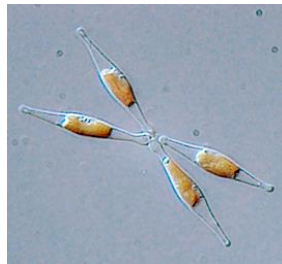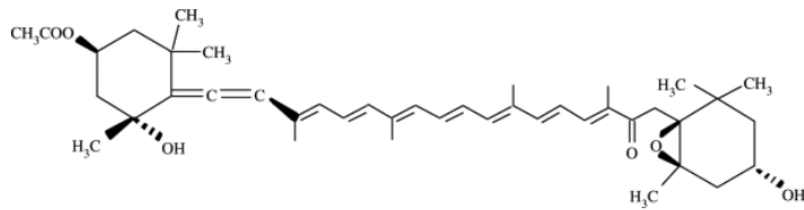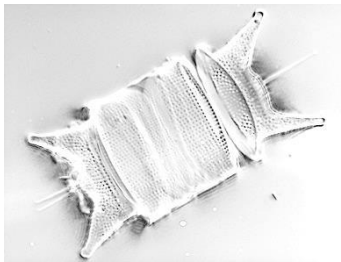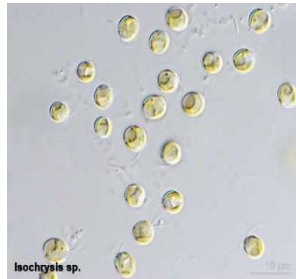# **Fucoxanthin:** a promising bioproduct to be derived from algae?

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Abstract

The carotenoid fucoxanthin has been the subject of research for several decades. The light-harvesting pigment is found in the fucoxanthin-chlorophyll light harvesting complexes of the photosystems of brown algae. Where research originally focused on the mechanistic aspects of the pigment, nowadays a lot of attention is on its possible health promoting benefits. This thesis looks at several aspects of fucoxanthin to determine whether or not the pigment could be a suitable candidate as a bioproduct derived from algae.

For micro-algae, the three species *Odontella aurita*, *Phaeodactylum tricornutum* and *Isochrysis gff galbana* were identified as the prime candidates for fucoxanthin production. Different lighting and nutrient schemes could be developed to increase production even further, while several down-stream processing extraction techniques could provide a simultaneous production of fucoxanthin and other desired products such as lipids in one batch. For macro-algae, the *Laminariceae, Sargassaceae* and *Alariaceae* are considered to be the best choices. The *Sargassaceae* for their relatively high content and the *Laminariaceae* and *Alariaceae* as these are currently already being consumed in high quantities, thereby having an advantage of existing infrastructure. Fucoxanthin content can mostly be influenced by time of harvest. The possibility of using normally discarded "waste" fractions in combination with the CO_2 supercritical fluid extraction technique would reduce environmental pressures of fucoxanthin production considerably in comparison to other methods.

The pigment was found to be a safe product that has a rather low bioavailability in humans. The addition of ingesting lipids together with fucoxanthin can increase the bioavailability. Fucoxanthin has potential health benefits as an agent in the categories of anti-cancer, anti-inflammatory and anti-obesity as shown in medical research. However, the lack of human trials and conflicting results in the few that are done, do not yet show its efficacy in the form of a supplement. This gives little hope that the pigment would be legally legitimized as a so-called functional food by the food health and safety authorities as of right now. A small market does already exist, using dubious health claims at best. Most of these powders are still derived from seaweeds. These uncertainties lead to the conclusion that for now, investing in the development of micro-algae for the production of fucoxanthin would not be worthwhile. Instead, using the usually discarded, "undesirable", fractions of macro-algae that are harvested already should be able to produce enough fucoxanthin until more evidence of its health-promoting effects in living humans is found.

Chapter 1: Introduction

1.1 Fucoxanthin, a new functional food ingredient?

The term "nutraceutical" was introduced by Dr. Stephen L. DeFelice, MD in 1989 (Bower, 2005) as a portmanteau of "nutrition" and "pharmaceutical". He defined the term as "a food or a part of a food that has medical or health benefits, including the prevention and/or treatment of disease". The term has found its way into the global food supplementation market, yet no clear, regulated definitions exist (Kalra 2003). Yet, the term can be found both in consumer marketing as well as in published scientific papers. Use of the term is not without question however, and not all scientists believe the term to be a genuine denominator (correspondence with Dr. HJ Woerdenbag, 2016).

Nevertheless, the health-claim based food market is thriving. Making estimates on the "nutraceutical market" is hard due to the above mentioned unclear official definitions. However, there have been official definitions of so-called "functional foods" or just nutritional supplements (encompassing also foods with added ingredients as opposed to just singular ingredients). Global estimates of the net value in 2008 range from 33 to 47.6 billion US Dollars (Siró *et al*, 2008).

Many different food types and groups are found within all these profitable functional foods, ranging from for example omega-3 fatty acids, vitamins and carotenoids to dairy products enhanced with probiotics. Out of all these possible types the carotenoids form a substantial portion of the global revenue, with estimations ranging from already 1.5 billion US Dollars in 2014 and reaching 1.8 billion dollars by 2019[1]. Carotenoids such as β-carotene, lycopene and in lesser extent violaxanthin and zeaxanthin are in high demand, traditionally as food colorants and for their anti-oxidative properties (Stahl 2005). Recently, another carotenoid has been garnering attention for its supposed "nutraceutical" value: Fucoxanthin.

Fucoxanthin is a main carotenoid pigment found in both micro-algae and in the macro-algae class of the phaeophytes (Kumar *et al*, 2013). The large abundance of this orange coloured pigment is responsible for the golden-brown color of the above mentioned species and groups.

Structurally, fucoxanthin (see fig 1) is comprised of several double bonds, an allenic bond and functional groups of epoxy, hydroxyl, carbonyl and carboxyl moieties, thereby subtly distinguishing itself from the structures found in other carotenoids such as the ubiquitous β-carotene (Peng *et al* , 2011). Fucoxanthin is bound together with chlorophyll a and chlorophyll c in the Fucoxanthin-chloropyll protein complex (FCP), in which the fucoxanthin aids as a light harvesting pigment for performing photosynthesis as well as a photoprotective

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[1] http://www.bccresearch.com/market-research/food-and-beverage/carotenoids-global-market-report-fod025e.html

agent in cases of excess light (Kuczynska *et al* , 2015).
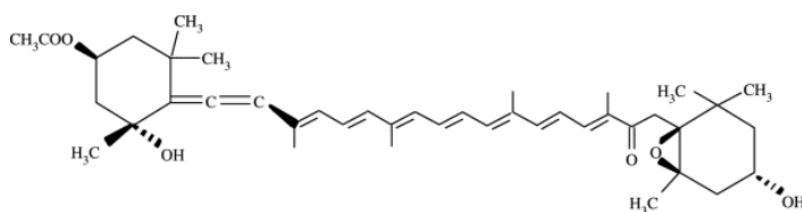
Fig 1: Structural formula of Fucoxanthin (Shiratori et al , 2015)

As a light-absorbing pigment, fucoxanthin displays a high absorption ratio of light frequencies between 460 and 570 nm (Kuczynska *et al*, 2015). This absorption of blue-green light that is poorly absorbed by chlorophyll is of special importance to marine organisms due to the absorption properties of seawater, in which red light is rapidly reduced (Siefermann-Harms, 1987). Fucoxanthin is unstable at higher temperatures and in light. When exposed to temperatures over 70^0C it degrades by about 30% each day while exposure to a 60W light bulb degrades it by about 50% (Shin *et al*, 2012). Short term exposure of temperatures of 80^0C-100^0C are generally well tolerated though (Shang *et al*, 2011). In general, carotenoids should be avoided from direct light exposure as much as possible during all stages of extraction, as this can cause them to break down rapidly (Davey *et al*, 2009).

In current years, lots of research has been published claiming a whole plethora of possible health benefits attributable to the administration of fucoxanthin, seemingly earning it the "nutraceutical" or "functional food" title. Amongst these benefits are anti-oxidative, anti-inflammatory, anticancer, anti-obesity, antidiabetic, anti-angiogenic and antimalarial properties, as well having a protective effect on the liver, blood vessels in the brain, bones, skin and eyes (Peng *et al*, 2011). The effects of fucoxanthin on the lipid metabolism especially are of high interest, in which fucoxanthin was able to attenuate body and visceral fat gain as well as regulating lipid levels in rodents fed with high fat diets (Muradian *et al* , 2015). In an experiment investigating the effects of β-carotene and fucoxanthin on the lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans,* it was found that both carotenoids increased the lifespan of *D. melanogaster*, yet only fucoxanthin did so for *C. elegans* (Lashmanova *et al* , 2015). This seems to indicate that Fucoxanthin does indeed have different biological properties than other carotenoids, yet this author points out that the observed effects in the lifespan study are marginal at best.

With these possible health effects in mind, one can ask the question of whether or not fucoxanthin might be a commercially viable bioproduct that taps into the lucrative functional food market while also aiding in public health. Fucoxanthin is already on the market, but mostly in the form of pure powder used for chemical analysis in the Asian online marketspaces, with just a few nutritional supplements available on marketplaces such as amazon.com or nature-based supplement internet –based providers.

Out of all the supplements, β-carotene and astaxanthin are currently the only two successfully being produced via algal biomass cultivation (Leu & Boussiba, 2014), utilizing the algae *Dunaliela salina* and *Haematococcus pluvialis* respectively. With fucoxanthin belonging to a similar functional group as these other pigments and possibly showing similar effects, the question arises whether micro-algal production of fucoxanthin could also be achieved in a similar manner.

However, fucoxanthin is also found in several macro-algae which are already being produced on a large scale as well. Harvesting directly from the sea could circumvent some of the other problems that micro-algal culturing encounters such as relatively high production and processing costs (Leu & Boussiba, 2014).

This paper will try to answer which of the algae are the most viable candidates and looks at fucoxanthin production as a whole by both investigating the options of production and whether or not the current studies on fucoxanthin provide a solid basis for legitimate health claims.

1.2 Research questions

Main question:
Can fucoxanthin currently be considered a viable bio-product for creating health-promoting supplements using extensive algal cultivation?

Subquestions:

-What is the current state of the medical literature on the effects of fucoxanthin?
- In what micro- and macro-algae can one find fucoxanthin?
- What is the inter- and intraspecies variability?
- What are the different extraction methods?

2. Medical properties of fucoxanthin

There are many different studies on the possible health effects of administering fucoxanthin to animals, animal- and human cell lines. This paper will focus on its anticancer and related anti-oxidative capabilities as well as its antidiabetic and anti-obesity effects as these fields seem to be the most promising, most studied and relevant for average human health. But before one can look at the potential positive effects of fucoxanthin there are two important barriers to cross: safety and bioavailability.

2.1 Safety

Humans have been eating seaweed since ancient times (Indergaard, 1983) with Saccharina latissima, a fucoxanthin containing seaweed, being one the most popular. It seems that in the very least, fucoxanthin in the form of eating seaweed does not provide a direct risk to human health. Yet that becomes different when thinking about food supplements and their relative dose. To assess the potential risk of fucoxanthin, Beppu *et al* (2009) administered several doses of fucoxanthin to living male and female mice. Both in the single dose study (1000 and 2000 mg/kg) and in a separate repeated dose study (500 and 1000 mg/kg/daily for 30 days) no increased mortality or abnormal growths were detected. In a follow-up study they administered 500, 1000 and 2000 mg/kg fucoxanthin to mice. After 24 hours bone marrow cells were harvested and examined. The study found no evidence of mutagenicity of fucoxanthin (Beppu *et al*, 2009). In combination with the established consumption of seaweed, the administration of fucoxanthin in normal doses is not expected to be directly harmful to human health.

2.2 Bioavailability

Fucoxanthin is broken down into its metabolite fucoxanthinol in both Caco-2 human intestinal cell lines, mice and rats (Sugawara *et al*, 2002. Asai *et al*, 2004). Additionally, in mice, rats and human HepG2 cells, fucoxanthinol can then be broken down further into amarouciaxanthin A (Asai, 2004, Sangeetha, 2010). In mice, the breakdown of fucoxanthin is very rapid, and the two metabolites are shown to build up in heart and liver for fucoxanthinol and in adipose tissue for amarouciaxanthin A, while fucoxanthin itself was only detectable in the lower limits (Hashimoto, 2009). However, the bioavailability and accumulation of fucoxanthin and fucoxanthinol in humans directly from a food source such as wakame was shown to be very low (Asai, 2008). If this was caused by the digestion of wakame itself and it was interfering with the accumulation of fucoxanthin or its metabolites could not be assessed.
In the same study, Akira Asai (2008) suggests that the incorporation of lipids might enhance the bioavailability of fucoxanthin as this is also the case in other carotenoids. For example in Lutein, a yellow carotenoid, glycolipids improve the bioavailability and accumulation of the

carotenoid in the eyes of mice (Gorusupudi & Vallikannan 2012). Which lipids are most useful is determined by the carotenoid itself (Nagao *et al*, 2013). A combination of fish oil and fucoxanthin for example was more effective in attenuating white adipose tissue weight gain in diabetic/obese KK-Ay mice than fucoxanthin alone (Maeda *et al* , 2007), indicating that fish oil is an effective delivering device for fucoxanthin . The types of oil used and the consequent absorption of fucoxanthin however are also dependent on whether or not the study itself is done in vitro and in vivo, highlighting the importance of distinguishing between these set-ups in future research (Salvia-trujillo *et al* 2015). Nevertheless, a nanogel carrier of fucoxanthin made of chitosan-glycolipds has also already been shown to be a non-toxic mode of delivery in rats (Ravi *et al*, 2015). Concluding, as the bioavailability of fucoxanthin from dietary sources is deemed to be very low, supplementation with the addition of certain lipids is a better method of administering fucoxanthin to ensure a better accumulation for a desired effect.

