet to the inhibitory effect on WAT, fucoxanthin and glycolipids were isolated and used in separate treatments. The results showed that fucoxanthin was responsible for the effect because treatment with the fucoxanthin-rich fraction (67.4% fucoxanthin) resulted in a marked decrease in WAT weight in mice, but no change was observed after treatment with fucoxanthin-free glycolipid fraction. Expression

of uncoupling protein 1 (UCP1), a key factor preventing excess fat accumulation, was also assessed to determine the mechanism of action of fucoxanthin during anti-obesity activity of the seaweed extract. UCP1 protein and mRNA expression in WAT was observed in mice fed the *Undaria* lipid or fucoxanthin-rich fractions, whereas only slight UCP1 expression was observed in the control and glycolipid-fed mice (Maeda et al., 2005). Fucoxanthin and its metabolite, fucoxanthinol, also exhibit suppressive effects on adipocyte differentiation of 3T3-L1 (murine preadipocyte) cells, which are closely related to the development of adipose tissue. Treatment with 25 µM fucoxanthin for 120 h reduced lipid accumulation during cell differentiation to 70% of that in control cells with no cytotoxic effect. Fucoxanthinol inhibited lipid accumulation significantly more than that of fucoxanthin. The accumulation of lipid in cells treated with 10 µM fucoxanthinol was only 14% compared to that of control cells after 120 h. Furthermore, glycerol-3-phosphate dehydrogenase (GPDH) activity, an indicator of adipocyte differentiation, and expression of peroxisome proliferator-activated receptor gamma, a factor regulating expression of adipogenic genes, were significantly inhibited by fucoxanthin and fucoxanthinol treatments; in these cases, fucoxanthinol also had stronger effects than fucoxanthin (Maeda et al., 2006). These authors also combined fucoxanthin and fish oil to verify if this combination

in the diet is more effective against obesity and diabetes (Maeda et al., 2007). The weights of uterine, mesentery, perirenal, and retroperitoneal WAT in mice fed a diet consisting of 0.1% fucoxanthin and fish oil were significantly lower than those in control mice, whereas a diet composed of 0.1% fucoxanthin or fish oil alone did not result in any difference in WAT weight compared to that of the control. The authors found that combining fucoxanthin and fish oil in the diet contributed additive effects on the treated mice but not synergistic effects. Other studies have revealed that fish oil is rich in eicosapentaenoic acid and docosahexaenoic acid (DHA), which can decrease serum and liver triglycerides, by reducing the triglyceride supply to adipose tissue (Berge et al., 1999; Gronn et al., 1992). A lower level of fucoxanthin (0.2% compared to 0.4% in a previous study) also markedly decreased WAT weight, blood glucose, and plasma insulin levels and significantly increased UCP1 expression compared to those in the control group. Moreover, this diet suppressed TNF-α mRNA and plasma leptin levels, which are elevated by fat accumulation in adipocytes and cause insulin resistance in obese animals (Maeda et al., 2007). The combination of fucoxanthin contained in *U. pinnatifida* lipids and scallop phospholipids in capsules also exhibited anti-obesity properties. Male KK-A(y) mice treated with these capsules in drinking water achieved a significant reduction in body weight compared to that in controls. In

addition, UPC1 and UPC1 mRNA expression in epididymal fat of these mice was considerably higher than that in the controls (Okada et al., 2011).

In the first report on the effect of fucoxanthin on hepatic DHA, which plays key roles preventing obesity and diabetes, the amount of hepatic DHA in KKAy obese/diabetic mice increased after feeding a fucoxanthin-containing diet. The amount of hepatic DHA in mice fed 0.1% and 0.2% fucoxanthin was 1.7 and 1.9 times higher than that of control mice, respectively (Tsukui et al., 2007). A clinical trial was carried out to investigate the effects of Xanthigen^{TM} (combination of brown seaweed fucoxanthin and pomegranate seed oil [PSO]) in obese women with and without non-alcoholic fatty liver disease (NAFLD) in Russia. Treatment with Xanthigen-600/2.4 mg (300 mg PSO + 300 mg brown seaweed extract containing 2.4 mg fucoxanthin) for 16 weeks markedly reduced body weight, liver fat content, and waist circumference in the NAFLD patient group compared to baseline and the placebo group (olive oil treatment). Furthermore, the mechanisms behind these decreases were also evidenced by significant decreases in serum triglycerides, plasma C-reactive protein (related to the development of insulin resistance), levels of plasma aminotransferase enzymes, such as gamma-glutamyl transferase and alanine aminotransferase, which are associated with accumulation of liver fat and decreased hepatic insulin sensitivity, respectively, and a marked increase in resting energy expenditure (an important factor affecting weight loss) (Abidov et al., 2010).

The anti-obesity effect of astaxanthin was clearly illustrated through a variety of parameters in obese-promoted mice treated with this carotenoid. Mice fed a high fat and high fructose diet (HFFD) showed significant weight gain, increased liver weight, liver lipids, and plasma glucose,

insulin, and lipids levels compared to those of control mice. However, the astaxanthin treatment (6 mg/kg body weight/day) significantly reduced body weight of HFFD-fed mice and returned levels of other parameters closely to values of control mice (Bhuvaneswari et al., 2010). These results are consistent with those of a previous study, which showed an obvious decrease in body, adipose tissue, liver weight, liver triglycerides, plasma triglycerides, and total cholesterol levels in obese mice fed a high-fat diet supplemented with astaxanthin (30 mg/kg) (Ikeuchi et al., 2007). Astaxanthin also has positive effects on a series of metabolic syndrome features in a SHR/NDmcr-cp rat model. Administration of astaxanthin (50 mg/kg/day) for 18 weeks lowered fasting blood glucose levels in treated rats compared to those in the control. In addition, the treated group exhibited significant decreases in plasma levels of triglycerides and non-esterified fatty acids, a reduction in WAT size, and an increase in high-density lipoprotein cholesterol. Improvements in adiponectin level and insulin sensitivity were also observed in the astaxanthin-treated group (Hussein et al., 2007). Anti-obesity and anti-diabetic effects of astaxanthin have been exhibited through improved insulin sensitivity in mice. In an *in vivo* study, the mechanisms of the insulin-sensitivity effect of this carotenoid were reported for the first time. A 60-day treatment of male Swiss albino mice with astaxanthin (6 mg/kg per day) showed that improved insulin signaling was caused by activation of insulin receptor-β and by decreases in secretion of oxidative stress and pro-inflammatory cytokine (Arunkumar et al., 2012).

