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Enzymatic Extraction of Fucoxanthin from Brown Seaweeds

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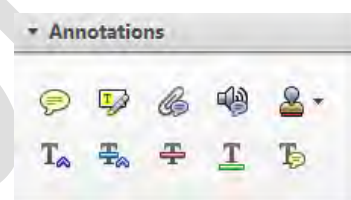


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Original article

Enzymatic extraction of fucoxanthin from brown seaweedsEmer Shannon  & Nissreen Abu-Ghannam*

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Summary Brown seaweeds contain a number of bioactive compounds. The xanthophyll, fucoxanthin, has *in vivo* efficacy against disorders such as type 2 diabetes, obesity and cancer. Organic solvents are traditionally employed to extract fucoxanthin, but carry a toxic chemical and environmental burden. The aim of this study was to optimise a fucoxanthin extraction method using enzymes, water, low-temperature dehydration and mechanical blending, to produce yields comparable to those achieved with an organic solvent (acetone). Response surface methodology was applied, using *Fucus vesiculosus* as a model species. A fucoxanthin yield of 0.657 mg g⁻¹ (dry mass) was obtained from *F. vesiculosus* blade using the enzymatic method, equivalent to 94% of the acetone-extracted yield. Optimum extraction parameters were determined to be enzyme-to-water ratio 0.52%, seaweed-to-water ratio 5.37% and enzyme incubation time 3.05 h. These findings may be applied to the development of value-added nutraceutical products from seaweed.

Keywords Brown seaweed bioactives, enzymatic extraction, extraction yield optimisation, fucoxanthin, green chemistry, liquid chromatography coupled with mass spectrometry.

Introduction

Fucoxanthin is a photosynthetic xanthophyll carotenoid found predominantly in brown macroalgal seaweeds (Phaeophyceae) and in microalgal diatoms (Bacillariophyceae) (Durnford, 2003). It also occurs at lower concentrations in golden algae (Chrysophyceae) and Raphidophyceae (Roy *et al.*, 2011; Larkum *et al.*, 2012). Fucoxanthin has many applications in human health as an inhibitor of tumour activity (Hussain *et al.*, 2016; Mei *et al.*, 2017), bacteria (Shannon & Abu-Ghannam, 2016), type 2 diabetes (Miyashita *et al.*, 2011), obesity (Abidov *et al.*, 2010; D'Orazio *et al.*, 2012), oxidative stress (Kong *et al.*, 2016), metabolic syndrome (Nishikawa *et al.*, 2012), Alzheimer's disease (Lin *et al.*, 2016) and UV light-induced damage (Matsui *et al.*, 2016). However, the majority of brown seaweed biomass harvested globally each year is used for animal feed, fertiliser, biofuel production or low-value human food products (Loureiro *et al.*, 2015; Wells *et al.*, 2017). An efficient, green chemical fucoxanthin extraction method may be useful in the

development of value-added nutraceutical products from brown seaweed biomass. The extraction of fucoxanthin is traditionally achieved with organic solvents such as acetone, hexane, ethanol, dimethyl sulphoxide or methanol which are yield effective but result in the production of chemical waste as environmental and economic burdens (Kerton & Marriott, 2013; Jose & Archanaa, 2017). The cell wall of brown seaweeds is composed primarily of cellulose (repeating units of β (1 \rightarrow 4)-linked D-glucose) and alginate (repeating units of two epimers β -(1 \rightarrow 4)-D-mannuronate (M) and α -(1 \rightarrow 4)-L-guluronate) which may be degraded with various enzymes such as cellulases or alginate lyase (Deniaud-Bouët *et al.*, 2014; Manns *et al.*, 2016). Enzymolysis, that is the hydrolysis of cell wall polysaccharides with enzymes, coupled with low-temperature drying, and mechanical blending has the potential to produce fucoxanthin yields comparable to those obtained with organic solvents by releasing pigment-containing thylakoids, while obviating the need for chemical waste disposal. Temperature, incubation time, pH and ratio of substrate to enzyme are important parameters to be considered.