2.3 Anti-cancer

In 1993, fucoxanthin was shown to inhibit chemical carcinogenises in mice. In a control group the known carcinogen N-ethyl-N'-nitro-N-nitrosoguanidine (0.01%) was added to drinking water, while in the experimental group fucoxanthin (0.005%) was also added to the diet. In the fucoxanthin group, both the total number of tumors as well as tumor size was significantly smaller than in the control group (Okuzumi *et al*, 1994).
Due to their double bond structure, carotenes are effective anti-oxidative agents (Stahl & Sies, 2005). Not surprisingly, fucoxanthin and its metabolite fucoxanthinol were also shown to exhibit a high radical quenching ability, but with β-carotene outperforming them in their singlet oxygen quenching ability (Sachindra *et al*, 2007). Yet when comparing the antioxidant potential of fucoxanthin and β-carotene in relieving lipid peroxidation caused by retinol defiency in rats, fucoxanthin was proven to be slightly more effective (Sangeetha *et al* , 2009). However, Kumar *et al* point out that while fucoxanthin may be an effective anti-oxidant, its realm of protecting against cancer is much wider than that. They identify 3 main ways by which fucoxanthin can possibly combat cancer: by inducing apoptosis in cancer cells, cancer cell cycle arrest and the suppression of metastasis (Kumar *et al*, 2013). In figure 2, all factors by which fucoxanthin might possibly affect cancer are summarized.
As examples of apoptosis inducing behavior, Kotake-Nara *et al,* (2005) found that a 48 hour treatment of 20µM carotenoids (fucoxanthin and neoxanthin) on PC-3 Human prostate cancer cell lines increased apoptotic cells by 30%. A similar effect was found by Hosokawa *et al* (2004) in which fucoxanthin reduced viability in human colon cancer cell lines Caco-2, HT-29 and DLD-1. The addition of 22.6 µM fucoxanthin for 24 hours increased DNA fragmentation in the Caco-2 cell lines ten-fold (Hosokawa *et al* , 2004). These two studies together give evidence that the apoptotic effects of fucoxanthin are specific for not only one cell line but that its workings differ amongst cell lines and cell types.
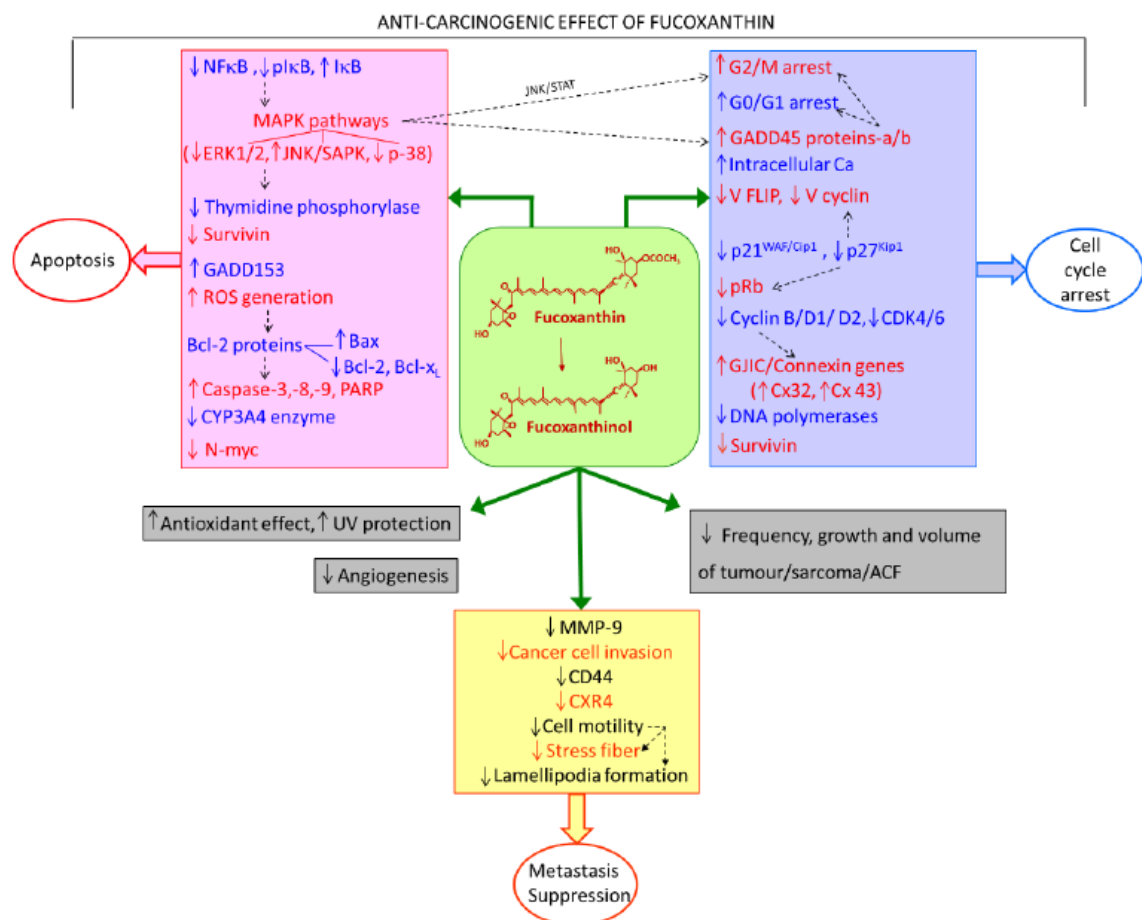
Fig 2- Factors (molecules and mechanisms) regulated by fucoxanthin, resulting in its anti-carcinogenic effects. Dashed lines indicate inter-relation/inter-effects between the factors; up and down arrows indicate up- and down-regulation (by fucoxanthin/fucoxanthinol, respectively). Figure and subtext by Kumar *et al* , 2013.

That fucoxanthin can work against cancer amongst different cell types can also be found in the ability of fucoxanthin to interfere with the cancer cell cyle. For instance, in both the human liver cancer cell line HepG2 and the human prostate cancer cell line DU145, fucoxanthin initiated cell cycle arrest in a dose-dependent manner at the G1-phase by inducing the GAD045A gene (Hoshiko & Hoyoku, 2007). It did however not induce apoptosis.

So it is clear that fucoxanthin and fucoxanthinol are able to combat different types of cancer in different ways, at least when administered in a dose-dependent manner directly to human cell lines. Yet while this is interesting from a medicinal standpoint, it is not yet evidence of the effectiveness of fucoxanthin as a preventative food supplement. For example, in relation to supplements and their effects on prostate cancer, Deeba N. Syed *et al* (2009) conclude that in the end, clinical trials are still the gold standard for determining prevention agents for cancer risk.

2.4 Anti-inflammatory & anti-obesity

Fucoxanthin has also been investigated intensively for its proposed anti-inflammatory and anti-obesity properties. The relationship between inflammation, insulin resistance and obesity in humans is slowly starting to be understood. In a review, Jean-Phillipe Bastard *et al* (2009) come to the conclusion that being obese is associated with a low-grade inflammation of white adipose tissue cells and that in conjunction with an increased infiltration of macrophages; the body produces more pro-inflammatory factors possibly leading to insulin resistance and diabetes. A similar line of thought can be found in the research on fucoxanthin. Of course, if fucoxanthin is able to interfere on different levels with the relationship between obesity and inflammation and thereby affect people with obesity positively this would be very promising. Both for public health and for profit potential, as weight-loss is a large market that is projected to reach a value of $206.4, - billion in 2019[2].

In both mice and rats evidence has been found of fucoxanthin regulating certain known inflammation factors. In one study, the effect of fucoxanthin on endotoxin-induced uveitis by lipopolysaccharides in rats was investigated. Immediately after inoculation, the rats were administered 0.1, 1 and 10 mg/kg^{-1} fucoxanthin and their cellular infiltration into the surrounding aqueous humour was measured. While untreated uveitis showed that $62.1\pm11.7\times10^5$ cells ml^{-1} infiltrated the aqueous humour, at 10mg/kg^{-1} fucoxanthin reduced this number to $12.8\pm3.5\times10^5$ cells ml^{-1}, an effect similar to 10 mg/kg^{-1} prednisolone ($12.8\pm8.0\times10^5$ cells ml^{-1}) (Hosokawa *et al*, 2010). In the same study, fucoxanthin also decreased pro-inflammatory and insulin-resistance inducing factors ((TNF-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2)) mRNA expression in mouse macrophage-like cells (RAW 263.7) (Hosokawa *et al,* 2010). Fucoxanthin does so in RAW 263.7 cells by inhibiting Nf-Kb protein complex activation and MAPK phosphorylation (Kim *et al*, 2010), both involved in stress and immune system responses in cells. However, In the study by Hosokawa *et al*, fucoxanthin reduced the same factors in obese/diabetic KK-A(y) mice, but not in lean C57BL/6J mice, with the same distinction also found in the macrophage-like cells (Hosokawa *et al*, 2010), indicating that the effect is more pronounced in already affected individuals.

In terms of a more direct effect on weight, a diet rich in fucoxanthin significantly reduced abdominal white adipose tissue (WAT) weight in rats and mice by upregulating the mitochondrial uncoupling protein 1 (UCP1) which is involved in metabolic thermogenesis to avoid excess fat storage (Maeda *et al* , 2005). This effect has been reproduced in multiple studies and seen as one of the main ways in which fucoxanthin interacts with obesity, with other studies also reporting effects on leptin regulation and liver-fat accumulation (Gammone & D'orazio, 2015).

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[2] http://www.marketsandmarkets.com/PressReleases/weight-loss-obesity-management.asp

In a follow-up study Maeda *et al* showed that a diet high in fucoxanthin can also alleviate problems such as hyperglycemia, hyperinsulinemia and hyperleptinemia in high fat fed mice thereby further linking fucoxanthin to possible anti-diabetic effects (Maeda *et al*, 2009).

Additionally, delivering the fucoxanthin alongside fish oil significantly increased the effectiveness of fucoxanthin supplements in attenuating WAT weight gain and decreasing blood glucose and plasma insulin concentrations in obese/diabetic KK-A(y) mice, more so than administering fucoxanthin on its own (Maeda et al, 2007).

All these results do point to fucoxanthin being an effective anti-obesity nutrient due to its ability to increase metabolic thermogenesis, its anti-inflammatory effect and subsequently linked insulin resistance inhibition. Yet this is also mostly in those who are already affected by obesity or diabetes, as was seen in the mouse macrophages. For a more comprehensive review on all the anti-obesity effects of fucoxanthin this author refers to the review by Gammone & D'orazio in the references.

Both the studies on bioavailability and the ones on fucoxanthin supplementation accompanied by fish oil in KK-A(y) mice gave rise to one of the first studies of direct application of a fucoxanthin supplement in humans. Abidov *et al* (2010) found that, "Xanthigen" (which is notably mentioned with a "trademark" continuously throughout the paper) a supplement consisting of both pomegranate seed oil and fucoxanthin in the form of brown seaweed extract managed to increase the resting energy expenditure rate (REE) and liver function while also reducing body and liver fat levels in obese non-diabetic premenopausal women with non-alcoholic fatty liver disease in a dose-respondent level (0.8 to 4 mg fucoxanthin).
Notably, the combination with the oils was much more efficient, thereby validating some of the bioavailability research (Abidov *et al* 2010).
In another study on the effect of Xanthigen, but on cell lines, Lai *et al* conclude that Xanthigen suppresses adipocyte differentiation and lipid accumulation through multiple mechanisms and more effectively then fucoxanthin or pomegranate seed oil alone (Lai et al, 2012); however this author did not have access to the whole article and specifics on concentrations are not known.

Yet a lot remains to be questioned in terms of true effects in the human body. In a follow up, random, placebo-controlled and double blind study done by Lisa Knecht, she investigated the effect of the same Xanthigen supplement on 60 obese people in rural America. No significant effects of Xanthigen were found on the reduction of body fat, nor in the increase of REE. Most of the fat loss observed in the study was attributed to a change in exercise and diet behavior due to frequent accountability (Knecht, 2012). There were however some indications that the supplement helped in lowering blood pressure and increasing fat utilization for energy expenditure, but no real definitive conclusions could be made on the

topic. Knecht also rightly points out several problems with the Abidov study such as very low sample sizes in each researched fucoxanthin level group (n=3 or 4) and underreported statistical methodologies (Knecht 2012).