Diabetes mellitus causes an imbalance in the antioxidant system of the salivary glands, reducing SOD activity and increasing catalase and glutathione peroxidase activities (Nogueira et al., 2005). Although submandibular saliva of diabetic rats induced by alloxane has lower SOD activity (60%) and higher catalase (184%) and glutathione (225%) peroxidase activities

compared to those of normal rats, an astaxanthin treatment (20 mg /kg body weight for 7 days) normalized all of these activities closely to levels of normal rats. These alterations in the salivary antioxidant system suggest that astaxanthin may be a therapeutic agent for diabetes (Leite et al., 2010).

In conclusion, a series of *in vivo* studies on mice and rats indicate that administration of astaxanthin-supplemented diets results in significantly beneficial effects on diabetic and obese models by decreasing body weight, adipose tissue, plasma triglyceride and glucose levels, and increasing insulin sensitivity. A list of *in vivo* anti-obesity and anti-diabetic effects of this marine carotenoid is presented in Table 3.

Anti-inflammatory Activity

Among marine carotenoids, fucoxanthin and particularly astaxanthin have attracted the attention of many scientists because of their anti-inflammatory potential.

Ohgami and coworkers investigated the anti-inflammatory activities of these carotenoids both *in vitro* using RAW 264.7 (mouse leukemic monocyte macrophage) cells and *in vivo* in an endotoxin-induced uveitis (EIU) rat model (Ohgami et al., 2003; Shiratori et al., 2005). One of their studies showed that fucoxanthin inhibits the development of lipopolysaccharide (LPS)-induced EIU in rats in a dose-dependent manner. Moreover, the mechanisms of this effect were also studied both *in vivo* and *in vitro*. Treatment with fucoxanthin markedly inhibited inflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE2) and suppressed the expression of pro-inflammatory markers, including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and TNF-α in the aqueous humor as well as in RAW 264.7 cells.

The inhibitory effects of fucoxanthin were comparable to those of prednisolone, a common anti-inflammatory drug, and higher than those of astaxanthin at the same dose (Shiratori et al., 2005). The anti-inflammatory effect of 100 mg/kg astaxanthin was as strong as that of 10 mg/kg prednisolone, whereas the effects of fucoxanthin were comparable to those of prednisolone (Ohgami et al., 2003). The inhibition of NO and iNOS and COX-2 expression by astaxanthin was reported in an *in vitro* study on LPS-stimulated BV2 microglial cells (Choi et al., 2008). The results of this study were consistent with a previous study, which showed suppressed serum levels of NO, PGE2, TNF-α, and interleukin-1β (IL-1β) in LPS-fed mice treated with astaxanthin. These suppressed levels were explained by the ability of astaxanthin to inhibit activation of nuclear factor-κB (NF-κB), which stimulates pro-inflammatory genes encoding the above factors (Lee et al., 2003). One study on LPS-stimulated neutrophils showed that astaxanthin significantly reduces the production of TNF-α and IL-6, which are pro-inflammatory cytokines. Treatment with 5 μM astaxanthin not only improved phagocytic and microbicidal capacity of neutrophils but also significantly decreased oxidative damage to lipids and proteins in human neutrophils (Macedo et al., 2010). The inhibitory activity of astaxanthin against secretion of IL-1β, IL-6, and TNF-α was confirmed in U937 (human lymphoma) cells induced with H_2O_2. Levels of these cytokines in cells pre-incubated with 10 μM astaxanthin before H_2O_2 stimulation were approximately or less than half of those of cells not pre-treated with astaxanthin. In addition, SHP-1 (a protein tyrosine phosphate) expression, an inhibitor of cytokine secretion, was restored by pre-incubation with astaxanthin and negatively correlated with NF-κB expression (Speranza et al., 2012). Another study indicated reduced gastric inflammation in *Helicobacter pylori*-infected mice treated orally with a micro algae

(*Haematococcus pluvialis)* extract containing astaxanthin. Treatment with 200 mg of the algal extract/kg body weight for 10 days significantly decreased inflammatory grade and mucosa-bacterial load in the mouse stomach. These reductions were associated with a shift in the T-lymphocyte response from a Th1-response to a mixed Th1/Th2-response. This change was demonstrated by suppressed IFN-γ release and enhanced IL-4 release in splenocytes of infected mice treated with astaxanthin (Bennedsen et al., 1999). Furthermore, clinical evidence has been reported about the antioxidative and anti-inflammatory effects of astaxanthin on young healthy South Korean females. The anti-inflammatory effect of this compound was demonstrated through decreases in plasma 8-OHdG (a DNA damage biomarker) and plasma C-reactive protein concentrations in subjects fed 2 mg astaxanthin. In addition, a diet containing astaxanthin also inhibited ROS, thereby reducing ROS-induced production of transcription factors, such as NF-kB and AP-1, which regulate genes controlling inflammatory cytokine production (Park et al., 2010).

Cardioprotective Effects

The first report on the antihypertensive effects of astaxanthin was carried out by Hussein et al. (2005) in spontaneously hypertensive rats (SHR). In that report, short-term administration of astaxanthin (50 mg/kg body weight/day) for 2 weeks led to a significant decrease in blood pressure. Long-term treatment with astaxanthin also reduced blood pressure and delayed the incidence of stroke. The rate of stroke in the control group was 60% on day 4, but no signs of stroke were observed in the astaxanthin-treated group at this time. These authors also illustrated the mechanisms of this effect in later studies. In one study, they observed a significant

