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Seaweed Carotenoid Fucoxanthin as Functional Food.

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Chapter 3

Seaweed Carotenoid, Fucoxanthin, as Functional Food

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Fucoxanthin: Overview and Sources

Fucoxanthin is a light-harvesting carotenoid pigment that occurs in the chloroplasts of the eukaryotic Chromalveolata (phylum Heterokontophyta, class Ochrophyta), including brown macroalgae (Phaeophyceae), and in unicellular microalgae, such as diatoms (Bacillariophyceae) (Cavalier- Smith and Chao

2006). Fucoxanthin is estimated to account for more than 10% of the total production of carotenoids in nature, and is responsible for the brown to yellow colour of brown macroalgae (seaweeds) and diatoms, where it masks green chlorophyll a and c (Hurd *et al*. 2014; Peng *et al*. 2011). Fucoxanthin was first isolated in Germany in 1914 from the brown seaweeds, *Dictyota*, *Fucus*, and *Laminaria* (Willstätter and Page 1914). Industrially, Japanese wakame (*Undaria pinnatifida*) is the seaweed most widely utilised for fucoxanthin extraction due to high concentrations of the pigment (≥10%) in the lipid extract (Billakanti *et al*. 2013). Other marine macroalgae known to contain fucoxanthin include the genera Ascophyllum, Fucus, Laminaria, Pelvetia, Ecklonia, Eisenia, Himanthalia, Sargassum, Saccharina, Ectocarpus, Schytosiphon, Petalonia, Carpophyllum, Hizikia, Padina, Dictyota, Myagropsis, Turbinaria, Cladosiphon, and Cystophora (Dominguez 2013; Haugan and Liaaen- Jensen 1994; Heo *et al*. 2010; Jaswir *et al*. 2011; Kanazawa *et al*. 2008; Mikami and Hosokawa 2013; Mise *et al*. 2011; Miyashita and Hosokawa 2007; Sangeetha *et al*. 2010; Shang *et al*. 2011; Yan *et al*. 1999).

Notable fucoxanthin-containing microalgae include the diatoms *Odontella aurita, Phaeodactylum tricornutum, Chaetoceros gracilis, Thalassiosira weissflogii*, and *Cyclotella meneghiniana*; the Prymnesiophyceae species *Emiliania huxleyi*, *Pavlova lutheri*, and *Phaeocystis pouchetii*; and the Chrysophyceae species *Pelagococcus subviridis* (di Valentin *et al*. 2013; Pyszniak and Gibbs 1992; United States Department of Agriculture 2015; Wright and Jeffrey 1987; Xia *et al*. 2013). These microscopic algae occur as marine plankton.

Globally, Japan, Korea, and China have the greatest seaweed production, consumption, and most developed fucoxanthin extraction industry (Ryan 2014). Irish coastlines also support the growth of fucoxanthin-containing brown seaweeds, such as bladderwrack (*Fucus vesiculosus*), channelled wrack (*Pelvetia canaliculata*), knotted wrack (*Ascophyllum nodosum*), oarweed (*Laminaria digitata*), sea rod (*Laminaria hyperborea*), sea spaghetti (*Himanthalia elon*gata), serrated wrack (*Fucus serratus*), and sugar kelp (*Saccharina latissima*; formerly *Laminaria saccharina*) (Dominguez, 2013; Morrissey *et al*. 2001; Stengel and Dring 1998).

Chemistry of Fucoxanthin

Carotenoids are tetraterpene pigments which occur in plants, algae, and photosynthetic bacteria. Carotenoids composed entirely of hydrogen and carbon belong to the subclass of carotenes, while those that additionally contain oxygen are classed as xanthophylls. Fucoxanthin is a xanthophyll, similar to the plant xanthophylls violaxanthin, neoxanthin, and lutein (Kotake-Nara and Nagao 2011). Xanthophylls share some chemical and physical properties with carotenes, such as lipophilicity and antioxidant activity due to their ability to quench reactive oxygen and nitrogen species (Kim and Chojnacka 2015). However, the presence of oxygen in the hydroxyl and epoxide groups of xanthophylls makes them more polar than carotenes (Landrum 2009). Fucoxanthin's systematic name is (3S,3′S,5R,5′R,6S,6′R,8′R)-3,5′- dihydroxy-8-oxo-6′,7′-didehydro-5,5′,6,6′,7,8-hexahydro-5,6- epoxy-β,β-caroten-3′-yl acetate.

It contains an allelic bond, an epoxy group, and six oxygen atoms, as shown in Figure 3.1 (Chemspider 2015).

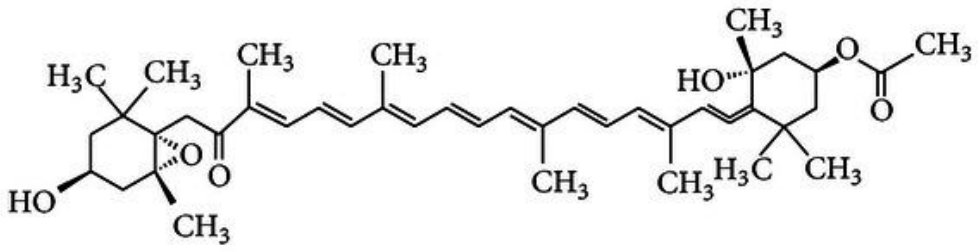

Figure 3.1 Fucoxanthin molecular structure (formula $C_{42}H_{58}O_6$).