The author of this paper concludes that with these contradicting studies in mind, the true value of fucoxanthin as a supplement in regards to fat and weight loss is still questionable. Secondly, while studies done on the effectiveness of fucoxanthin in combating cancer through inducing apoptosis and cell cycle arrest in human cancer cells do indicate a direct effect in those specific occasions, extrapolations to human health benefits are arguably promising at best. This author agrees with Deeba N. Syed that human trials should be the gold standard. Not only in terms of prostate cancer in the case of that article, but for all potential human health benefits of supplementation.

In the discussion we will come back to this topic when regulations and the needed proof on the medical benefits of supplements amongst countries are evaluated and discussed. We will now move on to the practical aspects of fucoxanthin production as a bioproduct; where is it found and how do we harvest it?

Fucoxanthin production and harvesting

Fucoxanthin is ubiquitous and accounts for almost 10% of the carotenoids found in nature (Liaaen-Jensen, 1978). As a functional pigment of the photosystem, it is found in the antenna complex of all heterokonts and haptophytes (Strange, 2016). As this encompasses a very large group of species this author decided to look at the micro- and macro-algae that were most researched separately. Intra- and interspecies differences in fucoxanthin as well as extraction methods were analyzed for both micro- and macro-algae.

Chapter 3: Micro-algae

3.1   Inter-species fucoxanthin content.

Relatively few studies have been done comparing fucoxanthin content under laboratory circumstances. In table 1 a short summary on fucoxanthin content (mg/gr dryweight) from relevant literature that had stable culture conditions is provided. The most promising species are: *Phaeodactylum tricornitum, Isochrysis aff. galbana* and *O. aurita*. It should be noted that *Cyllindrica closterium* might have a different fucoxanthin content, as acetone as an extraction method is not as efficient as ethanol, at least as was shown in *Phaeodactylum tricornitum* (Kim, 2012).

In one of the most recent studies on intra-species differences done by Guo *et al*, they looked at the fucoxanthin productivity of 13 different diatoms, which can be seen in figure 3.

Table 1 – Fucoxanthin content (mg/g) in several micro-algal species. References: 1 = Kim *et al*, 2012-1. 2= Kim *et al*, 2012-2. 3= Xia *et al*, 2013. 4= Pasquet *et al*, 2005.

Species	Fucoxanthin content (mg/g sample)	Sample condition	Extraction method	References
Phaeodactylum tricornitum	15,33	dried	Ethanol	1
Phaeodactylum tricornitum	8.55 ± 1.89	dried	Ethanol	2
Chaetoceros gracilis	2.24 ± 0.01	dried	Ethanol	2
Isochrysis galbana	6.04 ± 0.28	dried	Ethanol	2
Isochrysis aff. galbana	18.23 ± 0.54	dried	Ethanol	2
Nitzschia sp.	4.92 ± 0.11	dried	Ethanol	2
Odontella aurita	20,83	dried	Ethanol	3
Cylindrotheca closterium	5.23 ± 0.06	dried	Acetone	4

The diatoms were all kept in F2 medium, exposed to a light intensity of 30 µmol photons m^2 s^{-1} on a 12:12 light/dark cycle for 14 days. It was concluded in this study as well that *P. tricornitum* and *O. aurita* were the most efficient fucoxanthin producers with both achieving productivity rates of more than 0.20 mg/L^{-1}/d^{-1}.
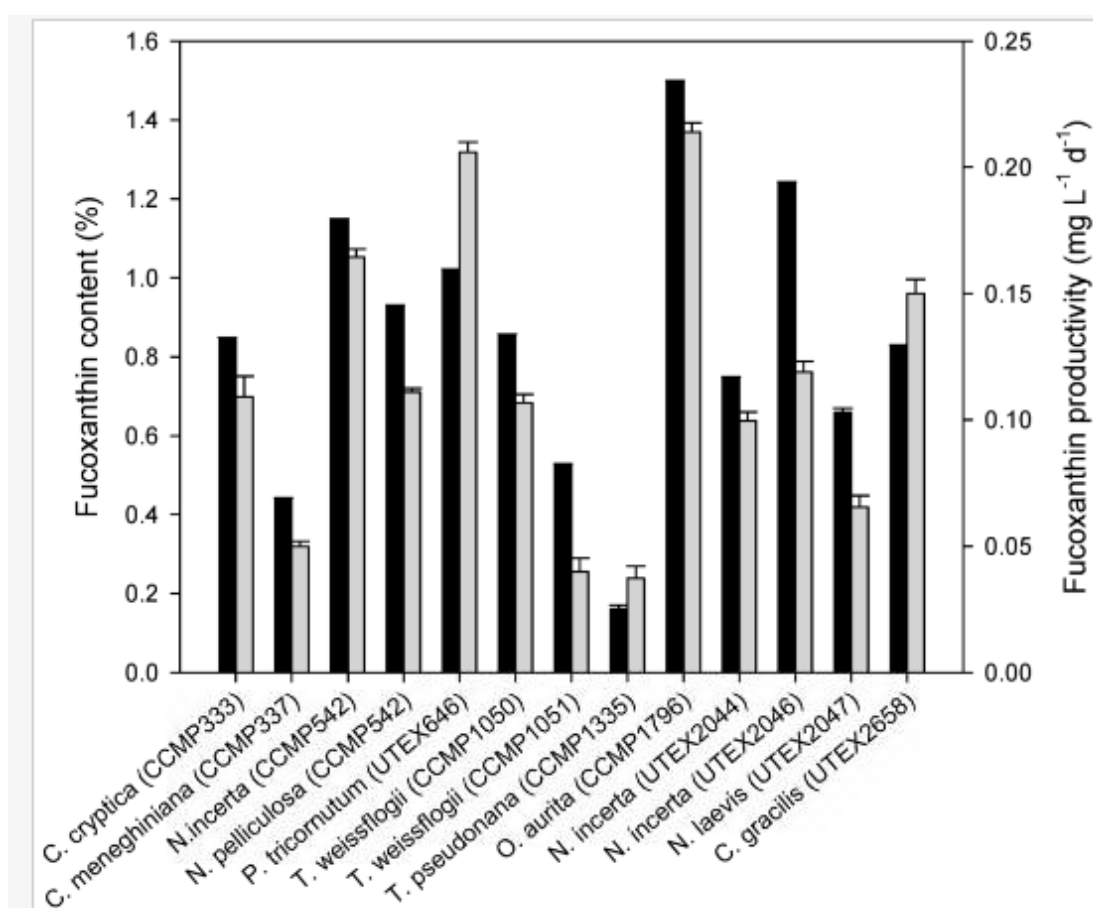
Fig 2 - Fucoxanthin content and productivity of thirteen diatom strains under photoautotrophic conditions. Cells on Day 14 were harvested for analysis. Dark bars represent the fucoxanthin content and grey ones represent the fucoxanthin productivity. The fucoxanthin productivity was expressed as fucoxanthin yield divided by culture days. Data are means of three replicates and error bars indicate standard deviations. (Figure and subtext: Gao *et al, 2016*).

It is worth mentioning that, although no direct content measurements were provided, in a study on the anti-proliferative effects of cultured algae extracts on human bronchopulmonary and epithelial cancer cell lines, *I. galbana* and *O. aurita* extracts were amongst the most potent as well, attributed to their fucoxanthin content (Moreau, 2006).

However, the fact that the entire *P. tricornitum* genome has been sequenced (Bowler et al, 2008) also makes this alga a prime candidate for being a viable bio-product producer. This author concludes all three algae are viable candidates. Increasing fucoxanthin production is not only accomplished by selecting the correct algae; environmental factors at play might also be manipulated.

3.2 Intra-species fucoxanthin content.

3.2.1 Intra-species variability – strains

The specific strain of an alga can have a large impact on pigment content. For example when comparing the percentage of fucoxanthin relative to its total amount of carotenoids in *Phaecystis globosa,* values ranged from 0 to 83% (Vaulot et al, 1994).So in the three previously mentioned algae, selecting the most productive strains would increase production efficiency.
No studies on differences between strains of *Isochrysis* or *Odontella* could be found.
In a comparison between 6 different *Phaeodactylum tricornitum* strains cultured under F2 medium conditions, an irradiance of 40 μmol photons $m^2 s^{-1}$ and 25^0C, Hualian *et al* found fucoxanthin content to range from 2.13±0.02 to 5.50±0.09. The lowest performer came from the Daya Bay, China and the highest from Fuzhou bay, China, a distance of around 750 km. While strains seem to differ in fucoxanthin content, the underlying causes for these differences are not yet thoroughly understood. Nevertheless, a complete screening of more different strains before selecting the optimum alga for fucoxanthin production seems prudent.

3.2.2 Intra-species variability – Environmental factors

3.2.2.1 Irradiance
As a light-harvesting pigment embedded in several fucoxanthin-chlorophyll complexes, it is hardly surprising that fucoxanthin content shows a dependence on irradiance levels. Culturing *Isochrysis* from a range of 50 to 1000 μmol photons $m^2 s^{-1}$ (with a self-reported potential light attenuation of 30-40% inside the flasks) resulted in a at first a short increase of 110% and subsequent loss of of almost 75% fucoxanthin relative to its value at 50 μmol photons $m^2 s^{-1}$ (Brown et al, 1994). In a more recent study, *I. galbana* and *P. tricornutum* fucoxanthin production was compared under irradiances ranging from 9.1 to 62.0 μmol photons $m^2 s^{-1}$. Both algae performed best at 13.5 μmol photons $m^2 s^{-1}$ and decreased in

fucoxanthin production thereafter (Loredo et al, 2016). *O. aurita* shows a similar curve with fucoxanthin decreasing at higher irradiance level. When grown under 100 µmol photons m^2 s^{-1} , at t=6 days fucoxanthin content reached 20.83 mg/ g^{-1} before gradually decreasing to 16 mg/g^{-1} at t=12 . This was in contrast to those grown under 300 µmol photons m^2 s^{-1} as they reached a maximum of around 18 mg/g^{-1} in 4 days and decreased to 9 mg/g^{-1} at t=12 (Xia et al, 2013). This is partly explained that, at least in *P. tricornutum,* fucoxanthin content decreases by age (Carreto & Cattogio, 1976). Additionally, as an accessory pigment, it is thought that up until a light saturation point is reached, fucoxanthin content will increase to aid in light harvesting. However, as irradiance levels exceed this point and starts damaging the photosystem, the accessory pigment diadinoxanthin is converted into diatoxanthin to dissipate excess energy into heat (Goss & Jakob, 2010). These newly formed xanthophylls are theorized to replace fucoxanthin in certain fucoxanthin-chlorophyll complexes to aid in photoprotection and as such lower total fucoxanthin content (Beer, 2006). Interestingly, culturing *Phaeodactylum* in an intermittent light schedule (5m L, 55m D, 40 µmol photons m^2 s^{-1}) did not decrease fucoxanthin content compared to a constant light schedule (16h L, 8h D, 40 µmol photons m^2 s^{-1}-) while increasing diadinoxanthin (Lavaud et a 2002), indicating that fucoxanthin is not replaced indiscriminately**.** The increase is thought to occur as the alga tries to capture more light in the short periods that it receives. This effect was seen earlier in *Isochrysis* when chl a concentrations increased during shorter periods of daylength (Hobson, 1979). In an unpublished study done Elzinga & Reuvers *et al* (2016) they showed that while fucoxanthin content in *P.tricornutum* is higher in low light cultures (10 µmol photons $m^2 s^{-1}$) then in high light (230 µmol photons $m^2 s^{-1}$) cultures, the improved growth rate in high light cultures ultimately provides a higher fucoxanthin content. They suggest a culturing set-up that would allow for the highest fucoxanthin production in which a high light growth phase is followed by a quick low light acclimation period.

3.2.2.2 Temperature
No studies of effects of temperature on fucoxanthin content in *O. aurita* or *P. tricornitum* could be found. Research suggests an optimal growth temperature between 10^0C (Roleda *et al*, 2013) and at least 24^0C (Pasquet *et al*, 2014). Yet optimum growth temperatures yield little information on cellular content. For example, increasing temperature in *O. aurita* increased polyunsaturated fatty acids while decreasing saturated fatty acids (Pasquet *et al*, 2014). For the strain *isochrisis galbana Parke*, Durmaz *et al* conclude that a temperature of 18^0C is ideal for carotenoid content, while exceeding this temperature decreases the total carotenoids (Durmaz *et al*, 2008). More research is needed on the effect of temperature on these algae to optimize fucoxanthin production while still encouraging growth to strike the optimal balance between total biomass and fucoxanthin content per algal cell.

3.2.2.3 Nutrients
No differences in fucoxanthin to chlorophyll a ratio were found in *P. tricornutum* when starved of both nitrogen and phosphorus, but it did decrease total growth (Zhao, 2015).

Another effect was shown for *I. galbana.* When lowered in or depleted of magnesium, silicate and nitrate and compared to a replete condition, no differences in fucoxanthin content were found in the first 5 days; after 9 days fucoxanthin increased in the silicate depletion condition while it decreased in the N depletion (Mulders, 2015). Additionally, growing in a "Conway" medium slight increased the maximum observed cell density by 1.45% in *I. galbana* compared to F/2 medium (Lananan 2013). For *O. aurita*, Xia *et al* determined that increasing nitrate from 6 (deficient) to 18 mM (replete) when grown under 100 µmol photons $m^2 s^{-1}$ increased total fucoxanthin from 6.67 mg/g^{-1} to 18.14 mg/g^{-1} respectively (Xia *et al*, 2-).