modulatory effect on NO-induced vasorelaxation and improved contractions of the thoracic aorta in SHR rats treated with astaxanthin. These effects were suggested to be associated with NO-related mechanisms based on a marked reduction in NO. In addition, the beneficial effects of astaxanthin on blood rheology were demonstrated based on a considerable decrease in transit time of whole blood in astaxanthin-treated SHR rats compared to control SHR rats. Astaxanthin administration also resulted in beneficial histopathological changes, including a reduction in the wall/lumen ratio of the coronary artery and fewer and straighter elastin bands in the aorta (Hussein et al., 2006; Hussein et al., 2005). Adding 25 mg astaxanthin/kg to the feed for 1 month significantly decreased systolic blood pressure of Zucker Fatty rats in a study by Preuss et al. (2011). This astaxanthin supplement also decreased renin-angiotensin system activity, suggesting that the renin-angiotensin system is involved in the blood pressure lowering ability of astaxanthin. Moreover, heart stress tests with heat resulted in > 60% death in the control group, whereas all rats in the astaxanthin-treated group survived. The cardioprotective effect of astaxanthin has also been demonstrated by an increase in heart mitochondrial membrane potential and contractility index in BALB/c mice treated with a diet containing 0.08% astaxanthin for 8 weeks (Nakao et al., 2010). A clinical trial of 24 adults showed the preventive properties of astaxanthin against atherosclerosis by prolonging oxidation lag time of low-density lipoprotein cholesterol. Participants consumed this marine carotenoid for 14 days at doses of 1.8, 3.6,14.4, and 21.6 mg and had 5.0, 26.2, 42.3, and 30.7% longer lag times, respectively, compared to those at day 0 (Iwamoto et al., 2000). A later study with 24 men showed the beneficial effects of astaxanthin isolated from *H. pluvialis* on human blood rheology. Ten days after ingesting 6 mg astaxanthin per day, the blood transit time of the treated group decreased

significantly (from 52.8 ± 4.9 s before treatment to 47.6 ± 4.2 s), and this value was also significantly lower than that of a placebo group (54.2 ± 6.7 s) (Miyawaki et al., 2008). Another human study with participants aged 25--60 years demonstrated that a 12 week administration of astaxanthin reduced serum triglycerides and increased high-density lipoprotein cholesterol significantly, whereas low-density lipoprotein cholesterol was unaffected. In addition, serum adiponectin also increased, and the changes in adiponectin were positively correlated with changes in high-density lipoprotein cholesterol independent of age and body mass index (Yoshida et al., 2010).

Fucoxanthin extracted from *U. pinnatifida* and its metabolite fucoxanthinol also exhibit cardioprotective effects *in vivo* by reducing blood triglyceride concentration, which is associated with atherosclerotic vascular disease (Matsumoto et al., 2010). Treatments with 2 mg fucoxanthin or fucoxanthinol resulted in significant decreases in triglyceride concentrations in jugular blood of non-pre-digested soybean oil-fed rats.

Bioavailability of Marine Carotenoids

Although many studies on the bioactivities and functions of marine carotenoids have been carried out, the number of reports on their bioavailability, an important factor influencing bio-efficiency in cells, is still limited. Almost all reports have focused on the most common marine carotenoids, including fucoxanthin, astaxanthin, and canthaxanthin.

The absorption of fucoxanthin from brown algae *(U. pinnatifida)* by human intestinal Caco-2 cells was compared with that of other carotenoids (Sugawara et al., 2001). The results showed that the amount of fucoxanthin absorbed by Caco-2 cells was very low and was approximately

25% of that of lutein. In a later *in vivo* study, a concentration of 10.4 ± 5.3 nM fucoxanthinol, a fucoxanthin metabolite, was detected in mice plasma 1 h after intubation of 40 nmol fucoxanthin (Sugawara et al., 2002). Another metabolite of fucoxanthin, amarouciaxanthin A, was found in the liver of mice orally administered fucoxanthin (Asai et al., 2004). Consistent results on detecting fucoxanthin metabolites in plasma and liver of rats administered fucoxanthin from brown algae (*Padina tetrastromatica)* were reported by Sangeetha et al. (2010). In a rare study on humans, fucoxanthin bioavailability was very low. The plasma concentration of fucoxanthinol in humans was < 1.0 nM (less than the high performance liquid chromatography quantification limit) after 1-week administration of a daily diet containing 6.1 mg fucoxanthin from brown algae (Asai et al., 2008).

Carotenoids are very lipophilic compounds, which causes their low bioavailability. Thus, the influences of various fat and lipid-based formulations have been investigated. The bioavailability of algal astaxanthin in healthy volunteers administered a dose of 40 mg astaxanthin increased from 1.5 to 3.7 times following administration of various lipid-base formulations (Mercke et al., 2003). Absorption of astaxanthin from different emulsions of olive and corn oil into the mesenteric lymph duct of rats was also determined. The average recovery of astaxanthin in lymph was 20% and 13% for olive and corn oil emulsions, respectively (Clark et al., 2000).

The effects of carotenoid concentration and luminal lipid on the absorption of canthaxanthin by lymph duct-cannulated rats were investigated (Clark and Furr, 2001; Clark et al., 1998). The results showed that the average efficiency of canthaxanthin absorption was 16% and did not

depend on the infused concentration. A linear increase was observed for canthaxanthin absorption when the amount of lipid in the emulsions increased.

These results suggest that the low bioavailability of marine carotenoids could be improved by flexible lipid-based formulations. Thus, further studies should be conducted to promote the functional efficiency of marine carotenoids by increasing their bioavailability.

Conclusions

In summary, the great therapeutic potential of marine carotenoids in various diseases, such as cancers, obesity, diabetes, and cardiovascular diseases, has been demonstrated. Among the variety of marine carotenoids, fucoxanthin from brown seaweed and astaxanthin from algae exhibit markedly stronger effects *in vitro* and *in vivo* and in clinical treatments of diseases. In addition, many reports demonstrated that marine carotenoids have higher bioactivities than those from plant sources or synthetic compounds. Although the physiological effects of marine carotenoids have been reported, almost all studies have concentrated on fucoxanthin and its metabolites, astaxanthin, and canthaxanthin. In contrast, few reports are available on the mechanism of these effects and biological functions of many other marine carotenoids. Consequently, it is necessary to carry out further studies to investigate the bioactivities of many marine carotenoids other than the major ones in this review and discover the underlying mechanisms of their activities.

References

Abdel-Fatth, G., Watzl, B., Huang, D., & Watson, R. R. (1993). Beta-carotene in vitro stimulates tumor necrosis factor alpha and interleukin 1 alpha secretion by human peripheral blood mononuclear cells. *Nutrition Research.* **13** : 863-871.

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 Obes Metab.* **12** : 72-81.

Arunkumar, E., Bhuvaneswari, S., & Anuradha, C. V. (2012). An intervention study in obese mice with astaxanthin, a marine carotenoid--effects on insulin signaling and pro-inflammatory cytokines. *Food Funct.* **3** : 120-126.