In industry, it is most commonly extracted with solvents such as *n*-hexane, methanol, DMSO, ethanol, petroleum ether, diethyl ether, dimethyl ether, acetone, or ethyl acetate, and dried to a powder (Kanda *et al*. 2014; Kim 2011b).

In algal cells, fucoxanthin is contained in the chloroplasts, within membrane-bound compartments called thylakoids. In the thylakoids, fucoxanthin binds with chlorophyll a, c, and apoproteins, forming complexes that absorb light in the blue-green region of the spectrum and transfer energy to the alga. At depths of several meters, this is commonly the only spectral wavelength available to marine algae (Kita *et al*. 2015). Fucoxanthin captures a broader spectrum of light (449–540nm) than chlorophyll a and c alone, which increases the efficiency of photosynthesis (Kim *et al*. 2011; Pyszniak and Gibbs 1992). It also protects the algal cells from damage by reactive oxygen species caused by constant exposure to high levels of oxygen and light in the ocean. Fucoxanthin generally

occurs more significantly in the blade of the seaweed thallus, which experiences the greatest light exposure, compared to the stipe and holdfast (Lobban and Wynne 1981).

Fucoxanthin can exist in a *trans* or *cis* configuration. The *trans* isomer is the more chemically stable and potent antioxidant of the two, and comprises ~90% of the fucoxanthin found in nature (Holdt and Kraan 2011; Nakazawa *et al*. 2009). Fucoxanthin content varies widely amongst macro- and microalgae. For example, the brown seaweed *Fucus serratus* has been reported to contain 0.56 mg/g (dry weight, DW) (Haugan and Liaaen-Jensen 1994) compared to 2.67 mg/g in *Undaria pinnatifida* (DW) (Mori *et al*. 2004). Most diatoms and other microalgae have a greater fucoxanthin content than brown seaweeds (Kawee-ai *et al*. 2013), but are less commonly used commercially for extraction due to the necessity for photobioreactors and strict culturing conditions. Xia *et al*. (2013) reported the fucoxanthin content in eight species of diatoms, ranging from 2.24 mg/g (DW) in *Chaetoceros grac*ilis to 18.47 mg/g (DW) in *Odontella aurita*. Seasonal and geographic variations hugely affect content. For example, brown seaweeds harvested from September to March, during the mature phase of the sporophyte, commonly contain higher concentrations of fucoxanthin (Fung *et al*. 2013; Terasaki *et al*. 2009). This has been attributed to the upregulation of the xanthophyll, or violaxanthin, cycle pathway in reduced levels of sunlight during the winter. Under environmental stressors such as reduced light exposure, the formation of fucoxanthin from zeaxanthin via the epoxidation of antheraxanthin, violaxanthin, and diadionoxanthin may be accelerated to regulate

photosynthetic pathways (Campbell *et al*. 1999; Goss and Jacob 2010; Mikami and Hosokawa 2013; Ramus *et al*. 1977).

Current Applications

Despite its discovery and isolation in seaweed over one hundred years ago, fucoxanthin has remained somewhat underutilised in food and pharmaceutical applications. Initial studies focused on quantification and extraction methods, structural elucidation, and biosynthetic pathways. It was not until the late 1990s that scientific papers began to emerge on fucoxanthin's potential as a functional food. This was most probably due to growing clinical evidence at the time of the role of antioxidants in the prevention of chronic diseases. Currently, fucoxanthin is available to retail consumers in the form of relatively expensive weight loss supplements, of varying purity and quality, from health stores and online. Unlike other carotenoids, such as β-carotene, which are commonly used as food colourants, 100% pure fucoxanthin is not sold as a bulk food ingredient (Hurst 2002). Its instability due to oxidation and high extraction costs have, to date, been prohibitive. Currently, fucoxanthin is available to food producers as a percentage of various seaweed extracts. Analytical grade fucoxanthin (≥ 95% pure) is produced for laboratories, but retails at €556 per 50 mg (Sigma-Aldrich 2015a).

Food and Pharmaceutical Regulations

In 2009, the European Food Safety Authority (EFSA) published its scientific opinion on fucoxanthin and the substantiation of health claims related to it and

maintenance or achievement of a normal body weight, in the case of *Undaria pinnatifida* thallus extract, under Article 13(1) of Regulation (EC) No 1924/2006. It determined that the extract, as a food constituent, had been sufficiently characterised to be consumed in an amount equivalent to 15 mg pure fucoxanthin per day. However, to date, the EFSA has not accepted that a relationship has been established between its consumption and the maintenance or achievement of a normal body weight (European Food Safety Authority 2009). Other health claims, such as antidiabetic or anticancer effects, relating to fucoxanthin have not yet been evaluated by the EFSA. In the Republic of Ireland, the Health Products Regulatory Authority does not list fucoxanthin as a controlled substance, or include it in the List of Medicinal Herbs considered acceptable as THMPs, under the European Traditional Herbal Medicinal Products Directive (2004/24/EC) which came into effect in Ireland in 2007 (Health Products Regulatory Authority 2015).