The aim of this study was to maximise the solid-liquid extraction yield of fucoxanthin from brown seaweeds using a prolifically available variety, *Fucus vesiculosus*, as a model species. A pretreatment of low-temperature oven drying and mechanical blending was

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Chemical compounds studied in this article: Fucoxanthin (PubChem CID: 5281239); Viscozyme (PubChem SID: 318693935); ammonium acetate (PubChem CID: 517165); sodium acetate trihydrate (PubChem CID: 23665404).

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1 followed by enzymatic extraction. Response surface
2 methodology (RSM) was applied using three extrac-
3 tion variables: enzyme-to-water ratio, seaweed-to-water
4 ratio and enzyme incubation time. Optimised
5 responses were applied to the holdfast, stipe and blade
6 of nine other commercially available and common
7 brown seaweeds. Preparative thin-layer chromatogra-
8 phy (P-TLC) was used to isolate fucoxanthin in the
9 crude extract before characterisation and quantifica-
10 tion by high-performance liquid chromatography cou-
11 pled with mass spectrometry (LC-MS). Fucoxanthin
12 yields were compared to those previously achieved
13 with the same ten species and harvest batch using ace-
14 tone extraction (Shannon & Abu-Ghannam, 2017).

15 Materials and methods

16 Chemicals

17 α -Amylase (from *Aspergillus oryzae* ATCC 14156) was
18 purchased from Megazyme (Ireland). Viscozyme (from
19 *Aspergillus aculeatus* ATCC 36411), Protease (from
20 *Bacillus licheniformis* ATCC 6346), and Celluclast
21 (from *Trichoderma reesei* ATCC 26921) were pur-
22 chased from Novozyme (Ireland). Ammonium acetate
23 was purchased from BDH Laboratory (UK), sodium
24 acetate trihydrate from Scharlau (Spain) and LC-MS-
25 grade ethanol, methanol and fucoxanthin standard
26 (all-*trans*-fucoxanthin) from Sigma-Aldrich (Ireland).

27 Samples

28 Ten common species of Irish brown seaweeds were
29 selected for the study. *Alaria esculenta*, *F. vesiculosus*,
30 *Laminaria digitata*, *Saccharina latissima*, *Ascophyllum*
31 *nodosum*, *Laminaria hyperborea*, *Himanthalia elongata*,
32 *Fucus serratus*, *Saccorhiza polyschides* and *Pelvetia*
33 *canaliculata* were purchased from Quality Sea Vegeta-
34 bles, Burton Port, Co. Donegal, Rep. of Ireland.
35 Authentication of species was provided by the sup-
36 plier. Samples were harvested in the mid of July 2015
37 from the north-western coast of Ireland (54.9823°N,
38 8.4343°W) at mean monthly air and seawater tempera-
39 tures of 14.5 °C.

40 Methods

41 Sample preparation and dehydration

42 Fresh, whole seaweeds were placed in a colander and
43 rinsed with cold tap water to remove epiphytes and
44 debris and then patted dry. Each thallus was separated
45 into holdfast, stipe and blade and then sliced into 2-
46 cm pieces. Samples were placed in a single layer on a
47 drying tray in an incubator oven (Innova 42; Mason
48 Technology, Ireland) with an air velocity of
49 $2.0 \pm 0.1 \text{ ms}^{-1}$ for 12 h at 40 °C in darkness.

Dehydration temperature and time were based on a
modified version of previously optimised parameters
for Irish brown seaweeds in terms of phytochemical
preservation (Gupta *et al.*, 2011).

49 Mechanical blending

Dehydrated blade, stipe and holdfast were ground sepa-
rately using a laboratory-scale blender (Salter EK2002,
1000 Watt, 25 000 RPM, 1 L capacity) for 20 s to a par-
ticle size of $1.0 \pm 0.2 \text{ mm}$ (VWR Digital calliper). Ini-
tial trials using blending times from 5 to 90 s found no
significant ($P \geq 0.05$) increase in fucoxanthin extraction
yields or reduction in particle size after 20 s.