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 Metab Dispos.* **32** : 205-211.

Asai, A., Yonekura, L., & Nagao, A. (2008). Low bioavailability of dietary epoxyxanthophylls in humans. *Br J Nutr.* **100** : 273-277.

Bennedsen, M., Wang, X., Willen, R., Wadstrom, T., & Andersen, L. P. (1999). Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. *Immunol Lett.* **70** : 185-189.

Berge, R. K., Madsen, L., Vaagenes, H., Tronstad, K. J., Gottlicher, M., & Rustan, A. C. (1999). In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *Biochem J.* **343** : 191-197.

Bhuvaneswari, S., & Anuradha, C. V. (2012). Astaxanthin prevents loss of insulin signaling and improves glucose metabolism in liver of insulin resistant mice. *Canadian Journal of Physiology and Pharmacology.* **90** : 1544-1552.

Bhuvaneswari, S., Arunkumar, E., Viswanathan, P., & Anuradha, C. V. (2010). Astaxanthin restricts weight gain, promotes insulin sensitivity and curtails fatty liver disease in mice fed a obesity-promoting diet. *Process Biochemistry.* **45** : 1406-1414.

Chew, B. P., Park, J. S., Wong, M. W., & Wong, T. S. (1999). A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo. *Anticancer Res.* **19** : 1849-1853.

Choi, S. K., Park, Y. S., Choi, D. K., & Chang, H. I. (2008). Effects of astaxanthin on the production of NO and the expression of COX-2 and iNOS in LPS-stimulated BV2 microglial cells. *J Microbiol Biotechnol.* **18** : -1996.

Clark, R. M., & Furr, H. C. (2001). Absorption of canthaxanthin by the rat is influenced by total lipid in the intestinal lumen. *Lipids.* **36** : 473-475.

Clark, R. M., Yao, L., She, L., & Furr, H. C. (1998). A comparison of lycopene and canthaxanthin absorption: using the rat to study the absorption of non-provitamin A carotenoids. *Lipids.* **33** : 159-163.

Clark, R. M., Yao, L., She, L., & Furr, H. C. (2000). A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. *Lipids.* **35** : 803-806.

Ghidalia, W. (1985). The Biology of Crustacea. Academic Press, New York.

Gronn, M., Christensen, E., Hagve, T. A., & Christophersen, B. O. (1992). Effects of dietary purified eicosapentaenoic acid (20:5 (n-3)) and docosahexaenoic acid (22:6(n-3)) on fatty acid desaturation and oxidation in isolated rat liver cells. *Biochim Biophys Acta.* **1125** : 35-43.

Gross, J. (1991). Pigments in Vegetables: Chlorophylls and Carotenoids. Van Nostrand Reinhold, New York.

Grubbs, C. J., Eto, I., Juliana, M. M., & Whitaker, L. M. (1991). Effect of canthaxanthin on chemically induced mammary carcinogenesis. *Oncology.* **48** : 239-245.

Hanusch, M., Stahl, W., Schulz, W. A., & Sies, H. (1995). Induction of gap junctional communication by 4-oxoretinoic acid generated from its precursor canthaxanthin. *Arch Biochem Biophys.* **317** : 423-428.

Hosokawa, M., Kudo, M., Maeda, H., Kohno, H., Tanaka, T., & Miyashita, K. (2004). Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARgamma ligand, troglitazone, on colon cancer cells. *Biochim Biophys Acta.* **1675** : 113-119.

Hosokawa, M., Okada, T., Mikami, N., Konishi, I., & Miyashita, K. (2009). Bio-functions of marine carotenoids. *Food Science and Biotechnology*, **18** : 1-11.

Hosokawa, M., Wanezaki, S., Miyauchi, K., Kurihara, H., Kohno, H., Kawabata, J.,... Takahashi, K. (1999). Apoptosis-inducing effect of fucoxanthin on human leukemia cell HL-60. *Food Sci. Technol. Res.* **5** : 243--246.

Huang, D. S., Odeleye, O. E., & Watson, R. R. (1992). Inhibitory effects of canthaxanthin on in vitro growth of murine tumor cells. *Cancer Lett.* **65** : 209-213.

Hussein, G., Goto, H., Oda, S., Sankawa, U., Matsumoto, K., & Watanabe, H. (2006). Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats. *Biol Pharm Bull.* **29** : 684-688.

Hussein, G., Nakagawa, T., Goto, H., Shimada, Y., Matsumoto, K., Sankawa, U., & Watanabe, H. (2007). Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. *Life Sci.* **80** : 522-529.

Hussein, G., Nakamura, M., Zhao, Q., Iguchi, T., Goto, H., Sankawa, U., & Watanabe, H. (2005). Antihypertensive and neuroprotective effects of astaxanthin in experimental animals. *Biol Pharm Bull.* **28** : 47-52.

Ikeuchi, M., Koyama, T., Takahashi, J., & Yazawa, K. (2007). Effects of astaxanthin in obese mice fed a high-fat diet. *Biosci Biotechnol Biochem.* **71** : 893-899.

Iwamoto, T., Hosoda, K., Hirano, R., Kurata, H., Matsumoto, A., Miki, W.,... Kondo, K. (2000). Inhibition of low-density lipoprotein oxidation by astaxanthin. *J Atheroscler Thromb.* **7** : 216-222.

Jyonouchi, H., Sun, S., Iijima, K., & Gross, M. D. (2000). Antitumor activity of astaxanthin and its mode of action. *Nutr Cancer.* **36** : 59-65.

Katsumura, N., Okuno, M., Onogi, N., Moriwaki, H., Muto, Y., & Kojima, S. (1996). Suppression of mouse skin papilloma by canthaxanthin and beta-carotene in vivo: possibility of the regression of tumorigenesis by carotenoids without conversion to retinoic acid. *Nutr Cancer.* **26** : 203-208.

Kim, J. M., Araki, S., Kim, D. J., Park, C. B., Takasuka, N., Baba-Toriyama, H.,... Tsuda, H. (1998). Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. *Carcinogenesis.* **19** : 81-85.

Kim, K. N., Heo, S. J., Kang, S. M., Ahn, G., & Jeon, Y. J. (2010). Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-mediated Bcl-xL pathway. *Toxicol In Vitro.* **24** : 1648-1654.