The clinical evidence supporting fucoxanthin's health benefits has not yet been evaluated by the US Food and Drug Administration. However, fucoxanthin can be sold in the United States as a food supplement with the disclaimer "These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease" on the product label (US Food and Drug Administration 2014). In Japan, Food for Specified Health Uses (FOSHU) under the Ministry of Health, Labour and Welfare have not evaluated fucoxanthin as a functional food ingredient or pharmaceutical; however, it can be sold as a food supplement.

Applications in Human Health

Although fucoxanthin has not been evaluated by some food regulatory bodies, in recent years it has been studied clinically for its antioxidant properties, which inhibit free radical damage in cells, reducing the risk of many chronic diseases. It has also been studied for its anticancer, anti-type 2 diabetes, antiobesity, anticholesterol, anti-inflammatory, antiangiogenic, antimalarial, and antihypertensive activities, and for the treatment of Alzheimer's disease (Gammone and d'Orazio 2015; Hosokawa *et al*. 1999; Ikeda *et al*. 2003; Kawee-ai *et al*. 2013; Kim 2011a; Kotake-Nara *et al*. 2001; Maeda *et al*. 2007; Rodrigues *et al*. 2012; Shiratori *et al*. 2005; Sivagnanam *et al*. 2015).

Antiobesity Effects

Obesity, type 2 diabetes, metabolic syndrome, and chronic inflammatory diseases are global health epidemics. The World Health Organization estimates that, globally, 2.3 billion people will be overweight and 700 million obese by 2015. To prevent and treat diseases such as these, natural, bioactive, functional compounds such as fucoxanthin are increasingly being studied as an alternative to, or as combination therapy with, orthodox medicines (Peng *et al*. 2011; Watson, 2014).

In obesity treatment, fucoxanthin has been shown to mediate the induction of uncoupling protein-1 (UCP-1) in abdominal adipose tissue mitochondria in murine studies, leading to the oxidation of fatty acids and heat production, resulting in a reduction in white adipose tissue (d'Orazio *et al*. 2012; Maeda *et al*. 2005). Abidov *et al*. (2010) conducted a double-blind placebo-controlled

study of 115 non- diabetic, obese, premenopausal women with a liver fat content above 11% at the Russian Academy of Medical Sciences. A daily supplement of 300 mg brown seaweed extract (species not specified) containing 2.4 mg fucoxanthin, combined with 300 mg pomegranate seed oil, was administered. An olive oil capsule was administered to the placebo group. The treatment group showed a significant increase in resting energy expenditure and mean weight loss of 4.9 kg after 16 weeks. In Japan, a kombu (*Saccharina japonica*) extract of fucoxanthin (3%) was evaluated for its antimetabolic syndrome effects in human clinical trials. A daily dosage of the extract, equivalent to 0.5–1.0 mg pure fucoxanthin/ day, was found to have a significant effect on blood serum parameters related to metabolic syndrome (Oryza 2015). However, no official RDA for fucoxanthin has been established by the World Health Organization.

Topical preparations as vehicles for fucoxanthin in the treatment of obesity have also been reported. Dai *et al*. (2014) developed a stable microemulsion containing 0.25% pure fucoxanthin using medium chain triglyceride as the oil phase, Tween 80 as a surfactant, and polyethylene glycol 400 as a co-surfactant.

Anticancer Effects

Fucoxanthin's anticancer activity is hypothesised to be due to its ability to induce apoptosis in tumour cells (Nakazawa *et al*. 2009). Hosokawa *et al*. (1999) found that fucoxanthin induced apoptosis in human promyelocytic leukaemia HL-60 cells by cleaving procaspase-3 and poly-ADP-ribose

polymerase. Kim *et al*. (2010) showed that fucoxanthin induced reactive oxygen species generation, inactivated the Bcl-xL signalling pathway, and induced caspase-3, -7, and poly-ADP-ribose polymerase cleavage, triggering the apoptosis of HL-60 cells, indicating that the generation of reactive oxygen species was a critical target in fucoxanthin- induced apoptosis of these cells. Wang *et al*. (2014) reported significant growth inhibition of cells in nine human cancer cell lines with extracts of *Undaria pinnatifida* containing fucoxanthin. Satomi and Nishino (2013) reported fucoxanthin extract to have a significant effect on the expression and enzymatic activity of the xenobiotic metabolising enzymes CYP1A1, CYP1A2 and CYP3A4, which are involved in the activation of pro-carcinogens. The study found that the inhibitory effect of fucoxanthin (≤ 45μM) on these enzymes in human hepatocellular carcinoma HepG2 cells and recombinant human CYPs could also attenuate the action of some anticancer drugs that are normally activated by CYP3A4.

Antidiabetic Effects

Fucoxanthin's antidiabetic activity has been studied in mice with induced type 2 diabetes. It has been found to improve insulin resistance and decrease blood glucose levels mainly via the regulation of cytokine secretions from white adipose tissue (Miyashita *et al*. 2011). Other studies found that a fucoxanthin-enriched diet promoted the recovery of blood glucose uptake to muscle by the upregulation of GLUT4 mRNA expression. Fucoxanthin has also been shown to affect the peroxisome proliferator-activated receptor γ (PPARγ) and promote gene expression related to lipid metabolism in adipocytes. In cultivated cells, fucoxanthin prevented inflammation and insulin resistance by inhibiting nitric

oxide and PGE2 production through the down-regulation of iNOS and COX-2 mRNA expression, as well as adipocytokine production in white adipose tissue (Hosokawa *et al*. 2010; Maeda *et al*. 2006; Miyashita *et al*. 2011).