50 Initial enzyme trials and RSM range determination

Trials with Viscozyme, α -amylase, Protease and Cellu-
clast determined Viscozyme to have significantly
greater extraction efficiency for fucoxanthin compared
to all other enzymes, and therefore, it was selected for
this study. Pre-RSM trials for three extraction variables
were carried out in the following ranges: seaweed-to-
water ratio 0.1% to 50.0%; enzyme-to-water ratio
0.1% to 50.0%; and enzyme incubation time 0.1 to
24.0 h. Quantification by HPLC determined that no
statistically significant increase in *F. vesiculosus* fucox-
anthin content occurred outside the following ranges:
seaweed-to-water ratio 0.5% to 5.0%; enzyme-to-water
ratio 0.5% to 5.0%; and enzyme incubation time 0.5 to
12.0 h. These ranges were therefore used as the upper
and lower limits for the RSM design of experiment.

51 Response surface methodology design and analysis

A 2^3 + star central composite design was applied using
Statgraphics Centurion XV (StatPoint Technologies
Inc., USA). Sixteen variable combinations in experi-
mental runs were generated by the design. The effects
of unexpected variability in the observed responses
were minimised by randomisation. Experimental data
generated from the design were fitted to a second-
order polynomial regression model (eqn 1) where Y is
the predicted response (fucoxanthin), and X_1 (enzyme-
to-water ratio), X_2 (seaweed-to-water ratio), and X_3
(enzyme incubation time) are the coded values of the
independent variables.

$$Y = B_0 + (B_1X_1) + (B_2X_2) + (B_3X_3) + (B_{11}X_1^2) + (B_{22}X_2^2) + (B_{33}X_3^2) + (B_{12}X_1X_2) + (B_{13}X_1X_3) + (B_{23}X_2X_3) \quad (1)$$

Statistical interpretation of RSM experimental data
generated by the model was evaluated by the analysis
of variance (ANOVA) and coefficient of determination,
 R^2 , measuring goodness of fit of the regression model.
Significance of the model and data was determined at
the 95.0% confidence level ($\alpha = 0.05$).

Enzymatic extraction procedure

Sodium acetate buffer (4 mL, 0.1 M, pH 4.5) was added to a flask. Dehydrated, ground seaweed was combined with ddH₂O in ratios from 0.5% to 5.0% to a total volume of 16 mL and added to the buffer. The flask was covered with Parafilm and incubated (Innova 42; Mason Technology) at 50 °C, 100 RPM for 10 min. Once the flask contents had reached 50 °C, liquid Viscozyme (100 fungal β -glucanase units g⁻¹) was added in ratios of 0.5% to 5.0% of the total solvent volume. Incubation was carried out at 50 °C, 100 RPM from 0.5 to 12.0 h. The reaction was halted by plunging the flask into a water bath (80 °C, 5 min). The flask was then cooled on ice (5 min). Flask contents were transferred to Nalgene tubes and centrifuged (12 min, 12000 \times g, 4 °C) (Sigma 2K15; Mason Technology). The supernatant was retained. The pellet was washed and centrifuged eight times (20 mL). Pooled supernatant was reduced by evaporation (Laborota 1002 Heidolph rotary evaporator, Germany) at 30 °C to 5 mL. Extracts were frozen to -80 °C and then lyophilised to a powder (24 h, 0.02 mbar, -52 °C) (Labconco freeze-drier, USA). The lyophilised extract was stored (-20 °C, in darkness) until HPLC analysis.

Quantification of fucoxanthin

Preparative thin-layer chromatography

Preparative thin-layer chromatography was used to isolate fucoxanthin from the crude enzymatic seaweed extract according to a protocol optimised in this laboratory by Rajauria & Abu-Ghannam (2013). Lyophilised, crude enzymatic *F. vesiculosus* extract was dissolved in ethanol and pipetted onto the base of a TLC silica plate precoated with fluorescent indicator (Macherey-Nagel ALUGRAM[®] SIL G/UV₂₅₄, 0.20 mm, 20 \times 20 cm, Germany). A solution of commercial fucoxanthin standard was pipetted alongside the crude extract as a reference. The plate was left in darkness (30 min, 25 °C) to dry. Mobile phase (chloroform:diethyl ether:ethanoic acid:n-hexane (10:3:1:1, v/v/v/v)) was added to a glass TLC developing chamber with lid. The silica plate was developed in the chamber (30 min, 25 °C) in darkness and then removed and allowed to dry (30 min, 25 °C). An orange band with an R_f corresponding to that of the fucoxanthin standard was visible for the enzymatic *F. vesiculosus* extract. The bands were collected separately by cutting with a scalpel and dissolving the silica fragments in methanol (20 mL). The solutions were vortexed (10 min, 100 RPM) in Nalgene tubes, then centrifuged (15 min, 10 000 \times g) and washed twice with fresh methanol (20 mL) to remove the fucoxanthin from the silica fragments. Pooled supernatants were syringe-filtered (Sigma-Aldrich Millex Durapore

PVDF 0.22- μ m pore) and lyophilised to a powder (24 h, 0.02 mbar, -52 °C) (Labconco freeze-drier, USA).