Konishi, I., Hosokawa, M., Sashima, T., Kobayashi, H., & Miyashita, K. (2006). Halocynthiaxanthin and fucoxanthinol isolated from Halocynthia roretzi induce apoptosis in human leukemia, breast and colon cancer cells. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology.* **142** : 53-59.

Kotake-Nara, E., Asai, A., & Nagao, A. (2005). Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells. *Cancer Lett.* **220** : 75-84.

Kotake-Nara, E., Kushiro, M., Zhang, H., Sugawara, T., Miyashita, K., & Nagao, A. (2001). Carotenoids affect proliferation of human prostate cancer cells. *J Nutr.* **131** : 3303-3306.

Kotake-Nara, E., Sugawara, T., & Nagao, A. (2005). Antiproliferative effect of neoxanthin and fucoxanthin on cultured cells. *Fisheries Science.* **71** : 459-461.

Kozuki, Y., Miura, Y., & Yagasaki, K. (2000). Inhibitory effects of carotenoids on the invasion of rat ascites hepatoma cells in culture. *Cancer Lett.* **151** : 111-115.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Mol Aspects Med.* **26** : 459-516.

Kurihara, H., Koda, H., Asami, S., Kiso, Y., & Tanaka, T. (2002). Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress. *Life Sci.* **70** : 2509-2520.

Lee, S. J., Bai, S. K., Lee, K. S., Namkoong, S., Na, H. J., Ha, K. S.,... Kim, Y. M. (2003). Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation. *Mol Cells.* **16** : 97-105.

Leite, M. F., Lima, A. M., Massuyama, M. M., & Otton, R. (2010). Astaxanthin restores the enzymatic antioxidant profile in salivary gland of alloxan-induced diabetic rats. *Arch Oral Biol.* **55** : 479-485.

Lyons, N. M., & O'Brien, N. M. (2002). Modulatory effects of an algal extract containing astaxanthin on UVA-irradiated cells in culture. *J Dermatol Sci.* **30** : 73-84.

Macedo, R. C., Bolin, A. P., Marin, D. P., & Otton, R. (2010). Astaxanthin addition improves human neutrophils function: in vitro study. *Eur J Nutr.* **49** : 447-457.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2005). Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem Biophys Res Commun.* **332** : 392-397.

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-Ay mice. *J Agric Food Chem.* **55** : 7701-7706.

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. *Int J Mol Med.* **18** : 147-152.

Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M.,... Hara, H. (2010). Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *Eur J Nutr.* **49** : 243-249.

Matsuno, T. (2001). Aquatic animal carotenoids. *Fisheries Science.* 67 : 771-783.

Mein, J. R., Lian, F., & Wang, X. D. (2008). Biological activity of lycopene metabolites: implications for cancer prevention. *Nutr Rev.* **66** : 667-683.

Mercke, O. J., Lignell, A., Pettersson, A., & Hoglund, P. (2003). Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. *Eur J Pharm Sci*. **19** : 299-304.

Miyawaki, H., Takahashi, J., Tsukahara, H., & Takehara, I. (2008). Effects of astaxanthin on human blood rheology. *J Clin Biochem Nutr*. **43** : 69-74.

Molnar, P., Kawase, M., Satoh, K., Sohara, Y., Tanaka, T., Tani, S.,... Molnar, J. (2005). Biological activity of carotenoids in red paprika, Valencia orange and Golden delicious apple. *Phytother Res.* **19** : 700-707.

Muto, Y., Fujii, J., Shidoji, Y., Moriwaki, H., Kawaguchi, T., & Noda, T. (1995). Growth retardation in human cervical dysplasia-derived cell lines by beta-carotene through down-regulation of epidermal growth factor receptor. *Am J Clin Nutr.* **62** : 1535S-1540S.

Nakao, R., Nelson, O. L., Park, J. S., Mathison, B. D., Thompson, P. A., & Chew, B. P. (2010). Effect of astaxanthin supplementation on inflammation and cardiac function in BALB/c mice. *Anticancer Res.* **30** : 2721-2725.

Nishino, H., Tsushima, M., Matsuno, T., Tanaka, Y., Okuzumi, J., Murakoshi, M.,... et al. (1992). Anti-neoplastic effect of halocynthiaxanthin, a metabolite of fucoxanthin. *Anticancer Drugs.* **3** : 493-497.

Nogueira, F. N., Carvalho, A. M., Yamaguti, P. M., & Nicolau, J. (2005). Antioxidant parameters and lipid peroxidation in salivary glands of streptozotocin-induced diabetic rats. *Clin Chim Acta.* **353** : 133-139.

Ohgami, K., Shiratori, K., Kotake, S., Nishida, T., Mizuki, N., Yazawa, K., & Ohno, S. (2003). Effects of astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. *Invest Ophthalmol Vis Sci.* **44** : 2694-2701.

Okada, T., Mizuno, Y., Sibayama, S., Hosokawa, M., & Miyashita, K. (2011). Antiobesity effects of Undaria lipid capsules prepared with scallop phospholipids. *J Food Sci.* 76 : H2-6.

Palan, P. R., Mikhail, M. S., Goldberg, G. L., Basu, J., Runowicz, C. D., & Romney, S. L. (1996). Plasma levels of beta-carotene, lycopene, canthaxanthin, retinol, and alpha- and tau-tocopherol in cervical intraepithelial neoplasia and cancer. *Clin Cancer Res.* **2** : 181-185.

Palozza, P., Calviello, G., Serini, S., Moscato, P., Luberto, C., & Bartoli, G. M. (1997). Antitumor effect of an oral administration of canthaxanthin on BALB/c mice bearing thymoma cells. *Nutr Cancer.* **28** : 199-205.

Palozza, P., Maggiano, N., Calviello, G., Lanza, P., Piccioni, E., Ranelletti, F. O., & Bartoli, G. M. (1998). Canthaxanthin induces apoptosis in human cancer cell lines. *Carcinogenesis.* **19** : 373-376.

Park, J. S., Chyun, J. H., Kim, Y. K., Line, L. L., & Chew, B. P. (2010). Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutr Metab (Lond).* **7** : 18.

Preuss, H. G., Echard, B., Yamashita, E., & Perricone, N. V. (2011). High dose astaxanthin lowers blood pressure and increases insulin sensitivity in rats: are these effects interdependent? *Int J Med Sci.* **8** : 126-138.

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). *Mol Cell Biochem.* **333** : 299-310.