Dietary Antioxidant Effects

The antioxidant capacity of seaweed s has been widely reported (Heffernan *et al*. 2014). As a dietary antioxidant, fucoxanthin has been shown to improve the antioxidant capacity of blood serum levels in mammals. Fucoxanthin is unusual in that it donates an electron to reactive oxygen species, instead of a proton (hydrogen), as most antioxidants such as ascorbic acid or β-carotene do. Fucoxanthin can also quench reactive oxygen species under hypoxic physiological conditions, unlike the majority of food-derived antioxidants (Nomura *et al*. 1997; Yan *et al*. 1999). A high-fat diet has been associated with obesity in humans and other mammals, and has been shown to cause overproduction of reactive oxygen species (Dandona *et al*. 2005). Reactive oxygen species are known to cause cellular damage, which is implicated in the pathogenesis of diseases such as type 2 diabetes, cardiovascular disease, cancer, and infectious illnesses (Uzun *et al*. 2004). Ha *et al*. (2013) reported that fucoxanthin supplementation improved the antioxidant capacity of blood serum levels in obese rats via activation of the nuclear erythroid factor like-2 pathway and its downstream target gene NQO1. A study by Zaragozá *et al*. (2008) on the antioxidant effect of fucoxanthin extract from *Fucus vesiculosus* found that the extract exhibited increased antioxidant activity in ex vivo assays of erythrocytes and plasma, after 4 weeks of daily oral administration in rats. Significant antioxidant activity was also observed in non-cellular systems and in

activated RAW 264.7 mouse leukemic monocyte macrophage cell lines. Therefore, supplementation of fucoxanthin may also reduce the risk of oxidative stress in humans.

Fucoxanthin has also been successfully used topically to protect against UV-B-induced cell damage in hairless mice, and in human fibroblast cell lines as an antioxidant against skin aging caused by free radical damage (Heo and Jeon 2009; Urikura *et al*. 2011).

Toxicity Studies

No toxicity of fucoxanthin extracts has been reported to date, making it a good candidate for functional food use. A number of clinical trials in animal models have shown no significant toxicity with short- or long-term dosage. For example, in Japan, a recently developed functional food ingredient, containing up to 5% fucoxanthin from kombu (*Saccharina japonica*) extract, was evaluated on rats. Toxicity and micronucleus tests were conducted. No toxicity or abnormalities were found after 14 or 90 days. The LD_{50} of the extract (3.0% fucoxanthin) was calculated to be 2000 mg/kg body mass for rats (Oryza 2015). Maeda *et al*. (2005) supplemented a murine diet with 0.27% fucoxanthin, equivalent to ~0.25 mg/kg body mass per day for 4 weeks, and found no side effects or abnormalities. Kadekaru *et al*. (2008) conducted a toxicity study on the repeated oral dosing of fucoxanthin (95% purity) to rats for 28 days, and found that it showed no apparent toxicity. Zaragozá *et al*. (2008) found no ill effects following a 4-week, acute toxicity test in rats, where a daily treatment of 0.0012% pure fucoxanthin extract from *Fucus vesiculosus* was administered. A single dose

toxicity study (Beppu *et al*. 2009) was conducted with doses of 1000 and 2000 mg/kg body mass and a repeated oral dose toxicity study with doses of 500 and 1000 mg/kg for 30 days on purified fucoxanthin (93% purity) in ICR mice. No mortality, abnormalities, abnormal changes in liver, kidney, spleen, or gonadal tissues were found in either study.

Human toxicity studies are required to assess both the toxicity levels and daily dosage for efficacy against any of the disorders discussed above.

Fucoxanthin as a Functional Food: Challenges and Opportunities

Fucoxanthin faces chemical, organoleptic, and bioavailability challenges as a functional food ingredient. Due to its chemical structure, fucoxanthin in its pure form is easily oxidised by high temperatures, low or high pH, UV light, and long storage periods (Kawee-ai *et al*. 2013; Mise *et al*. 2011). This may lead to chemical or enzymatic interactions with other ingredients over time. Organoleptic attributes, such as texture, taste, appearance, or smell, may deteriorate as a result of these interactions. Sensitivity to heat poses a problem for bakery products or sauces that require boiling, and foods that contain fruit or probiotic cultures may be too acidic. The natural brown colour, savoury flavour, and powdery texture of fucoxanthin itself may also make it unsuitable for some food products. The idea of eating seaweed derivatives may be unpalatable to some consumers, even at undetectable levels. This may be encountered in Western cultures where seaweed is rarely part of the diet, and misconceptions of a potential “fishy” or “sea” taste could arise.

Like other non-polar pigments, such as chlorophyll and lycopene, fucoxanthin is insoluble in water. Incorporation into water-based beverages or sauces would require emulsification or dispersion in appropriate colloids (Socaciu 2007). The lipid-soluble nature of fucoxanthin also affects its bioavailability in mammals (Sangeetha *et al*. 2010). Fucoxanthin is metabolised in the intestine into fucoxanthinol by cholesterol esterase and lipase, then converted to amarouciaxanthin A in the liver (Bagchi and Preuss 2012; Dominguez, 2013). The presence of some form of dietary lipid is required when fucoxanthin is consumed for solubility and absorption (Peng *et al*. 2011). Another consideration for fucoxanthin as a functional food is the high cost, due to the energy required for the extraction and freeze-drying process (Billakanti *et al*. 2013) and the lack of an artificial synthesis method for fucoxanthin. Seasonal variations in fucoxanthin nutritional content (Fung *et al*. 2013) could also affect health claims and nutritional efficacy.