Preparation of P-TLC seaweed extract stock solutions

Stock solutions of seaweed extracts were prepared for HPLC and LC-MS analysis by dissolving lyophilised P-TLC enzymatic extract of seaweed in LC-MS-grade ethanol.

HPLC-guided identification

Initial identification of fucoxanthin in the lyophilised P-TLC enzymatic *F. vesiculosus* extract band corresponding with the fucoxanthin standard was carried out according to a method developed by Sugawara *et al.* (2002). Separation was achieved with HPLC (Alliance-Waters e2695 Separations Module, 400 atm pressure, at 4 °C), using a C18 reverse-phase column (Phenomenex, Luna 4.6 mm \times 250 mm, 5 μ m particle size) and a UV photodiode array detector (Waters 2998). The mobile phase was acetonitrile:methanol:water (75:15:10, v/v/v) containing ammonium acetate (0.1%). Mobile phases were filtered (Merck Millipore HVLP 0.45 μ m) and sonicated (Branson 5510 Ultrasonic Cleaner). A 25-min isocratic programme was used with a flow rate of 1.0 mL min⁻¹, injection volume 10 μ L and 25 °C constant column temperature. Detection was performed at 449 nm. Separation of fucoxanthin was achieved at 14.937 min. The concentration of fucoxanthin in the seaweed extracts was extrapolated from commercial fucoxanthin standard solutions.

LC-MS characterisation of fucoxanthin

The HPLC-generated peaks that corresponded with commercial fucoxanthin standards were further characterised by their positive ions. Molecular characterisation of fucoxanthin in the seaweed extracts was carried out according to a protocol optimised in this laboratory for fucoxanthin by Rajauria *et al.* (2017) using LC-MS (Agilent Technologies 6410 Triple Quad LC-MS, with 1200 series LC and MassHunter Workstation software, USA). The liquid chromatographic conditions were as described in the preceding section (*HPLC guided identification*). Operating conditions for mass spectrometry were as follows: positive ionisation mode, fragmentor voltage 120 V, capillary voltage 3.5 kV and collision energy 10 eV. The nebulising and drying gas used was nitrogen, at a pressure of 50 psi, flow rate 10 L min⁻¹ and drying temperature 350 °C, with a capillary current of 35 nA. Mass spectral data were recorded in the mass range of m/z 100–1000 on ESI interface mode.

Moisture content determination

Raw, and after incubator-dried, moisture contents were determined by drying control samples in an oven (Binder, Germany) at 105 °C until a constant mass was obtained.

Statistical analysis

All experiments were conducted in triplicate ($n = 3$) and replicated at least twice. Results are expressed as mean values \pm standard deviation. All statistical analyses and data were fitted to models using Statgraphics Centurion XV. The coefficient of determination (R^2) and mean square error (MSE) were used as criteria for adequacy of fit. Multiple range tests were used to determine least significant differences between samples at the 95.0% confidence level ($\alpha = 0.05$).

Results and discussion

Mathematical modelling and RSM optimisation of enzymatic extraction

A regression equation fitted to the experimental data is shown in eqn 2, where Y is the predicted response (fucoxanthin), and X_1 (enzyme-to-water ratio), X_2 (seaweed-to-water ratio), and X_3 (enzyme incubation time) are the coded values of the independent variables.

$$Y = 0.504431 - (0.0474112 * X_1) + (0.0143882 * X_2) + (0.0289971 * X_3) + (0.00332511 * X_1^2) - (0.000958862 * X_1 * X_2) + (0.00202725 * X_1 * X_3) + (0.0023388 * X_2^2) - (0.0015828 * X_2 * X_3) - (0.00286578 * X_3^2) \quad (2)$$