3.2.2.4 Genetic manipulation

As the entire genome of *P. tricornitum* is mapped, this gives rise to more possibilities of increasing fucoxanthin production. By specifically targeting the overexpression of the genes encoding for 1-deoxy-d-xylulose 5-phosphate synthase (DXS) and phytoene synthase (Psy), most strains increased their fucoxanthin content compared to the wild-type (Eilers et al, 2015). While it did so in an irregular pattern so the exact efficacy is still uncertain, it does open up new ways of thinking about enhancing fucoxanthin production.

The above mentioned factors create possibilities for a range of fucoxanthin production strategies specifically targeted for a certain strain. For example cultures are first encouraged to create a high cell density and size before being starved of silicate to increase fucoxanthin content in the case of *I. galbana*. Mapping the genomes of *I. galbana* and *O. aurita* and research in genetic manipulation could also yield higher production rates; but the question is whether the benefit will outweigh the cost. And this is in turn dependent on the true value of fucoxanthin as a supplement or as an addition to regular foodstuffs.

3.3 Extraction processes

3.3.1 Extraction processes - focused on fucoxanthin

No in-depth extraction studies on *O.aurita* could be found. However, in two different studies, Kim *et al* determined the most efficient solvents and methods for pigment extraction in both *P. tricornutum* and *I. galbana* (Kim et al, 2012-1 & Kim et al, 2012-2). The results of these studies have been compiled by this author in 1 graph which can be seen in fig 4. No data for methanol extraction was obtained in the study on *Phaedactylum tricornutum*.
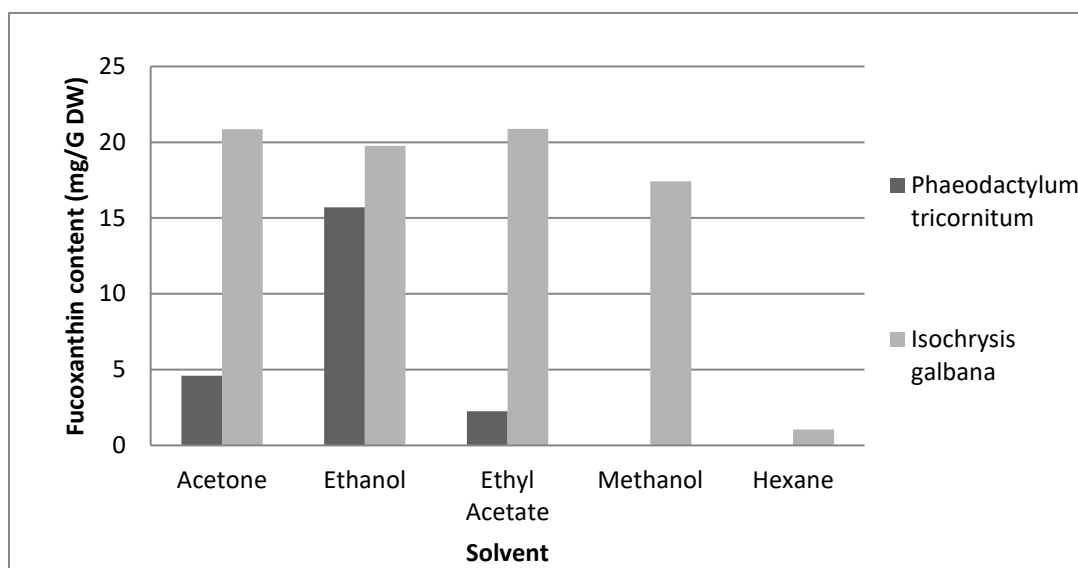
Fig 4 – **Effects of solvents on fucoxanthin extraction in two micro-algae. Extraction times for *Phaeodactylum sp.* and *Isochrysis sp.* were 1h and 30m respectively. Data is compiled from 2 different studies (*Phaeodactylum*: Kim et al, 2012. *Isochrysis*: Kim et al, 2012).**

Gomez-Loredo observed a 70% recovery of fucoxanthin and a removal of more than 60% of other low molecular weight compounds when they used an ethanol-salt (potassium) aqueous two-phase system (Gomez-Loredo *et al,* 2014). Their proposed method and ATPS ethanol/potassium concentrations for each alga can be seen in figure 6. They argue that this system, while still in need of increasing efficiency, is feasible as the required components are relatively low cost. In a follow-up study they investigated the effect of ultra-filtration and more ethanol as a solvent of the ATPS top-stream to further purify the streams.
At a 44.15% and 54.15% aqueous ethanol solution, around ±80 and ±90% respectively of the fucoxanthin content remained within the retentate while a 74.15% solution recovered over 85% in the permeate (Gomez-Loredo *et al,* 2015). The end product contained 16% less impurities such as protein while still retaining about 63%, which in itself could also be recovered for more processing (Gomez-Loredo *et al,* 2015). While this method does involve several steps and a possible small loss of fucoxanthin, it will increase purity of the end product over time.
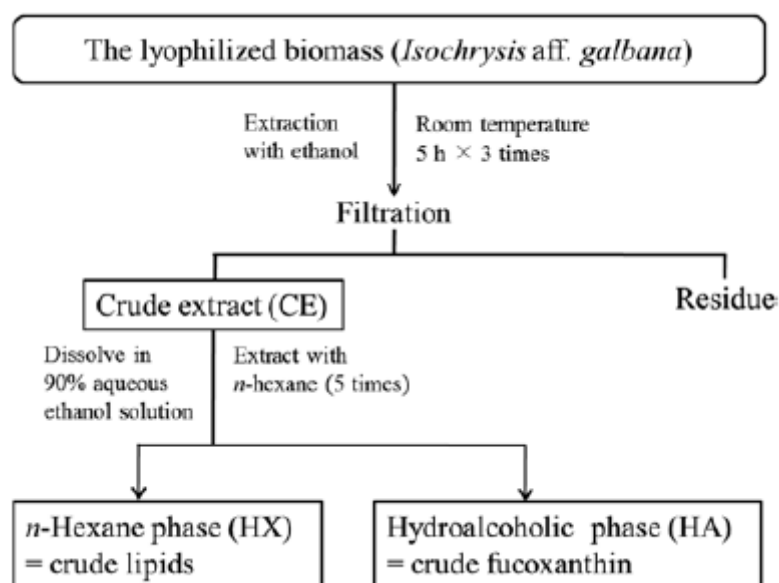
Fig. 3 - Process for the preparation of crude fucoxanthin and lipids from the microalga *Isochrysis aff. galbana*. (Kim et al, 2012-b)

3.3.2 Extraction processes – fucoxanthin as a part:

While the above-mentioned extraction processes account for pure fucoxanthin recovery, one can also look at downstream-processing, in which several different valuable products are harvested from one alga through several different steps. As fucoxanthin has a higher bioavailabilty when combined with lipids, Kim *et al* further investigated a method (see fig. 5) of harvesting both fucoxanthin and the lipid fraction in *I.galbana.* They found that with this method 75% of the fucoxanthin was present in the HA phase and only 7% was found in the CE phase. The remaining loss of fucoxanthin was attributed to degradation.

While the total fucoxanthin found was less than through regular extraction processes, it does show the methods' feasibility for producing both lipids and fucoxanthin simultaneously. In a similar study on downstream-processing of on *I.galbana*, Gilbert-Lopez *et al* investigated a four-sequential step extraction process, with each step using the residue of the preceding step. In each step they used (1) supercritical CO_2 (ScCO_2), (2) ScCO_2/ethanol (Gas Expanded Liquid), (3) pure ethanol and (4) pure water.
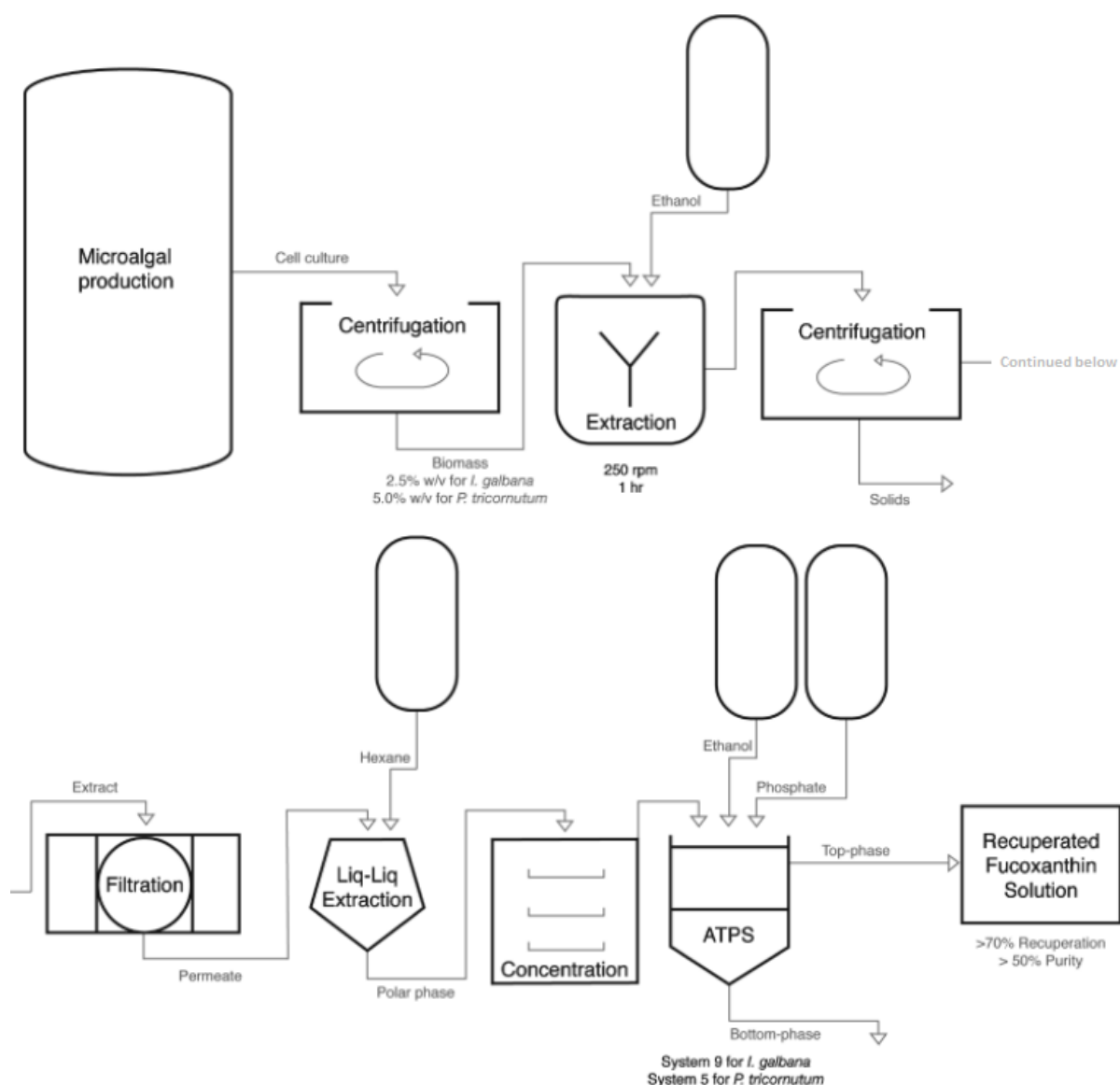
Fig 4. - **Proposed process diagram for recovery of fucoxanthin from *P. tricornutum* and *I. galbana* wet biomass. "System 9" equals 30.5% ethanol/16% phosphate while "System 5" equals 18% ethanol/24.75% potassium. After Gomez-Loredo *et al*, 2014.**

This was done to separate carotenoids, non-polar lipids, chlorophylls, mid-and-highly polar lipids protein and sugars, respectively. The highest amount of total fucoxanthin isotopes was found in the $ScCO_2$/ethanol extraction with 75% ethanol and steps 3 and 4 still providing extracts enriched with lipids and carbohydrates (Gilbert-Lopez *et al*, 2015). More specific information on supercritical CO_2 extraction can be found in chapter 4.3.4.

3.4 Conclusions

The prime candidates for fucxoanthin production in micro-algae are *Phaeodactylum tricornutum*, *Isochrysis aff. Galbana* and *Odontella aurita*. Differing environmental factors to optimize fucoxanthin is possible, with irradiance probably being the most important factor. A possible trade-off between total growth and increasing fucoxanthin content such as the one found in *P.tricornutum* by altering environmental factors could be investigated further in other species to increase efficiency.

As of right now, due to the ease of extraction with multiple solvents *I.galbana* seems to be alga with the most promise. While few studies have been done looking into newer methods of fucoxanthin extraction without the need of organic solvents within micro-algae, some of the newer methods for macro-algae described in chapter 4. 3 could also apply. This has been the case in the supercritical CO_2 fluid extraction method proposed by Gilbert-Lopez.