Despite these potential hurdles, fucoxanthin has several intrinsic, beneficial properties. Fucoxanthin, along with other carotenoids such as astaxanthin, is a more powerful antioxidant than many other natural and synthetic antioxidants (Miyashita and Hosokawa 2007). Using chemiluminescence detection, Nishida *et al*. (2007) reported that fucoxanthin had stronger singlet oxygen-quenching activities than ascorbic acid, α-tocopherol, quercetin, resveratrol, (-)-epigallocatechingallate, lutein, lycopene, gallic acid, pyrocatechol, α-lipoic acid, and the synthetic antioxidant butylated hydroxytoluene. Consumer awareness and interest in naturally antioxidant-rich foods have grown in recent years (Kim 2013; Rodrigues *et al*. 2012). Seaweed extracts such as carrageenan, alginate,

and other hydrocolloids are already accepted by consumers as widely used ingredients in the food, pharmaceutical, and cosmetic industries (Venugopal 2011). Marine-derived functional foods such as fucoxanthin have the potential to be marketed as a more potent and sustainable alternative to many natural and synthetic antioxidants, particularly in countries such as Japan, China, and Korea, where seaweed is a common part of the diet. Fucoxanthin is also Kosher, Halal, and suitable for vegetarians and vegans.

As discussed later in this chapter, little has been reported in peer- reviewed journals regarding the use of fucoxanthin as a functional ingredient. Most fucoxanthin research has focused on medical rather than functional food applications. However, a study conducted by Prabhasankar *et al*. (2009) at the Central Food Technological Research Institute, Mysore, in India reported the successful addition of fucoxanthin and fucosterol as a constituent of wakame (*Undaria pinnatifida*) powder extract into semolina (wheat)-based pasta. Fucosterol is a structural component of algal lipid membranes which has been shown to have antioxidant, anticancer, antidiabetic, and hepatoprotective properties in animal trials (Alasalvar *et al*. 2011; Jung *et al*. 2013; Lee *et al*. 2003). Prabhasankar *et al*. (2009) investigated the effect of different percentage additions of wakame powder on the sensory, cooking, nutritional, and biofunctional quality of pasta. Blends of semolina and seaweed were combined by replacement method in ratios of (semolina/wakame, w/w) 100:0, 95:5.0, 90:10, 80:20, and 70:30. In sensory analysis of taste, mouth-feel, appearance, and strand quality, 15 semi-trained panellists, who were regular consumers of wakame, found no significant (P>0.05) or discernible organoleptic differences

between the control and the wakame pasta up to 10% total ingredient mass, with acceptance decreasing after 10% up to 20% wakame content. Above 20%, panellists reported saltiness and a seaweed taste. Since wakame is composed of other components such as polysaccharides, proteins, lipids, and minerals, 10% wakame, in this study, equated to 0.04 mg/g (DW) of fucoxanthin and 1.25 mg/g (DW) of fucosterol of the dry ingredient portion. HPLC analysis of the pasta after processing/ kneading, and after cooking (25g of raw pasta in 250mL boiling water for 8 min) showed a loss of less than 10% for both fucoxanthin and fucosterol. The authors hypothesised that this remarkable preservation occurred due to stability of fucoxanthin/fucosterol in the protein matrix of gluten. To the authors' knowledge, the stability of fucoxanthin in a food system has not been reported in any other studies. To date, these lab-scale pilots have not been reported as developed to commercial scale.

A recent Irish study reported the successful incorporation of ethanol and water extracts of *Fucus vesiculosus* and *Ascophyllum nodosum*, at 0.25% and 0.50%, into yoghurt and fluid milk (O'Sullivan 2013). The fucoxanthin content of the seaweed extracts was not reported. However, it is probable that both contained fucoxanthin, due to its presence being widely reported in both species and their extracts (Stengel and Dring 1998; Zaragozá *et al*. 2008). Sensory analysis found that overall acceptability of the yoghurts was governed by appearance and flavour, with the 100% water extract of *A*. *nodosum* having the greatest panel preference and least yellowness. Overall acceptability of the milk was governed by perception of a fishy flavour. Again, the 100% water extract of A. nodosum (0.50% addition) was found to be the most acceptable in terms of

taste, and the least green/yellow. *In vitro* antioxidant analysis found no deterioration of antioxidant activity, shelf-life, or pH in the seaweed-supplemented milk and yoghurt formulations.

Fucoxanthin, like many natural food extracts, is widely available wholesale online from many companies, primarily based in China. It is sold in the form of dried seaweed extract, generally from wakame or kombu, with stated percentages of fucoxanthin purity ranging from 10% to 98%. Price ranges widely, from less than $1 to $2000 per gram, as do claims of purity and certification (Alibaba 2015; Kyndt and d'Silva 2013).