All three variable effects were found to be significant. Enzyme-to-water ratio was found to have the most significant effect ($P = 0.0057$) on fucoxanthin yield, followed by seaweed-to-water ratio ($P = 0.0192$) and enzyme incubation time ($P = 0.0340$). The combination of factorial levels required to maximise fucoxanthin yield from *F. vesiculosus* blade was determined to be enzyme-to-water ratio 0.52%, seaweed-to-water ratio 5.37% and enzyme incubation time 3.05 h. An optimum value of 0.706 mg g⁻¹ (dry mass of *F. vesiculosus* blade enzymatic extract) was predicted. The greatest observed value obtained was 0.657 mg g⁻¹. This is 93.06% of 0.706 mg g⁻¹ which is in good agreement with the predicted value. The R-squared statistic (adjusted for degrees of freedom) indicated that the model as fitted explained 91.16% of the variability in fucoxanthin yield. The standard error of the estimate showed the standard deviation

of the residuals to be 0.031, with the average value of the residuals expressed as a mean absolute error of 0.014.

A response surface plot (Fig. 1) was constructed according to the modelled experimental data. The effects of two variables (enzyme-to-solvent ratio and seaweed-to-solvent ratio) on fucoxanthin yield are depicted in a three-dimensional surface plot, while the third variable (time) was kept at 6.25 h, the midpoint of the range.

Fucoxanthin quantification of enzymatic extract by HPLC

Using Viscozyme, a fucoxanthin yield of 0.657 mg g⁻¹ (dry mass of *F. vesiculosus* blade) was achieved using the enzymatic method. This was equivalent to 93.99% of the previously optimised organic solvent (acetone) extraction yield 0.699 mg g⁻¹ (dry mass of *F. vesiculosus* blade) (Shannon & Abu-Ghannam, 2017). RSM was an effective technique for optimising enzymatic extraction conditions as the fitted model explained 91.16% of the variability in fucoxanthin yield. Optimised responses applied to *F. vesiculosus* stipe and holdfast produced fucoxanthin contents equivalent to 90.43% and 88.02%, respectively, of their acetone extracted yields. The comparative Viscozyme-extracted fucoxanthin content of the blade, stipe and holdfast of the ten seaweeds under study is presented in Fig. 2, as quantified by HPLC. The previously optimised acetone extraction yield for each species and thallus region is included in the graph for comparison. Values are the mean of three replicates \pm standard deviation. Letters denote least significant difference between columns ($P \leq 0.05$).

In the other nine seaweeds under study, fucoxanthin content of the enzymatic extracts ranged from

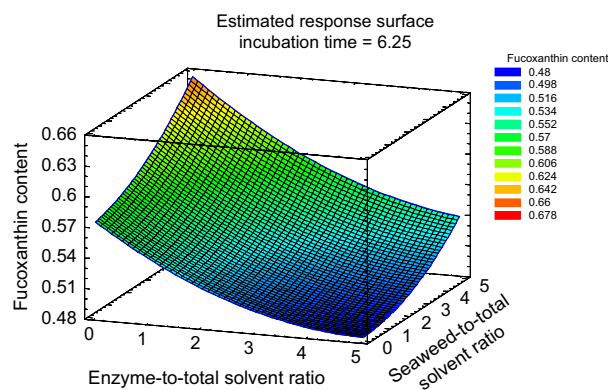


Figure 1 Multiple response surface optimisation plot showing the effects of enzyme and solvent ratios on fucoxanthin yield.