For now though, when going for pure fucoxanthin production the ATPS with ultrafiltration method is reported to be a relatively cheap and easy method. However, down-stream processing techniques will be able to utilize more from 1 algal source thereby increasing the sustainability of algal culturing. A downside to downstream processing is that all other valuable products one wants to harvest probably also react differently to different environmental parameters during the growth stage thereby altering the content of the end product. Ultimately the question of which method is preferable will also rely on the demand of the potential market in whether or not they rely more on purity or bulk extracts.

Chapter 4: Macro-algae

4.1 Inter-species fucoxanthin content.

Few studies have been done directly comparing fucoxanthin content in macro-algae under the same conditions such as the time and place of harvesting or culturing methods. Additionally, fucoxanthin content measurements differ between wet and DW notifications. These factors make direct evaluations of fucoxanthin content between species from the current literature difficult. As an indication, some of the data from the literature has been summarized in table 2. Additionally, fucoxanthin content in several Northern European species can be found in figure 7; these values were not added to the table directly as they are divided into content per part of the plant.

Despite the difficulties there are some observations to be made. Notably, that macro-algae contain much less fucoxanthin per gram DW in absolute terms than micro-algae. While as mentioned micro-algae can reach fucoxanthin content values of 20 mg/g^{-1} DW or more, the highest values in macro-algae this author found were in the family of *Sargassacea*, ranging from 0.065 to 3.7 mg/g^{-1} DW (Terasaki, 2009).
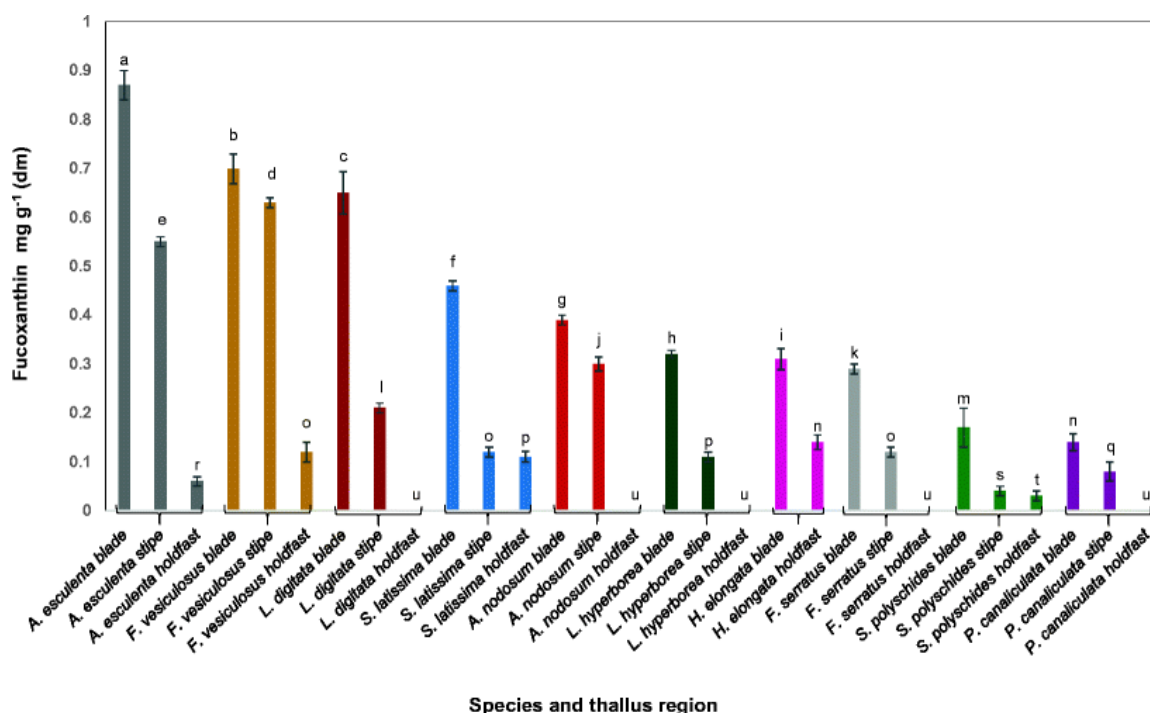
Fig 5 - Comparative fucoxanthin content (mg g−1 dry mass) per species and thallus region of ten Irish brown seaweeds. Values are the mean of three replicates ± standard deviation. Letters denote least significant difference between columns (P ≤ 0.05) (Shannon & Abu Ghannan, 2016).

Interestingly, the highest ranking species, *Sargassum horneri* is also known as "Devils Weed" and seen as an invasive species in California[3] while eaten in Japan (Terasaki et al, 2016). This is in contrast to the more traditionally eaten seaweeds such as *Laminaria saccharina* ("Kombu" and also known as *Saccharina latissima*) and *Undaria pinnatifida* ("Wakame") which contain lower amounts of fucoxanthin, ranging from 0.243 mg/g^{-1} and 0.87 mg/g^{-1} DW respectively.

It is clear that there are large differences in fucoxanthin content within macro-algae. And looking at table 2 for *U. pinnitifada* as an example, these same differences are also apparent between different strains of the same species, indicating ecotype differentiation. Determining what the specific causes of these differences are in this table is as said difficult from this data, as an ecotype differentiation encompasses many factors such as the place of origin (latitude/longitude), harvesting depth and local nutrient supply.

To be able to derive some information on how environmental factors can influence fucoxanthin production, three groups of species of macro-algae were identified for further analysis: the *Sargassaceae (s*everal species)*, Laminariaceae* (*Saccharina sp* in particular) and Alariacea (*Undaria pinnatifida*). They were chosen due to both their relatively high

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[3] https://blogs.scientificamerican.com/expeditions/usc-dornsife-scientific-diving-an-analysis-of-sargassum-horneri-ecosystem-impact/

fucoxanthin content in case of the Sargassaceae and their long history as edible seaweeds or as traditional medicine.

Table 2 –Fucoxanthin content in different seaweeds grouped on family and species level. References: 1= Terasaki *et al*, 2009. 2= Seely *et al*, 1979. 3= Kanazawa *et al*, 2008. 4= Kim *et al*, 2012. 5= Mori *et al*, 2004. 6= Kim *et al*, 2004.

Family	Species	Fucoxanthin content (mg/g sample)	Sample condition	References
Sargassaceae	Sargassum horneri	3.7 ± 1.6	Dry	1
	Sargassum thunbergii	1.8 ± 1.0	Dry	1
	Sargassum fusiforme	1.1 ± 0.6	Dry	1
	Cystoseira hakodatensis	2.4 ± 0.9	Dry	1
	Sargassum confusum	1.6 ± 0.8	Dry	1
	Sargassum muticum	0,293	Dry	2
	Sargassum binderi	0,73 ±0,39	Dry	6
	Sargassum duplicatum	1,01 ±0,10	Dry	6
	Sargassum fulvellum	0,065	Fresh	3
Fucaceae	Hizikia fusiformis	0,022	Fresh	3
	Silvetia babingtonii	0.7 ± 0.2	Dry	1
	Fucus distichus	0.9 ± 0.3	Dry	1
	Fucus distichus	0,156	Dry	2
Laminariaceae	Saccharina sculpera	0.7 ± 0.4	Dry	1
	Laminaria saccharina	0,243	Dry	2
	Laminaria japonica	0,187	Fresh	3
	Laminaria religiosa	0.24	Fresh	5
Alariaceae	Alaria crassifolia	1.1 ± 0.4	Dry	1
	Undaria pinnatifida	0,111	Fresh	3
	Undaria pinnatifida	0.32	Fresh	5
	Undaria pinnatifida	0.87	Dried	6
Asperococcaceae	Melanosiphon intestinalis	1.9 ± 0.9	Dry	1
Ralfsiaceae	Analipus japonicus	1.4 ± 1.0	Dry	1
Leathesiaceae	Leathesia difformis	0.3 ± 0.1	Dry	1
Chordariaceae	Sphaerotrichia divaricata	0.2 ± 0.1	Dry	1
Scytosiphonaceae	Scytosiphon lomentaria	0.5 ± 0.1	Dry	1
	Scytosiphon lomentaria	0.24–0.56	Fresh	5
	Petalonia binghamiae	0.43–0.58	Fresh	5
Desmarestiaceae	Desmarestia viridis	0.1 ± 0.1	Dry	1
Lessoniaceae	Eisenia bicyclis	0,077	Fresh	3
	Eisenia bicyclis	0.26	Fresh	4

4.2   Intraspecies fucoxanthin content

4.2.1   Water depth

Water depth was found to be a significant factor in total fucoxanthin content in a study comparing different tropical *Sargassum* species (*S. aquafolium, S. cristaefolium* and *S.polusystum*). Those species that were living in deeper waters (3-6 m) contained on average 53.64 ± 5.91 µg g^{-1} DW, while those living near the surface (0-1 m) reached 148.21 ± 5.971 µg g^{-1} DW. However, no significant differences were found between low and intermediate depths. (Le Lann *et al,* 2012). A similar effect was seen in two possible ecotypes of *S. filipendula* C. AG. sampled in North Carolina near the coast (2-3 m) or an

offshore site (30 m): $1.44 \pm .06$ mg/g^{-1} and $.82 \pm .07$ mg/g^{-1} fucoxanthin, respectively (Peckel & Ramus, 1985).

Too shallow waters can also be detrimental to the alga itself due to ultraviolet radiation. In *Saccharina latissima,* Aprill & Lesser conclude that exposure to visible and UV-R irradiance results in both dynamic and chronic photoinhibition, which in turn cause a decrease in gross primary production in shallow waters (Apprill & Lesser, 2003).

As irradiance and UVR (Ultraviolet radiation) are attenuated by depth they might be a large factor in total fucoxanthin content. Also note that the tropical *Sargassum* species compared to the more northern species in these two studies contain far less fucoxanthin, suggesting temperature can also be a factor.

4.2.2   Irradiance

An increase in irradiance from 20 to 180 µmol photons m^2 s^{-1} decreased total fucoxanthin content in *S. thunbergi* (Jiang et al, 2009). These results are however read solely from an abstract as no access to the article could be obtained, therefor making it unable to look into specifics. No other direct irradiance effect studies on fucoxanthin content in *Sargassaceae* could be found.

The mechanism of lowered fucoxanthin content at higher irradiance levels was also found in several *Laminariacaea.* In *Saccharina latissima,* increasing culturing irradiance from 15 µmol photons m^2 s^{-1} to 150 µmol photons m^2 s^{-1} decreased fucoxanthin content significantly in 5^{0}C water, while no significant differences were found between the low and high light conditions in 17^{0}C water (Machalek *et al*, 1996). In *Laminaria hyperborea*, pigment content was highest at 20 µmol photons m^2 s^{-1}, while significantly lower at both 5 and 100 µmol photons m^2 s^{-1}(Dring, 1986).

As is the case with micro-algae, too much sunlight can actually be detrimental to the photosystem and precautionary reactions in terms of photoprotection lowering fucoxanthin content in favour of more potent photoprotective pigments can be expected. When talking from a culturing standpoint, managing irradiance in the open sea is not as precise as with micro-algae, as the effects of total exposure intensity is affected by planting depth, cloud cover, turbidity and seasonality.

4.2.3   Temperature

In *S. horneri*, dropping to 15^{0}C for 3 months after being cultured at 20^{0}C for 9 months reached a fucoxanthin content of 12.0 ± 0.9 mg/g^{-1} DW, which was significantly different from those that were continuously subjected to 20^{0}C with a content of 3.9 ± 1.2 (Terasaki, 2016). In contrast, the only other laboratory controlled study of *S. thunbergi* actually found an increase of fucoxanthin content due to an increase in temperature from 10^{0}C to 25^{0}C (Yiang et al, 2009), but again no specifics can be provided as this is from the same abstract as mentioned before. For *S. lattisima,* samples cultivated at 17^{0}C showed an overall significantly higher fucoxanthin content than those cultivated at 5^{0}C (Machaelek *et al*, 1996). These however did not receive a similar sudden decrease in temperature but were instead cultivated under constant temperatures. The observation that specimens grown under low

temperatures did show a dependency on irradiance while those grown under higher temperatures did not, suggesting a form of interaction at some point. Taking into account the observations for *S. horneri,* a change in *t*emperature might be functioning as a so-called "zeitgeber" for the circadian clock in these algal species, promoting a change in pigment composition.