However, in Japan, a government-certified functional food ingredient has recently been commercially developed from kombu (*Saccharina japonica*) containing 1–5% fucoxanthin. The powder and oil products contain only cyclodextrin, or triglyceride, in addition to the kombu extract, and natural tocopherol. Stability testing of the products found the fucoxanthin fraction to be thermostable up to 80 °C for 1 hour, and stable in solution from pH 3.0 to 10.0, with the greatest loss being 6% after 1 week at pH 3.0. The addition of the antioxidant preservative tocopherol to the products is most likely the stabilising factor in this case. The triglyceride and cyclodextrin may also offer a protective matrix for the fucoxanthin, combined with photo-protective, vacuum packaging. The powder and oil products were reported to have been successfully incorporated into beverages, cakes, shortbread, puffed rice biscuits, spreads, and potato snacks. However, the percentage of fucoxanthin extract used was not specified, nor have any sensory evaluation results been published to date (Oryza 2015).

Outside Asia, in the USA, a project was initiated in 2010 by the Research, Education, and Economics Information System of the Department of Agriculture and the National Institute of Food and Agriculture, Auburn University, Alabama, to optimise large-scale fucoxanthin extraction from the diatom *Chaetoceros gracilis*. The effects of the extracts on energy balance in an animal model of obesity are being used to develop a functional food for the public health treatment of obesity to reduce the risk of developing cardiovascular disease, type 2 diabetes, and some forms of cancer. The project intends the functional food industry to be the immediate beneficiary of the study, but also aims to target the wider food industry, nutritionists, scientists, and engineers (United States Department of Agriculture 2015). There have been no publications from the project to date.

This significant project, in the country with the highest global rates of obesity, along with the success of the pasta study and Japan's government-sponsored development of stable oil- and water-soluble fucoxanthin products, is very encouraging for further exploration of fucoxanthin as a functional food ingredient on a large commercial scale.

Approaches to Overcome Adverse Reactions in Functional Food Models

Micro and Nanoencapsulation

The principal obstacles to the incorporation of fucoxanthin in a food or beverage matrix are water insolubility, pH instability, sensitivity to oxidation, and impaired

bioavailability. These properties are an issue with existing food ingredients, such as other carotenoids and polyphenolic compounds, but can be overcome with various technologies and approaches. Microemulsions composed of a water phase, lipid phase, and an amphiphilic compound are widely used to combine lipid solutes, such as carotenoids, into a hydrophilic matrix for food and pharmaceutical purposes (de Campos *et al*. 2012).

Indrawati *et al*. (2015) encapsulated an acetone extract (primarily trans fucoxanthin) of Indonesian Sargassum species in maltodextrin and Tween 80 (~70:1). The Sargassum extract was combined with canola oil and homogenised in the water-based maltodextrin/Tween 80 emulsion. After freeze drying, the fucoxanthin was found to be stable within the microencapsulates for 63 days at 28°C under inert atmosphere. Encapsulating fucoxanthin in this manner increases its suitability for incorporation into dried food products. Suhendra *et al*. (2012) succeeded in formulating a clear, stable, oil-in-water microemulsion for fucoxanthin, capable of delivering this hydrophobic antioxidant in aqueous food systems. Virgin coconut oil was used as the lipid phase with a combination of Tween 20, Tween 80, and Span 80 as non-ionic surfactants. The ratios were oil:surfactants (3:17); oil + surfactants:water (35:65); Tween 80:Tween 20:Span 80 (92.0:2.5:5.5). The microemulsion remained stable after exposure to pH 3.5–6.5, 105 °C for 5 hours, and centrifugation at 4500rpm for 30 minutes. Quan *et al*. (2013) reported a water-soluble fucoxanthin food application using fucoxanthin-loaded microspheres, composed of a gum arabic/fish gelatin coacervate shell cross-linked by tannic acid, with a solid lipid core of acetyl palmitate and canola oil. Wet and freeze-

dried forms of the solid lipid core microspheres were developed successfully. Stability of encapsulated fucoxanthin during long-term storage was significantly increased, as was sustained release in a simulated gastrointestinal environment.

Nanogels have been used extensively in the pharmaceutical industry to protect acid-labile bioactive compounds from the acidic stomach environment before reaching the intestinal tract (Liechty *et al*. 2010; McClements *et al*. 2009a). This technology easily translates to functional food delivery. For example, the biological availability and stability of fucoxanthin were reported to have significantly increased after encapsulation with chitosan-sodium-tripolyphosphate-glycolipid nanogels, prepared by ionic gelation (Ravi and Baskaran 2015). The authors were inspired by a study (Gorusupudi and Baskaran 2013) reporting the improved bioavailability of a similar compound, lutein, in mice, by solubilising the extract first in the glycolipid fraction of wheat germ oil. Apart from protecting the core material (fucoxanthin) against degradation, encapsulation prevents reactions with other ingredients (McClements *et al*. 2009b), and any seaweed flavour or brown colour is prevented from leaching into the food.

Current Trends in Fucoxanthin Research

Most extraction methods currently practiced are based on the utilisation of organic acids and solvents to break down the cell walls of seaweed or microalgae. Current trends are leaning towards greener chemical or physical extraction methods.