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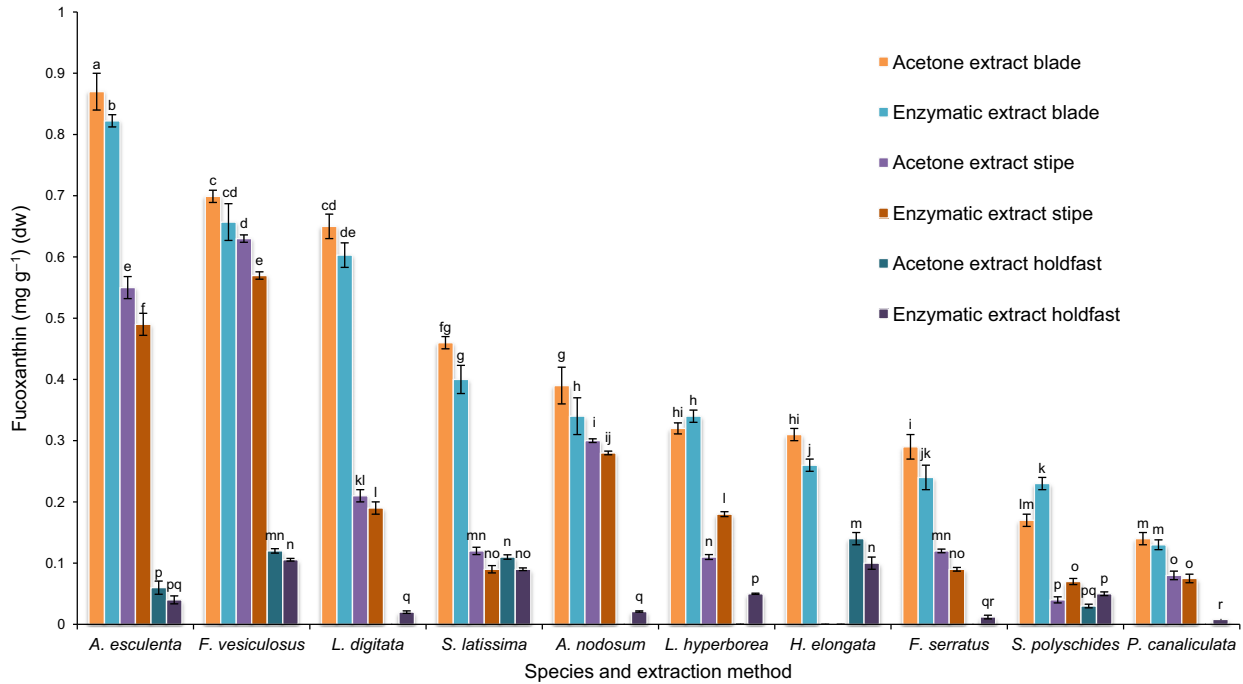


Figure 2 Comparative fucoxanthin content per species, thallus region and method of extraction as quantified by HPLC.

0.008 mg g⁻¹ in *P. canaliculata* holdfast to 0.822 mg g⁻¹ in *A. esculenta* blade. As expected, the greatest fucoxanthin content was found in the blade of all ten seaweeds, followed by the stipe, and least in holdfast. This is due to the photosynthetic function of the blade and the primarily structural functions of the stipe and holdfast, which contain lower concentrations of light-harvesting pigments.

On average, among all ten species, the optimised Viscozyme extraction yields of fucoxanthin were equivalent to 93.56% (blade), 94.19% (stipe) and 107.96% (holdfast) of their acetone extracted yields. In the case of eight of the ten species, the enzymatic fucoxanthin yield was slightly lower in all thallus regions. However, two of the species, *L. hyperborea* and *S. polyschides*, had enzymatic yields significantly greater than those achieved by acetone extraction. In addition, fucoxanthin was extracted from the holdfasts of five species (*L. digitata*, *A. nodosum*, *L. hyperborea*, *F. serratus* and *P. canaliculata*) in which none was extracted using acetone. This increase is most likely due to the physically impervious nature of some species and thallus regions and the ability of enzymes to hydrolyse bonds that hold cellulose-encased cell components within. *L. hyperborea* and *S. polyschides* were the two most resistant seaweeds in terms of physical texture and experienced the greatest increase in fucoxanthin extraction yield.

The suitability of Viscozyme for cell wall degradation is due to its composition of cellulase, arabanase, β-glucanase, hemicellulase and xylanase which catalyse the cleavage of bonds between β(1→4)-linked D-glucose units (cellulose) while also reducing the viscosity of the reaction mixture (Park *et al.*, 2004; Gupta, 2016).