No studies on the direct effect of temperature on *U. innatifida* fucoxanthin content could be found.
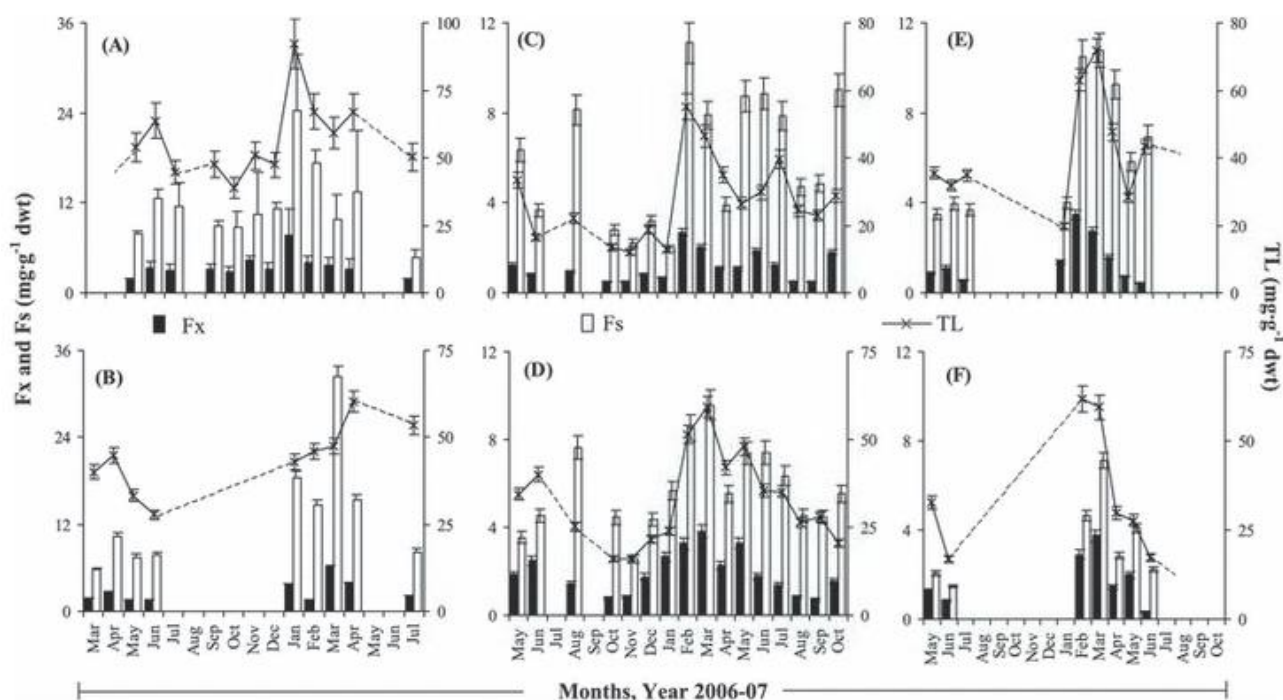
4.2.4   Seasonality

Seasonal variability of fucoxanthin content was investigated in (A) *Sargassum horneri*, (B) *Cystoseira hakodatensis*, (C) *Sargassum fusiforme,* (D*) Sargassum thunbergii*, (E) *Analipus japonicas*, and (F) *Melanosiphon intestinalis* (See fig 8) by Terasaki *et al*. They found that for all species, fucoxanthin content reached its peak in the period between winter and spring (Terasaki *et al*, 2009). This seems to be mostly in accordance with the studies on environmental parameters, as both irradiance and sea temperatures are lower during that period. A high pigment content in *S. muticum* during winter has also been attributed as a mechanism for allowing large growth during spring (Lewey & Gorham, 1984).

The reason as to why Yiang *et al* report higher fucoxanthin content in *S. thunbergi* with an increasing temperature while this study of Terasaki found the opposite is hard to elucidate due to the lack of access to data, but might be caused by interactive effects in the field study having a greater effect on fucoxanthin than temperature alone.

A somewhat similar pattern was found for *S. horneri* with a maximum fucoxanthin content of 4.49±0.6 mg/g^{-1} DW in January 2009 (Nomura *et al*, 2009). These samples were cultivated under identical laboratory conditions and cultivated in the same area, but initially were collected in Nesaki, Hokkaido, Japan (41°45' N, 140°49' E) and Matsushima, Miyagi, Japan (38 23' N, 141 04' E). The highest performer as mentioned was collected in Matsushima; its counterpart from Hokkaido reached its highest value in 3.23 mg/g^{-1} DW in December 2008. These results also indicate that even after a similar acclimation regime, ecotype differences may persist.

Seasonal variability was also found for *Saccharina latissima* in a simulated tidal cycle under laboratory circumstances: spring populations increased their total xantophylls, while winter adapted populations increased their antenna size and fucoxanthin content (Gevaert *et al*, 2002). The total carotenoid fraction of *S. latissima* harvested at Vattenholmen in Sweden also increased significantly at June to October from 0.10±0.01 to 0.39±0.17 mg/g^{-1} wet weight (Vilg *et al*, 2015).

Fig 6 - **Monthly variations in fucoxanthin (Fx, closed bars) and fucosterol (Fs, open bars) contents (mg · g⁻¹ dry weight [dwt]) of (A)** *Sargassum horneri*, **(B)** *Cystoseira hakodatensis*, **(C)** *Sargassum fusiforme*, **(D)** *Sargassum thunbergii*, **(E)** *Analipus japonicas,* **and (F)** *Melanosiphon intestinalis*. **All the values are mean ± SD of a minimum of three samples (period when seaweeds were not collected is indicated by dashed line). TL: total lipid (crosses). Figure by: Terasaki** *et al* **(2009)**

As is the case with the differences of fucoxanthin content in *S. thunbergi*, Machalek's observations that fucoxanthin content increased with temperature in *S. latissima* are also contradictory to those found in temporal studies. The same conclusion as before in terms of interactive effects could apply.

U. innatifida cultured in Southern New Zealand also showed a preference for winter, with fucoxanthin reaching its maximum value of 8.32 ± 0.78 nmol/cm² in July (Dean & Hurd, 2007). In another study, *U. innatifida* also reached a very high maximum value of around 6.2 mg/g⁻¹ DW in July (Fung *et al*, 2013).

4.2.5   Nutrients

Boderskov *et al* (2015) found that a high nutrient availability, while exposed to ambient temperature and light from fall to early winter in Denmark, increased total fucoxanthin content by 21.7-53.7% and in contrast a net loss of 7.1-17.2% at low nutrient availability. In a different study, the enrichment of ambient seawater with 50 µM NO_3 increased total fucoxanthin content from around 1.3 to 3.1 µmol/gr⁻¹ WW.

No studies on any effects of nutrient availability on total carotenoid or fucoxanthin content could be found for species from the *Sargassaceae* could be found. Surprisingly this was also the case for *U.pinnitifada*, even though its extract is used widely in the medical research so

one would assume more information on nutrient dependability would be known.

4.2.6 Fucoxanthin content related to morphology and age

There are also other factors at play when determining fucoxanthin content. In *U.pinnitifada* for example, both female and male gametophytes can exhibit a 5 to 8.2-fold increase (Mori et al, 2004). Also, while both juvenile and adult sporophytes usually exhibit lower fucoxanthin content during spring months, the relative decrease was much less in the juveniles (Campbell *et al*, 1996). In *Saccharina latissima,* age as determined by frond size (3 to 30 cm) in same size tissue disks increased fucoxanthin content from 57.4± 14.1 to 181.5 µmol/m^{-2} fucoxanthin (Hanelt *et al*, 1997).

Yet it is not only age that can be of influence, as morphology itself also does play a part. In *Sargassum confusum,* the most fucoxanthin could be found in the vesicles or leafs (2.9 and 2.8 mg/g^{-1} DW, respectively) while the least was found in the main axis (0.9 mg/g^{-1} DW) (Terasaki *et al,* 2009). However, when looking at *Saccharina japonica*, Kanazawa *et al* (2008) found that fucoxanthin distribution within either the whole leaf, the upper middle and lower parts of the leaf or the root of the plant were all similar, ranging from 17.8 to 19.6 mg/100 gr FW. Interestingly, this was not the case in almost all of the measured fucoxanthin content in the European species described by Shannon and Abu-Ghannan in figure 7, including the closely related *Saccharina latissimi.* In these species, the blade contains the highest amount of fucoxanthin compared to the stipe and the holdfast (Shannon & Abu-Ghannan, 2016). This author is inclined to believe that overall it is to be expected that fucoxanthin content will be more pronounced in the blades than the root-structures due to its function as a photosynthetic pigment. The occurrence of at least some fucoxanthin in the roots however is of interest in the extraction process.

All of the above show that there is still a lot of uncertainty on how fucoxanthin relates to its environment, as most species exhibit strong ecotype differentiation making comparisons difficult. Generally spoken, the traditionally eaten seaweeds seem to be good candidates for fucoxanthin production, when harvested at the end of winter. Additionally, farming gametophytes in similar ways such as micro-algae might also be an option as indicated by the high fucoxanthin reported in the Mori study.

4.3   Extraction

4.3.1   Organic solvents

In 1989, Haugan & Liaaen-Jenssen (1989) came up with a novel way of extracting fucoxanthin from seaweeds. They identified several problems with using fresh seaweeds, such as the large volumes of solvents needed, pre-drying grinding of fresh algae can initiate enzymatic reactions, isomerization and due to the sensitivity of fuxoxanthin to alkali's saponification is also not an option. Their new method involved drying the algae for 2 days

at 40^0 before grinding the dry matter, and subsequently using acetone-methanol solvent and chromatography for the isolation (Haugan & Liaaen-Jenssen, 1989).

A methanol based extraction process using HPLC-ODS more recently reported a fucoxanthin content of 1.01±0.10 mg/g^{-1} DW and 0.73±0.39 mg/g^{-1} DW in *Sargassum duplicatium* and *Sargassum binderi* respectively with an end stage purity of up to 99% (Jaswir *et al*, 2011).

Pretreatment before using organic solvents can increase fucoxanthin content. Washing the algae beforehand is needed to reduce the amount of salt in the final extracts, while shortly preheating and afterwards cutting and freezing the biomass strips preserves or increases fucoxanthin content (Kanazawa *et al,* 2009). By using these techniques and ethanol as a solvent Kanazawa *et al* demonstrated that it is possible to derive a total of 1490 grams of fucoxanthin from 10 tonnes of wet biomass, or more specifically those parts of the biomass that are usually discarded during harvesting as they are considered as waste (Kanazawa *et al*, 2009). This use of discarded waste fractions is probably a very sustainable approach to fucoxanthin production, which will be discussed later on in chapter 5.3 as well.

Using organic solvent techniques however is usually very time consuming and due to the volumes required in macro-algae can also be detrimental to the environment (Kadam *et al*, 2013). One main cause of these effects is the polysaccharide make-up of the cell walls of brown seaweeds. Many of the novel methods are aimed at reducing the required organic solvent by breaking down the polysaccharides before extraction or using newer techniques to increase permeability of the solvent. Some of these methods have also been used as ways of optimizing fucoxanthin extraction in *U.pinnatifida* and species from the S*argassaceae* and *Laminariacea,* which will be briefly discussed*.*

4.3.2   Enzyme-assisted Extraction (EAE)
Naturally occuring enzymes such as carbohdrases and proteases are effective agents in breaking down cell walls under the right environmental conditions (Temperature, pH), as well as being relatively cheap (Kadam *et al*, 2013). In a study done on *U.pinnatifida,* the algal extract was pretreated with alginase lyase enzymes at an optimized temperature of 37 °C, and pH of 6.2 prior to an extraction using dimethyl ether (DME). Results showed an increase of up to 50% in fucoxanthin extraction (Billakanti *et al,* 2012). For *Sargassum horneri*, various carbohydrases and proteases were used as a pretreatment. Results showed that extracts prepared with Alcalase and Viscozyme showed the highest antioxidant activity afterwards (Park *et al*, 2003), indicating that these enzymes are efficient in extracting the carotenoid fraction in *S.horneri.* Relatively few studies have been done looking at carotenoid extraction via EAE, but these results and some on other algal species do support EAE as an environmentally friendly way of increasing efficiency (Wijesinghe & Yeon, 2012). However, availability of enzymes on a larger scale, as well as keeping the bioreactors clean can cause some problems in upscaling this method (Kadam *et al*, 2009).

4.3.3 Pressurized liquid extraction (PLE)

PLE is an extraction method that uses high temperature (50–200 °C) and high pressure (3.5–20 MPa) conditions (see fig 9) to increase the boiling point of a solvent, along with increasing solubility and mass transfer rate (Kadam *et al*, 2009). This method has been investigated for fucoxanthin extraction in *Eisenia bicyclis* also known as "Arame" or "Sea Oak", edible kelp from Japan and South-Korea. Using a statistical method called response surface methodology the study came to the conclusion that at an optimum of 110°C and 90% ethanol (pressure was not found to be a significant factor), a maximum of 0.42 mg/g^{-1} fucoxanthin could be derived immediately from wet biomass (Shang *et al*, 2011).
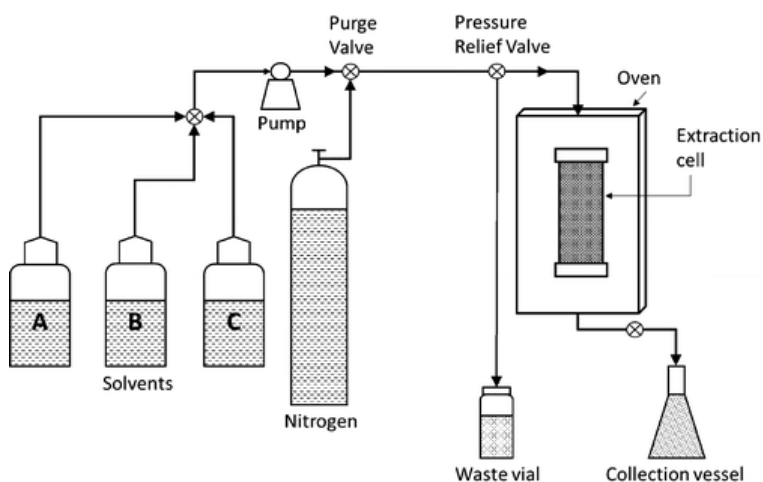
**Fig 7 -** Schematic diagram of pressurized liquid extraction (PLE). Figure by: Kadam *et al*, 2009

Even though temperatures of up to 100^{0}C could potentially be detrimental to the stability of fucoxanthin, no such evidence could be found in the study possibly due to the relatively short exposure time of 5-10 minutes. In fact, shortly heating raw *Saccharina japonica* to a boiling temperature as a pretreatment has been shown to actually increase fucoxanthin extraction, possibly by preventing the enzymatic decomposition of the carotenoid (Kanazawa *et al*, 2008). Concluding, PLE is a very promising technique, due to both a decreased solvent use and its quick running time, but care should be taken when used in a more down-stream method of extraction as the high temperatures could interfere with thermosensitive compounds.