Sharoni, Y., Agemy, L., Giat, U., Kirilov, E., Danilenko, M., & Levy, J. (2002). Lycopene and astaxanthin inhibit human prostate cancer cell proliferation induced by androgens. Paper presented at *the 13th Int. Carotenoid Symp*, Honolulu.

Shiratori, K., Ohgami, K., Ilieva, I., Jin, X. H., Koyama, Y., Miyashita, K.,... Ohno, S. (2005). Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. *Exp Eye Res.* **81** : 422-428.

Speranza, L., Pesce, M., Patruno, A., Franceschelli, S., de Lutiis, M. A., Grilli, A., & Felaco, M. (2012). Astaxanthin treatment reduced oxidative induced pro-inflammatory cytokines secretion in U937: SHP-1 as a novel biological target. *Mar Drugs.* **10** : 890-899.

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. *J Nutr.* **132** : 946-951.

Sugawara, T., Kushiro, M., Zhang, H., Nara, E., Ono, H., & Nagao, A. (2001). Lysophosphatidylcholine enhances carotenoid uptake from mixed micelles by Caco-2 human intestinal cells. *J Nutr.* **131** : 2921-2927.

Sugawara, T., Yamashita, K., Sakai, S., Asai, A., Nagao, A., Shiraishi, T.,... Hirata, T. (2007). Induction of apoptosis in DLD-1 human colon cancer cells by peridinin isolated from the dinoflagellate, Heterocapsa triquetra. *Biosci Biotechnol Biochem.* **71** : 1069-1072.

Tanaka, T., Kawamori, T., Ohnishi, M., Makita, H., Mori, H., Satoh, K., & Hara, A. (1995). Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase. *Carcinogenesis.* **16** : 2957-2963.

Tanaka, T., Makita, H., Ohnishi, M., Mori, H., Satoh, K., & Hara, A. (1995). Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. *Cancer Res.* **55** : 4059-4064.

Tanaka, T., Morishita, Y., Suzui, M., Kojima, T., Okumura, A., & Mori, H. (1994). Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. *Carcinogenesis.* **15** : 15-19.

Tsukui, T., Konno, K., Hosokawa, M., Maeda, H., Sashima, T., & Miyashita, K. (2007). Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAy obese/diabetic mice. *J Agric Food Chem.* 5**5** : 5025-5029.

Yoshida, H., Yanai, H., Ito, K., Tomono, Y., Koikeda, T., Tsukahara, H., & Tada, N. (2010). Administration of natural astaxanthin increases serum HDL-cholesterol and adiponectin in subjects with mild hyperlipidemia. *Atherosclerosis.* **209** : 520-523.

Yoshida, T., Maoka, T., Das, S. K., Kanazawa, K., Horinaka, M., Wakada, M.,... Sakai, T. (2007). Halocynthiaxanthin and peridinin sensitize colon cancer cell lines to tumor necrosis factor-related apoptosis-inducing ligand. *Mol Cancer Res.* **5** : 615-625.

Zhang, Z., Zhang, P., Hamada, M., Takahashi, S., Xing, G., Liu, J., & Sugiura, N. (2008). Potential chemoprevention effect of dietary fucoxanthin on urinary bladder cancer EJ-1 cell line. *Oncol Rep.* **20** : 1099-1103.

Table 1. Anti-cancer activities of fucoxanthin and its metabolites (halocynthiaxanthin and fucoxanthinol)

Treatments	Cells/Models	Effects	References
Fucoxanthin			
20 µM, 72 h	PC-3, DU 145, and LNCaP cells	Cell viability: PC-3 (14.9%), DU 145 (5.0%), LNCaP (4.8%) Higher activity than phytoene, canthaxanthin, β-cryptoxanthin, and zeaxanthin	Kotake-Nara et al. (2001)
15.2 µM, 72 h	Caco-2, DLD-1, and HT-29 cells	Cell viability: Caco-2 (14.8%), DLD-1 (29.4%), HT-29 (50.8%) Higher activity than β-carotene and astaxanthin	Hosokawa et al. (2004)
10 µM, 72 h	MRC-5, HUC-Fm, B16, Caco-2, HCT116, and PC-3 cells	Reduction in cell viability of 5 cell lines (except MRC-5) Higher activity than lycopene	Kotake-Nara et al. (2005)
20 µM, 72 h	EJ-1 cell	More than 93% apoptotic cells	Zhang et al. (2008)
30 µM, 72 h	HL-60 cells	Mechanisms of fucoxanthin-induced apoptosis: Generation of reactive oxygen species (ROS) Cleavage of caspases -3 & -7 and poly-ADP-ribose polymerase	Kim et al. (2010)

		Decrease in Bcl-xL levels	
Drinking water 0.01%, 7 weeks	Colon cancer-induced mice	25% reduction in putative preneoplastic aberrant crypt foci	Kim et al. (1998)
Fucoxanthinol 48 h incubation	PC-3 cell	IC_{50} = 2.0 µM Higher activity than fucoxanthin	Kotake-Nara et al. (2005)
Halocynthiaxanthin 12.5 µM, 48 h	HL-60, MCF-7 and Caco-2 cells	Cell viability: HL-60 (12%), MCF-7 (62%), Caco-2 (35%) Higher activity than fucoxanthin	Konishi et al. (2006)

Table 2. Anti-cancer activity of canthaxanthin

Treatments	Cells/Models	Effects	References
10 µM, 48 h	WiDr and SK-MEL-2 cells	Ratio of apoptotic cells: WiDr (18%), SK-MEL-2 (20%)	Palozza et al. (1998)
5 µM, 24 h	AH109A hepatoma cells	- Significant inhibition on AH109A cell invasion - Greater effect than β-carotene, lycopene, astaxanthin, β-cryptoxanthin, α-carotene, zeaxanthin, and lutein	Kozuki et al. (2000)
50 ppm in water 20 weeks	Urinary bladder carcinogenesis of mice	Smaller incidence of preneoplastic lesions and neoplasms	Tanaka et al. (1994)
500 ppm/day 34 weeks	Colon carcinogenesis of male F344 rats	- Reduction in incidence and multiplicity of neoplasms in large intestine - Inhibition on the development of aberrant crypt foci	Tanaka et al. (1995)
200 mg/kg bw/day 14 days	Skin papillomas in mice	- Reduction of 30% in size of skin papillomas - Higher effect than β-carotene	Katsumura et al. (1996)
14 mg/kg bw/day 15 days prior to tumor inoculation	BALB/c mice bearing thymoma cells	- Reduction of 60% in number of tumoral thymocytes - Prolonged survival of treated mice	Palozza et al. (1997)
3.4 g/kg bw/day	Chemically induced mammary cancer	Reduce 65% of frequency of cancer	Grubbs et al. (1991)