Fucoxanthin is a carotenoid. Carotenoids are divided into two classes: carotenes and xanthophylls. Carotenes, for example lycopene, are composed of carbon and hydrogen and are non-polar molecules. Xanthophylls, such as fucoxanthin, are composed of carbon, hydrogen and oxygen. The presence of oxygen in xanthophylls makes them more polar than carotenes. Fucoxanthin has six oxygen atoms, within hydroxyl and epoxy groups, allowing partial solubility with polar solvents such as water (Landrum, 2009). The acidic water (pH 4.5) used with Viscozyme in the present study may also have enhanced extraction. Acidified water has previously been used to enhance the extraction of bioactive compounds from brown seaweed. Charoensiddhi *et al.*, 2016b used pH-adjusted water (pH 4.5) to extract laminarin, fucoidan and other polysaccharides from *Ecklonia radiata*. There was a significant increase in total polysaccharide yield at pH 4.5, compared to that obtained at pH 6–8. Similarly, with non-algal biomass, a reduction in pH has been shown to improve the extraction of compounds

1 such as flavonoids and phenols from plants. For exam-
 2 ple, Ingrid & Santoso (2016) and Chumsri *et al.*
 3 (2008) found that acidified water ($\text{pH} \leq 3.0$) ruptured
 4 vacuoles in plant cell walls, allowing cell-bound com-
 5 pounds to be released. As vacuoles also occur in the
 6 cell wall of brown seaweeds (Hurd *et al.*, 2014), the
 7 acidic solvent used in the present study may have had
 8 a similar effect. It is also probable that the pretreat-
 9 ment of dehydration and mechanical blending aided in
 10 releasing fucoxanthin from within chloroplasts due to
 11 physical rupturing of the algal cell walls.

12 Enzymes have been used in the extraction of useful
 13 bioactives from algae. Wang *et al.* (2010) used Uma-
 14 mizyme, an endo- and exo-peptidase complex derived
 15 from *A. oryzae*, to enhance the recovery of polyphen-
 16 ols and other antioxidant compounds from the red
 17 seaweed *Palmaria palmata*. Heo *et al.* (2005, 2003)
 18 used inexpensive commercial enzyme preparations such
 19 Celluclast, Viscozyme, Flavourzyme, Alcalase and
 20 Kojizyme to produce antioxidant hydrolysates from
 21 brown seaweeds. They found that several of the hydro-
 22 lysates had a greater free radical scavenging activity
 23 and lipid peroxidation inhibitory effect than synthetic
 24 commercial antioxidants α -tocopherol, BHA and
 25 BHT.

26 However, little has been published on the use of
 27 enzymes for fucoxanthin extraction specifically. A
 28 study by Billakanti *et al.* (2013) reported a 9.3%
 29 increase in fucoxanthin yield, compared to solvent-
 30 only treatment, from *Undaria pinnatifida* using an
 31 enzyme pretreatment of alginate lyase, derived from
 32 *Flavobacterium multivorum*, followed by dimethyl ether
 33 and ethanol extraction. Optimum parameters for the
 34 alginate lyase pretreatment were 37 °C, for 2 h, pH
 35 6.2, 5% (w/v) solids, with 0.05% enzyme. However,
 36 the same volume of dimethyl ether and ethanol used
 37 for solvent-only extraction was required after enzy-
 38 matic pretreatment to achieve the 9.3% fucoxanthin
 39 increase. Qin *et al.* (2013) used an equal ratio of cellu-
 40 losase [*sic*] and pectinase to increase fucoxanthin yields
 41 from *Laminaria japonica*. Extraction was carried out at
 42 pH 5.0, 50 °C, 0.30% enzyme, for 80 min. A fucoxan-
 43 thin yield of 0.183 mg g⁻¹ (wet mass) was achieved,
 44 which was 26.5% greater than traditional organic sol-
 45 vent extraction yield; however, the organic solvent or
 46 method used was not specified.