Green Extraction Technologies

Pressurised Liquid Extraction

Shang *et al*. (2011) utilised pressurised liquid extraction with ethanol to optimise fucoxanthin yields from the seaweed Eisenia bicyclis. A yield of 0.39 mg/g was achieved with 90% ethanol, 110°C, 1500 psi, for 5 minutes of extraction static time. This was close to the predicted statistical experimental yield of 0.42 mg/g. Supercritical Carbon Dioxide Extraction Supercritical carbon dioxide extraction is another green chemical method with potential for commercial seaweed applications. Sivagnanam *et al*. (2015) extracted fucoxanthin from the brown seaweeds *Sargassum horneri* and *Saccharina japonica* using supercritical CO_2 with ethanol as a co-solvent (SC-CO_2E) for 2 hours at 45 °C in a semi-batch flow extraction process. SC-CO_2E extraction yield of fucoxanthin from *S*. *horneri* was 0.77 mg/g, compared to only 0.71 mg/g from traditional acetone- methanol extraction. SC-CO_2E extraction fucoxanthin yield from *S*. *japonica* was 0.41 mg/g, close to the acetone-methanol extraction yield of 0.48 mg/g. An *in vitro* hydrogen peroxide scavenging antioxidant activity assay showed the SC-CO_2E fucoxanthin extracts from *S*. *horneri* and *S*. *japonica* had significantly greater activity, or maximal inhibitory concentration (IC_{50} value), than acetone-methanol, hexane, or ethanol extracts. The IC_{50} values of the SC-CO_2E extracted S. horneri fucoxanthin (686 µg/mL) and *S*. *japonica* (600 µg/mL) were significantly greater than that of an ascorbic acid standard (448 µg/mL). SC-CO_2E fucoxanthin extracts from both species were also found to exert

angiotensin I-converting enzyme inhibitory effects *in vitro* comparable with those of the acetone-methanol extract.

Quitain *et al*. (2013) used supercritical carbon dioxide extraction with *Undaria pinnatifida*, combined with a microwave pre-treatment, to disrupt the cell membrane. Yields equivalent to 80% of those obtained with traditional solvent-based methods were reported using 40MPa, for 180 minutes, at 40 °C, without producing any chemical waste. Roh *et al*. (2008) extracted fucoxanthin and polyphenols from *Undaria pinnatifida* using supercritical carbon dioxide, with ethanol as a co-solvent. However, fucoxanthin yields were significantly lower than those obtained with solvent extracts, ranging from 0.00048 to 0.00753 µg/g. Optimum supercritical extraction was achieved after 50 minutes with a flow rate of 28.17 g CO_2/min, 2 mL/min ethanol, at a pressure of 200 bar, and a temperature of 323 K (49.85°C). Subramanian *et al*. (2013) optimised a novel, ultrasound-assisted extraction method, using *Sargassum muticum*. Fucoxanthin yields of 0.613 mg/g were reported with optimum parameters of 70% ethanol, at 80 °C, for 10 minutes, at an amplitude of 78 W, with a solid to solvent ratio of 1:5 w/v g/mL.

Microwave-Assisted Extraction

Xiao *et al*. (2012) developed a rapid (75 minute) microwave-assisted extraction method combined with high-speed counter-current chromatography, with a two-phase solvent system of hexane-ethyl acetate-ethanol-water (5:5:6:4, v/v/v/v). Fucoxanthin yields close to those of traditional methods were obtained from *Saccharina japonica* (0.83 mg/g), *Undaria pinnatifida* (1.09 mg), and *Sargassum*

fusiforme (0.20 mg/g). HPLC showed the extracts to have a minimum purity of 90%.

Enzymatic Extraction

Fucoxanthin extraction through the application of targeted enzymes, such as cellulases, has the potential to increase yield and safety. The presence of branched, sulphated, or complex polysaccharides, such as alginate and laminarin, in algal cell walls limits the efficiency of classic extraction methods (Kim 2011c; Kim and Chojnacka 2015). Enzymolysis, i.e., the hydrolysis (of cell wall polysaccharides) with enzymes, should, in theory, aid in degradation of the wall and release of the pigment-containing chloroplasts within. Enzyme-assisted extraction has already been used successfully to increase carotenoid yields from terrestrial plants, such as lycopene from tomatoes. Zuorroa *et al*. (2011) reported an 8 to18-fold increase in lycopene yield from tomato processing waste (skins) in pectinase and cellulase pre-treated samples, compared to hexane extraction alone. Barzana *et al*. (2002) reported a similar result for the extraction of carotenoids (primarily lutein) from marigold flowers. Typically, as much as 50% of total carotenoid yield is lost in the traditional drying and hexane extraction of marigold flowers. A pre-treatment of Pectinex, Viscozyme, Neutrase, Corolase, and HT-Proteolytic commercial enzymes was used to significantly reduce the volume of hexane required, and completely eliminate the drying and silage steps that cause degradation of the carotenoids. Carotenoid yields of ≥85% were recovered from the marigold flowers in simple stirring vessels. Barzana *et al*. (2002) hypothesised that if cost were no barrier,

the hexane could be omitted entirely, and enzymes alone, with the correct parameters, would achieve the same yield.