4.3.4 CO$_2$ Supercritical fluid extraction (CO$_2$ SFE)

CO$_2$-SFE is comparable to PLE as it uses temperature and pressure to create a supercritical state of CO$_2$ as a co-solvent so it behaves as a fluid with low surface tension, viscosity and a high diffusivity (Roh *et al*, 2008). An example of the set-up can be found in figure 10. Another distinct advantage is that it requires much lower temperatures than PLE (Kadam *et al*, 2009), thereby possible circumventing the possibility of degeneration of the valued compounds. In a study comparing fucoxanthin CO$_2$-SFE with ethanol as a co-solvent under

various temperatures and pressures in *U.pinnatifida*, optimum extraction efficiency was found at 323K (49.85⁰C) and 200 bar for a total of 0.00753 µg/g^{-1} (Roh *et al*, 2008). This author points at that these are very low values for total content, but little data on the origin and time of collection of the biomass is provided in the study. However, if CO_2-SFE itself was responsible for the low amount is unlikely, as it has shown to be an effective extraction method in both *Saccharina japonica* and *Sargassum horneri.* At operating conditions of 250 bar (higher than the previous study) and 45 ^{0}C (comparable), the fucoxanthin content yields in the two algae were 0.41 ± 0.05 and 0.77±0.07 mg/g^{-1} DW, respectively (Sivagnanam, 2015).

CO_2-SFE seems to be a valid extraction method for fucoxanthin in several species. It is rather quick and for some extractions it can even be used with no organic co-solvents required for an even better environmental footprint (Kadam, 2009). For fucoxanthin however, it was shown that the addition of a co-solvent increases yield significantly (Conde *et al*, 2015). Another downside is the high investment cost of CO_2-SFE (Kadam, 2009).
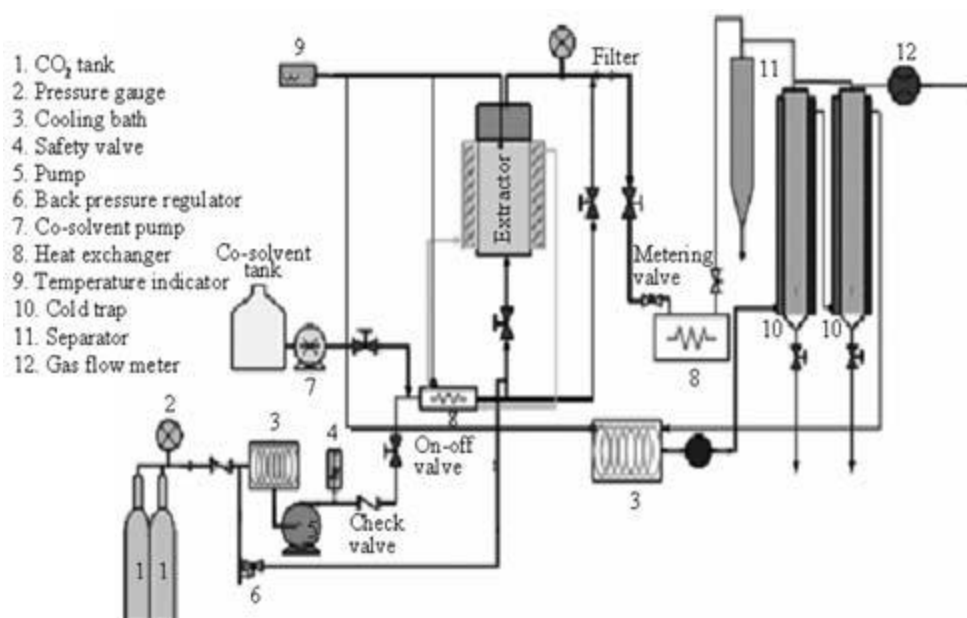
Fig 8 - **Flow diagram of a supercritical fluid extraction system. Figure by: Roh *et al*, 2008**

4.4   Conclusions

It is clear that fucoxanthin content in micro-algae differs wildly amongst species. Different times of harvesting, culturing methods and ecotype all influence the fucoxanthin content, making a list of the better performing species hard. As of now, the traditionally eaten species are probably fine producers of fucoxanthin and searching for the most optimized seaweed while there is an existing infrastructure in place seems futile. Additionally, fucoxanthin content within species can also differ immensely with time, temperature, irradiance and age. Especially irradiance can have a large influence on the yield, mostly when explained in the

context of seasonal variations. Trying to influence fucoxanthin content within macro-algae for large culture practices is therefor an unlikely strategy, as seedlings are usually hung in the ocean for maturation and environmental parameters can no longer be controlled as strictly. Harvesting time is probably the best predictor of optimized fucoxanthin yield. Delivering a stable yield on a timely basis however can as of now not yet be guaranteed. For extraction, traditional organic solvent methods are fine for laboratory determination as the retention rate of both ethanol and methanol is very high. Yet when looking at the industrialization of fucoxanthin production, more environmentally friendly methods such as CO_2-SFE are probably preferred. The realization that fucoxanthin can also be harvested from the usually discarded fractions of already farmed seaweeds such as *Saccharina japonica* (See chapter 4.3.1) is also very promising, as this would eliminate the need for a new infrastructure altogether, if supply could keep up with future demand.

Chapter 5 Discussion

As seen there are many ways of producing fucoxanthin, and in the future also ways of optimizing yields by using different culturing strategies. While the medical research is very promising, much of the evidence seems to be based on non-human experimentation. Even though the functional food market is booming, how many of these products are legitimately aiding in human health remains to be seen as regulations on health claims are, while stringent, also slow in "catching" and evaluating new products. This author is of the opinion that from a moral and probably also investment standpoint, fucoxanthin production should only be scaled up if the supplementation of fucoxanthin is proven to actually be beneficial to human health. A similar sentiment was uttered by Christopher P. F. Marinangeli and Peter J. H. Jones in a review on the functional food and the marketplace:

"Thus, without scientific endeavor, few functional foods and nutraceuticals would probably secure health claims and develop into profitable product lines."   (Mariangelli & Jones, 2013)

For these reasons, this paper also looked more in-depth into the rules and regulation concerning health claims for products.

5.1 Health claims for humans and its scientific backbone

Regulations on health claims differ amongst countries. Generally speaking, governmental agencies make a distinction between "*drugs"* and "*dietary supplements*". Drugs are those substances intended to treat a certain malady such as a headache or heart disease, while dietary supplements are seen as foodstuffs that contain one or more specialized ingredients in certain concentrations intended to be consumed next to the regular meal as a general way of supporting health. Drugs are a highly protected category, whilst dietary supplements can

vary wildly in the permitted claims.

The country or region of origin where the product is sold places certain regulations on how one can make these health claims (Correspondence with dr Oldenbach, 2016). In Japan it is the Food for Specfified Health Uses (FOSHU) administration, in China the Health Food (HF) protocol, in the EU the European Food Safety Authority (EFSA) and in the USA the Food & Safety Administration (FSA) that are the regulators. There can be large differences in these regulations: for example, the HF does not see clinical trials as mandatory but only needed on a per-product basis (Patel *et al*, 2008), while the other 3 do (Patel *et al* 2008, Aggett *et al* 2005). Another marked difference between the FH and the FOSHU is price: FOSHU approval will cost up to $1.500.000,- which is in sharp contrast with the FH at $35.000,- (Patel *et al* , 2008).

This author will focus on the FUSHO, EFSA and FDA as they together comprise most of the functional food market, with an explicit use of the EFSA protocols as they are deemed as the most relevant for this author.

While methodologies vary, for the FOSHU, FDA and EFSA consumer safety is always top priority. As fucoxanthin is mostly safe as mentioned in the safety chapter and has also been administered orally historically in the form of edible seaweeds for a long time there are no indications that fucoxanthin will be damaging to human health. Yet the absence of human clinical trials is something to be considered when talking about safety of nutritional supplements as these are usually in much higher doses than found in naturally occurring seaweeds while addressing the low bioavailability as well. This lack of human trials throughout fucoxanthin research also has consequences further down the chain of proof in health claims.

The second most important criterion is being able to substantiate the claim that the product is *actually* health promoting via supplementation.

While each safety agency has their own terminological distinctions, they do all require scientific proof. In the EFSA this is done via the PASSCLAIM protocol (Asp & Bryngellson, 2008) the FDA only gives recommendations to the industry[4] and the FUSHO uses a standardized method with science evidence as a part of It (Patel *et al* , 2008). What is found in all methodologies is that when it comes to health claims, human studies in a clinically appropriate population are seen as the most important evidence of health promoting benefits, with animal and in vitro studies merely as support. If human studies are hard to do due to many confounding variables, properly researched biomarkers can also sometimes be used (Agget, PJ, 2009). This idea on human trials and/or potential biomarkers was reinforced when at the 26th Hohenheim conference a large international group of nutrition scientists came together and set forth guidelines on grading scientific evidence (Biesalski *et al* , 2011). They came to the same conclusions as the governmental agencies which are summarized in Fig.11, with the addition that the judgement of governmental agencies themselves is also included in the grading system.

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[4] http://www.fda.gov/OHRMS/DOCKETS/98fr/07d-0125-gdl0001.pdf

The EFSA distinguishes between herbs and non-botanicals (e.g. proteins, vitamins, carbohydrates etc.). For most non-botanicals a list has already been made public that can be seen at the KAG/KOAG website, the Dutch joint commission of industry and government that oversees health claims which adheres to the EFSA guidelines. In a correspondence with the KAG/KOAG they mentioned not to be familiar with fucoxanthin (E-Mail KAG/KOAG, 2016). Neither could fucoxanthin be found on their list. Yet this could also mean that the claim was still pending. Sadly, no public access to the FOSHU database could be found on their English language site.

While screening the publicly available database for proposed health claims on foods in the EFSA, no mention was found of fucoxanthin[5]. Thus, on a European level one cannot produce a fucoxanthin supplement that can legally claim health benefits as of yet. But this might change in the future, and being a first-mover in a yet untapped market can be very profitable. One can ask the question then, how likely is it that fucoxanthin will get a certain status in the near future?

In an evaluation done by the author of this paper the referenced articles in one of the largest review papers on fucoxanthin, done by Feng *et al* (2011), were all scored on what kind of study they pertained; be it either in vitro and in vivo animal studies, in vitro human studies, small and large scale human studies and review articles. By comparing this with the evidence grading method as set by the 26th Hohenheim conference (fig 11) an idea on the state of the evidence on Fucoxanthin and its health benefits could be formed. Only studies that claimed a direct health effect of Fucoxanthin and its metabolites fucoxanthinol and Amantherien A were scored, while mechanistic studies of the molecule itself were omitted.
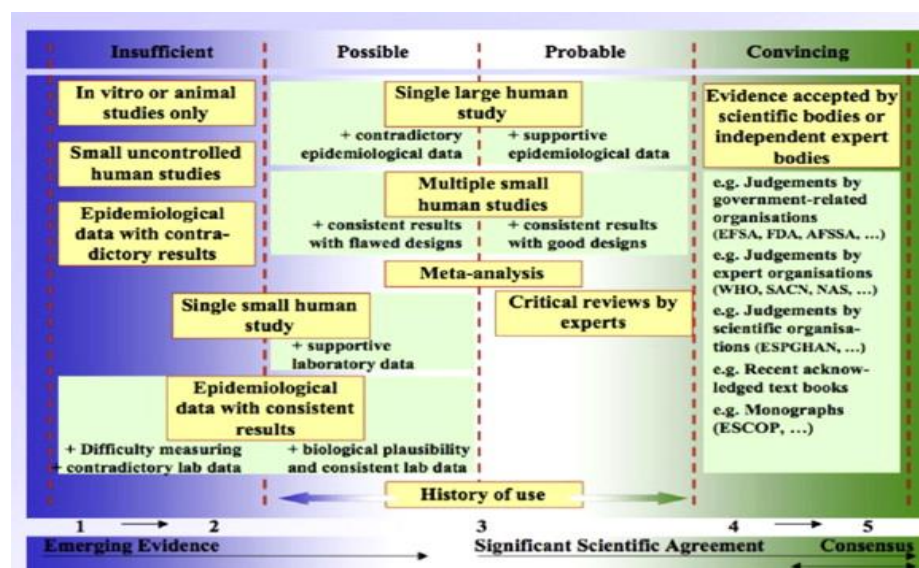
Fig 9 - Grading of the evidence as set by the 26th Hohenheim conference (Biesalski *et al*, 2011)

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[5] http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search (Filter search for "Fucoxanthin")

This was done due to the fact that while the mechanistic effect is important for its functionality, it does not say something on health effects per se and is also not necessary in health claims in the EU (Asp & Bryngelsson, 2008). It should also be noted that not all of the mentioned references were thoroughly analyzed per case for their scientific rigor. The results for this evaluation can be seen in table 3.