3 weeks	of rats			
0.4% diet 3 weeks before tumor inoculation	Mammary tumors in female BALB/c mice	Significant decrease in mammary tumor volume		Chew et al. (1999)
Human plasma canthaxanthin level	Women with diagnosis CIN or cervical cancer	Plasma canthxanthin level: 3.33 µg/dl	- Control group: - CIN group: 1.71-2.48 µg/dl - Carcinoma group: 1.91 µg/dl	Palan et al. (1996)

Table 3. *In vivo* anti-obesity and anti-diabetic activities of astaxanthin

Treatments	Models	Effects	References
30 mg/kg bw/day 60 days	High-fat diet induced obese mice	Decreases in body, adipose tissue, liver weight, liver triglycerides, plasma triglycerides, and total cholesterol levels	Ikeuchi et al. (2007)
50 mg/kg/day 18 weeks	SHR/NDmcr-cp rat models	- Reductions in size of WAT, fasting blood glucose, plasma triglycerides and non-esterified fatty acids levels - Increases in high-density lipoprotein cholesterol, adiponectin levels and insulin sensitivity	Hussein et al. (2007)
6 mg/kg bw/day 60 days	High fat & fructose diet (HFFD) induced obese mice	- Reduce in body weight - Normalizing liver weight, plasma glucose, insulin, and lipids	Bhuvaneswari et al. (2010)
20 mg /kg bw/day 7 days	Alloxan induced diabetic rats	- Increase SOD activity - Decrease catalase glutathione peroxidase activities to normal levels	Leite et al. (2010)
6 mg/kg bw/day 60 days	(HFFD) induced mice	Mechanisms of insulin sensitivity improvement: - Activation of insulin receptor-β - Decreases in oxidative stress and pro-inflammatory cytokine secretion	Arunkumar et al. (2012)
2 mg/kg bw/day	(HFFD) induced obese mice	Mechanisms of insulin sensitivity improvement:	Bhuvaneswari et al. (2012)

45 days	- Normalizing hexokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase activities - Increasing tyrosine phosphorylation and glycogen reserves in the liver - Decreasing serine phosphorylation of insulin receptor substrates 1&2 and HFFD-induced serine kinases

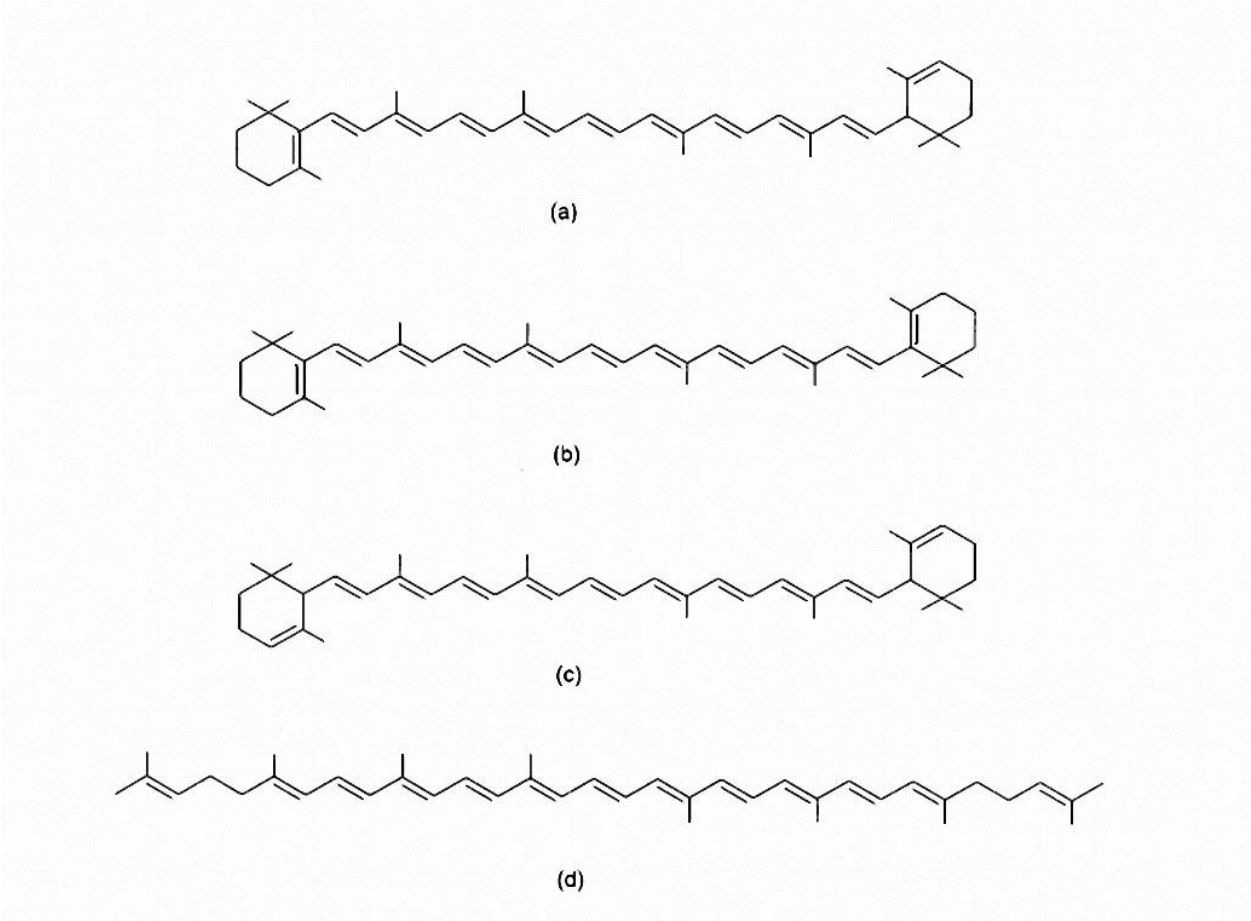

Figure 1. Chemical structures of the carotenes α-carotene (a), β-carotene (b), ε-carotene (c), and lycopene (d).