In the extraction of algal bioactives such as antioxidants, polysaccharides, carotenoids, and polyphenols, the use of enzymes has shown significant potential as a viable alternative, or addition, to pure solvent methods (Je *et al*. 2009; Rhein-Knudsen *et al*. 2015; Wijesinghe and Jeon 2012). Heo *et al*. (2005) extracted water-soluble antioxidant compounds from *Sargassum*, *Ecklonia*, *Ishige*, and *Schytosiphon* genera using proteases (Neutrase, Protamex, Alcalase, Flavourzyme, and Kojizyme) and carbohydrate- degrading enzymes (Celluclast, Viscozyme, AMG, Ultraflo, and Termamyl). *In vitro* hydrogen peroxide scavenging activity assays showed the majority of the enzyme extracts had significantly greater activity than the commercial synthetic antioxidants α-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). For example, the hydrogen peroxide scavenging activity of Ultraflo-extracted *Sargassum horneri* was 92.69%, and Kojizyme-extracted *Ishige okamurae* 96.27%, compared to only 50.32% for BHT, 67.37% for BHA, and 64.11% for α-tocopherol.

Ko *et al*. (2010) significantly increased the antioxidant activity of *Sargassum coreanum* extracts using Celluclast and Neutrase commercial enzymes. Celluclast is a cellulase derived from the fungus *Trichoderma reesei* which hydrolyses (1,4)-β-D-glucosidic linkages in cellulose and other β-D-glucans, forming cellobiose glucose and other glucose polymers (Sigma-Aldrich 2015b). Neutrase is a metalloprotease derived from *Bacillus amyloliquefaciens*. Similar

to thermolysin, it hydrolyses proteins into peptides (Nagodawithana and Reed 2013). The *S*. *coreanum* Neutrase and Celluclast extracts had significantly greater DPPH and hydrogen peroxide radical scavenging activities compared to eight other proteases and cellulases studied. The *S. coreanum* extracts were screened for cancer cell inhibition and found to suppress the growth of HL-60 cells through apoptosis (Ko *et al*. 2012). Ahn *et al*. (2012) reported obtaining antioxidant-rich extracts from the chlorophyte (green alga) *Enteromorpha prolifera* using the protease Protamex and the carbohydrase mix Viscozyme. The extracts contained up to 8.4 mg/g total flavonoids and 4.5 mg/g total polyphenols. Other *E*. *prolifera* extracts were produced using Flavourzyme, an exopeptidase that hydrolyses N-terminal peptide bonds, and Promozyme, a pullulanase (α-dextrin endo-1,6-α-glucosidase) derived from *Bacillus acidopullulyticus* (Mehta *et al*. 2012). The Flavourzyme and Promozyme extracts were found to have a significant angiotensin-converting enzyme inhibitory effect at concentrations of 1.0 mg/mL. No toxicity was exerted by any of the enzyme-assisted extracts in RAW264.7 cell cytotoxicity tests.

Little has been published on the use of enzymes for fucoxanthin extraction specifically. Billakanti *et al*. (2013) reported a significant increase (9.3%) in fucoxanthin extraction yields from wakame (*Undaria pinnatifida*) using alginate lyase derived from *Flavobacterium multivorum* as a pre-processing step, followed by dimethyl ether and ethanol extraction, compared to untreated pre-processing. Optimum enzyme pre-treatment parameters were found to be 37 °C, for 2 hours, at pH 6.2, 5% (w/v) solids, with 0.05 wt% enzyme using continuous mixing. Centrifugation was used to separate hydrophilic hydrolysis

products from the residual seaweed biomass. Alginate lyase, also known as mannuronate lyase, may have succeeded here where other enzymes have little effect due to its ability to catalyse the hydrolysis of alginate in the cell wall into smaller oligosaccharides, aiding extraction of fucoxanthin from the chloroplasts. Alginate lyase cleaves β-(1-4)-D-mannuronic bonds to yield oligosaccharides with 4-deoxy-α-L-erythro-hex-4-enopyranuronosyl groups at the non-reducing terminus (Sigma-Aldrich 2015b). Enzymes such as cellulose, protease or pectinase cannot break the glycosidic bonds specific to the β-D-mannuronate in alginate polysaccharides that occur in macroalgae (Sho *et al*. 2010).

Currently, this research institute (Dublin Institute of Technology, Ireland) is screening a number of commercially available Irish seaweed species for their fucoxanthin content, using novel extraction technologies. The species under investigation include wild Atlantic wakame (*Alaria esculenta*), bladderwrack (*Fucus vesiculosus*), channelled wrack (*Pelvetia canaliculata*), knotted wrack (*Ascophyllum nodosum*), oarweed (*Laminaria digitata*), sea rod (Laminaria hyperborea), sea spaghetti (*Himanthalia elongata*), serrated wrack (*Fucus serratus*), furbellows (*Saccorhiza polyschides*), and sugar kelp (*Saccharina latissima*).

Conclusion

Fucoxanthin is a bioactive compound found in one of the most prolific and sustainable organisms on the planet, alga (Mohamed *et al*. 2012; Werner *et al*. 2004). Its efficacy and potential in terms of health applications have been widely reported in clinical studies. However, further human clinical trials are necessary

to determine the safety and required daily dosage of fucoxanthin. Technical modifications, such as encapsulation, and sensory trials must be undertaken before fucoxanthin can be successfully utilised as a functional food ingredient. Factors to consider include solubility in the food matrix, organoleptic effects, stability, preservation against oxidation, consumer acceptability, bioavailability, and toxicity risk. The current, prohibitive cost of fucoxanthin must also be addressed. Possible solutions may include the development of more efficient and greener extraction technologies, which require shorter extraction times and less solvent, and have a more specific and higher extraction yield. The sustainability of potential seaweed cultivars must be assessed before large-scale harvesting, to ensure the preservation of this precious marine resource.

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