Table 3 – **References mentioned in Feng *et al* (2011) scored on their methodology**

	In vitro animal	In vivo animal	In vitro human	Small human	Large human	Review	Total
Total # studies:	18	31	28	2	0	3	82
...in %:	21,95	37,80	34,15	2,44	0,00	3,66	100

The evaluation shows that as of right now most of the evidence that is presented on the health benefits of fucoxanthin can be related back to either in vitro/in vivo animal studies (mice and rats) and in vitro human studies (liver, prostate and adipose tissue cell lines), accounting for a total of 93.9% of the studies. With only 2.44% of the total studies being referenced as human studies by 2011, according to the guidelines set by the Hohenheim conference the effects can at most be judged to be around level 2, or "emerging evidence". This means that as of now Fucoxanthin is probably not yet ready to be labelled as a true functional food supplement. The author of this paper would also like to address that at the time of the Feng review, the second and contradicting human trial on Xanthigen as mentioned in the anti-obesity chapter was not yet performed, while directly disputing the positive effects found in the other small human trials that were mentioned.

Obtaining a better scientific consensus on fucoxanthin in the future will rely heavily on the willingness of researchers and producers to start larger randomized and controlled human studies, with an effective delivery system to increase the bioavailability. One such an example of increasing the uptake of the pigment is done in Asia. Mok *et al* (2016) are working on reinforcing milk with fucoxanthin, thereby combining the pigment with a lipid which has shown to be a more effective delivery method then just fucoxanthin. It should be noted that much of these larger human trials are usually very costly and as such money provides a functional gap in the transference of theory to practice. Initiatives like those with the milk do provide a novel way of letting people easily incorporate a novel product in their daily diet instead of using pills.

Despite the as of yet mostly emerging evidence status, and some doubts on the validity itself of direct human health benefits of fucoxanthin, the structure of the pigment *is* highly related to the other carotenoids commonly associated with anti-oxidative capabilities such as β-carotene , lycopene and vitamin A. But β-carotene, zeaxanthin and lycopenes have also been denied true health-promoting status by the EFSA due to the lack of power of the

scientific evidence[6]. β-carotene is known to be a precursor to vitamin A and despite it well-established anti-oxidative properties, β-carotene supplementation in a large human controlled study in a smoking population showed a possibility of actually increasing the chance of lung cancer mortality in certain situations (Virtamo *et al* , 2013). In fact, the entire efficacy of antioxidants as always being health promoting has been put into question, as antioxidants could possibly prevent the development of the bodies' own stress response to oxidative stress (Ristow & Zarse, 2010). These kinds of studies are indicative of the problems that arise when one extrapolates small scale studies to human trials; the environment and dose the nutrient end up in are just not the same as a petri-dish. However, In her extensive book "Carotenoids in nature biosynthesis: regulation and function", in which dr. Claudia Strange sums up current knowledge on carotenoids, she comes to the following conclusion regarding carotenoids, weight and the human intervention studies done::

"In summary, the literature reviewed herein supports a role of specific carotenoids and carotenoid derivatives in the prevention of excess adiposity, and suggests that carotenoids requirements may be dependent on body composition, among other factors."

(Strange, 2016)

So while fucoxanthin its relation to other carotenoids might give some merit to the possibility of health promoting claims in the absence of controlled human trials, this evidence remains emergent and promising at best. However, the market in carotenoids is booming despite this apparent absence of proof. Thus the question also becomes, does one want to be a producer that navigates the borders of truth for profit? "No harm, no foul" seems to be a risky position to take, taking into account the findings of Virtamo *et al*.

5.2 Current market: Xanthigen and its competitors

Several supplement powders already exist in today's market despite the as of yet mostly unsubstantiated health claims. Not surprisingly, Xanthigen, the one mentioned in the Abidov study is one of the most widely available supplements. The Spanish producer of supplements "Polinat" has recently obtained the patent for Xanthigen's combination of pomegranate seed oil and fucoxanthin from seaweed extract[7]. Additionally, "Omega-Pharma", a large consumer health corporation also owning "Davitamon", supplies "Xanthigen" from a Belgian website[8]. Xanthigen can apparently be sold by other vendors as well, as Polinat does not seem to do so directly; prices range from $64.99,- to an astonishing $279,- dollars for 90 pills.
Interestingly, Xanthigen does not use "Fucoxanthin" as its label for marketing, but only uses

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[6] http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search (Searches for "carotene", "Zeaxanthin", lycopenes)

[7] http://www.plthealth.com/news/articles/xanthigen%C2%AE-now-patent-protected

[8] http://xanthigen.be/nl/

the moniker "brown sea-algae supplement". To this author it seems like the manufacturers are trying to circumvent the use of the term fucoxanthin.

But Xanthigen is not the only fucoxanthin supplement that can be found online. In a short query on the internet this author found several more producers who did directly mention fucoxanthin. Websites and packaging usually refer back to the Abidov study to claim an increase in metabolic resting rate, even promising up to 400 Kcal's loss daily after extended use in all supplements.

An oversight of the official Xanthigen and 3 other producers as well as the amount of fucoxanthin per pill and cost per pill as calculated from the labels found is given in table 4. For Xanthigen pricing, the "biggest hit" found on the Amazon marketplace was used.

Table 4 - Several providers of fucoxanthin supplements, all with the addition of green tea and other natural extracts. Values are per capsule and calculated from the content statements by the providers. Reference sites can be found in the appendix.

Company	Name	Fucoxanthin content	Other extract content	Fucoxanthin (%)	Cost per capsule	Serving size	Reference
PoliNat Inc	"Xanthigen"	2,4 mg	597,3 mg	0,400	€0,7355,-	1	1
Best Naturals	"Fucoxanthin with fucoplast"	1.665 mg*	500 mg	0,332	€0,0858,-	3	2
Newton-Everett Biotech	"Xanthadrene"	5 mg	370 mg	1,176	€0,4982,-	1	3
BRI Nutrition	"Fucoxanthin maximum strength"	5 mg	283 mg	1,736	€0,1986,-	1	4

Fucoxanthin content per serving size is usually amongst 2.5-5 mg, the same amount reported in the Abidov study. Much of the price of the supplements is probably determined by the other ingredients and marketing as fucoxanthin itself is at most 1.7% of the total capsule. In some supplements, caffeine is added by natural means as well. These are all confounding variables, making the true efficacy of fucoxanthin within the supplements unclear. This is also reflected in user reviews, as these vary greatly from no effect to very good effects. While user reviews are no evidence per se, they do indicate the same doubts on the efficacy of fucoxanthin supplements from a customer perspective. It is also worth noting that all current fucoxanthin supplements are derived from macro-algae.

These are by far not the only supplements on the market, but they are the ones most pronounced on the large internet seller Amazon. Many other shady sites offer similar fuxoxanthin supplements, usually claiming even more health benefits then the "regulars" mentioned above.

Interestingly, while the other providers all state that the production is in the USA under FDA

law, hidden away in the bottom of the marketpage or on their labels they do have a legal disclaimer concerning the health claims:

"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."

In a recent article of The Independent, a spokesman for the American Food & Trade Commission had the following to say with regards to new legislature in the United States concerning health claims made by homeopathic medicine:

"The FTC said that a homeopathic drug claim that is not substantiated by competent and reliable scientific evidence "might not be deceptive if the advertisement or label where it appears effectively communicates that: 1) there is no scientific evidence that the product works; and 2) the product's claims are based only on theories of homeopathy from the 1700s that are not accepted by most modern medical experts."

So it is possible, at least in the US, to claim without proper evidence, as long as one clarifies that there is no scientific evidence. The use of the disclaimer alongside the citations of at least some scientific studies is apparently a legal construction. This is somewhat similar to the EFSA regulations in which certain claims are allowed as long as the evaluation by authorities is on hold and one adheres strictly to the wording of claims that are allowed pertaining to that particular foodstuff.

Concluding on the current market situation, there is a wide range of different products and prices available, and a lot of conflicting information for consumers as well. Many of the purported health claims are based on the Abidov study, and the largest producer Xanthigen has shied away from using "Fucoxanthin" directly in favour of "Seaweed extract". Many more manufacturers seem more than willing to exacerbate their health claims in selling their product. As a consequence, even when fucoxanthin manages to become validated by governmental bodies in the future, differentiating a new product with legitimate health claims would still be very hard as consumers have been misled or let down by its efficacy in the meantime. It may have even have had its "hype" moment already, as a brown seaweed extract with the specific mention of fucoxanthin was already praised in the American TV-Show "Dr Oz". And with 39% of all his claims being unsubstantiated (Korownyk, 2013), this can hardly be seen as positive marketing for fucoxanthin if results on the user end are also low.

5.3 Conclusions

Concerning future production, as of right now, this author is not convinced that culturing micro-algae specifically for fucoxanthin production is worthwhile. There are numerous hurdles still to be overcome in micro-algal culturing on a larger scale such as efficiently controlling CO_2 levels, nutrients and temperature along with the high investment and operating costs (Murray et al, 2013). Not until more evidence on the efficacy of fucoxanthin as a true functional food is presented, finding investors will be difficult. The possible co-production of fucoxanthin and lipids in a controlled manner for further downstream-processing might be of a higher interest and gives micro-algal culturing an advantage over macro-algal culturing, but this is still in its infancy. As such fucoxanthin production from micro-algae will probably stay in the laboratory in the foreseeable future.

Even though the fucoxanthin content in macro-algae is much harder to control, the fact remains that there is a current infrastructure in place. The study done using waste parts of *Saccharina japonica* in particular is promising. According to the United Nations, over 1.000.000 tons (WW) of seaweeds are harvested each year (UN, 2016), and in Japan alone around 79,000 tons are discarded due to thinning (Kanazawa, 2009). Kanazawa calculated that out of 10 tons of discarded fractions, 1490 grams of fucoxanthin could be harvested. Keeping in mind that the reported fucoxanthin values were very high in this study and might be lower in actuality, extrapolating those figures to the supplement "Xanthigen" with its 216 mg fucoxanthin per package (2.6 mg per capsule x 90 capsules), 6.898.1 pots of "Xanthigen" could be produced from 10 tons of the discarded fractions from seaweed harvesting alone. Concerning there is even much more of this kind of "waste" material available, this should be more than sufficient in the foreseeable future as a means of production of new supplements or products that can then be tested in humans. Additionally, this method is also much more environmentally friendly and cost effective than running several microbiological reactors or culturing macro-algae specifically for fucoxanthin production, thereby harvesting more wild seaweeds or creating more sea farms. In the end, only when fucoxanthin will be used as a medicine directly, purity and reliability of production might become important enough to merit the more costly micro-algal approach.

As a whole, fucoxanthin does deserve the scientific attention it is getting in the medical field as there are many promising effects that the pigment is able to accomplish. However, this is not yet enough evidence to merit specific production on a larger scale.
Using waste-streams of discarded macro-algae fractions as a means of production can suffice to support human trials to elucidate whether or not fucoxanthin is truly helpful from a nutritional standpoint.
Distinctions should also be made between "pure" fucoxanthin supplements and fucoxanthin content as a percentage of regular algal extract to discern whether fucoxanthin on its own works better or worse than when in combination with its original source as well. But in the

meantime, overestimating the effect of fucoxanthin will only create a hype that will be long gone before true production can start.

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Appendix

EFSA information and regulations regarding functional foods:

1. Basic information: https://www.efsa.europa.eu/en/topics/topic/nutrition
2. Regulatory files: http://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32006R1924

Reference list of table 3 "Comparisons between supplements":
1: http://pubs.acs.org/doi/full/10.1021/jf204862d (Fucoxanthin content calculated from article as "xanthigen" was used)
2:
https://www.amazon.com/gp/product/B006QSZJ3I/ref=s9_simh_hd_bw_bFnIn_p121_d0_i1?pf_rd_m=ATVPDKIKX0DER&pf_rd_s=merchandised-search-4&pf_rd_r=ZK9GT4W6ESMTGT0GPG5G&pf_rd_t=101&pf_rd_p=b82f3e4e-77c4-593d-b28e-a3135efe5502&pf_rd_i=3764441

3:
https://www.biovea.net/nl_nl/product_detail.aspx?PID=7526&TI=GGLNLNL&C=N&gclid=CJnqxs3zltACFcW4GwodXFkFxg

4: https://www.amazon.com/BRI-Nutrition-Fucoxanthin-Strength-Capsules/dp/B00LLLRUSW