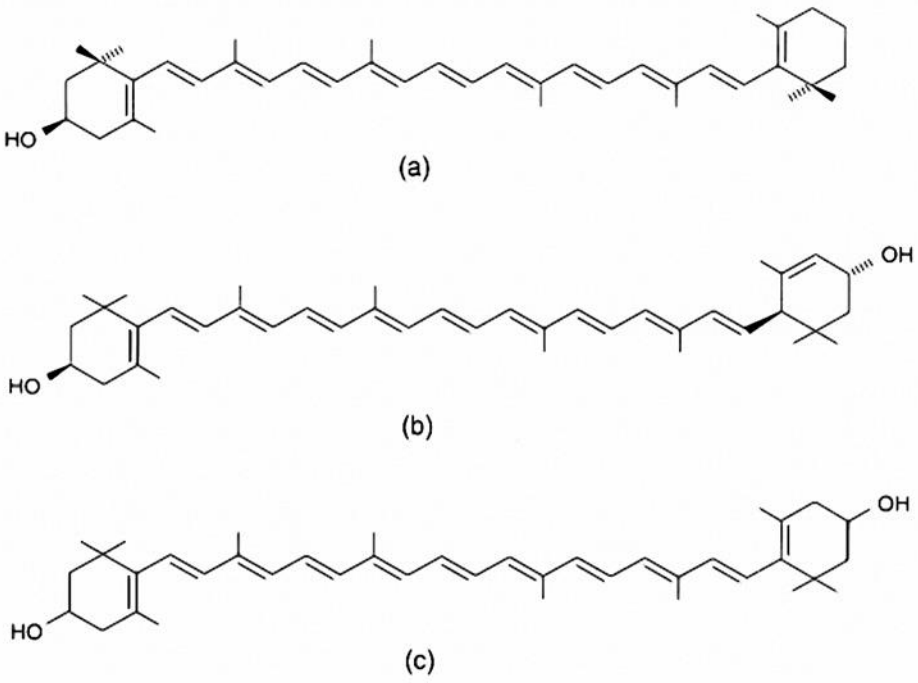

Figure 2. Chemical structures of the xanthophylls cryptoxanthin (a), lutein (b), and zeaxanthin (c).

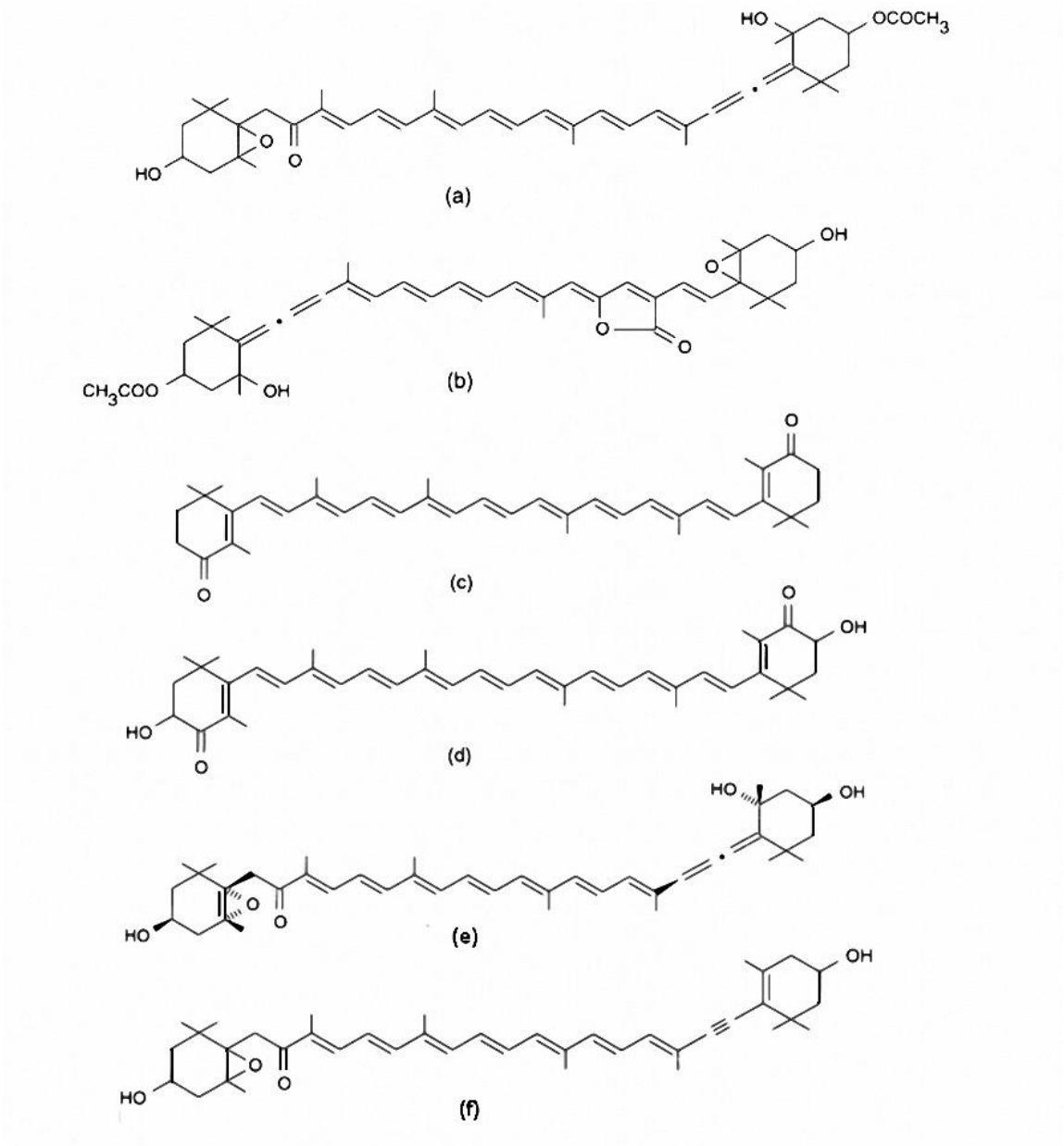

Figure 3. Chemical structures of some major marine carotenoids fucoxanthin (a), peridinin (b), canthaxanthin (c), astaxanthin (d), fucoxanthinol (e), and halocynthiaxanthin (f).