47 Extraction of algal bioactives through the applica-
 48 tion of enzymes has the potential to increase yield and
 49 safety (Charoensiddhi *et al.*, 2016a; Abu-Ghannam &
 50 Shannon, 2017). The presence of cellulose in the cell
 51 walls of brown seaweed limits the efficiency of organic
 52 solvent extraction (Kim, 2011; Kim & Chojnacka,
 53 2015). The fucoxanthin yields of less than 1 mg g⁻¹ in
 54 the present study are in line with, and in some cases,
 55 greater-than-published results for fucoxanthin in the
 56 ten species under study (Ramus *et al.*, 1977; Stengel &

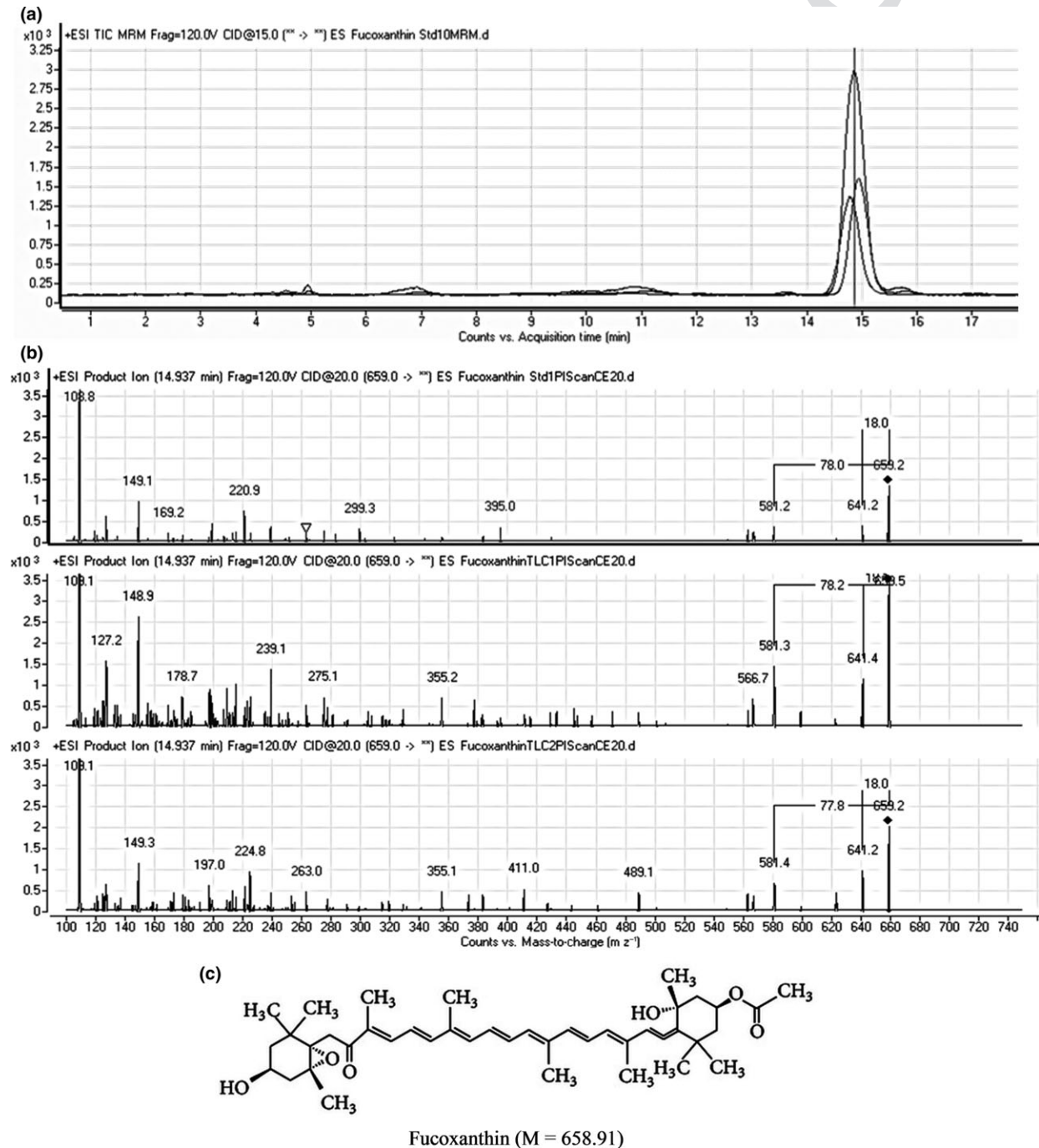
Dring, 1998; Schmid & Stengel, 2015). Human clinical
 trials have found doses of only 0.5–2.4 mg per day to
 be effective in the treatment of diseases such as obesity
 and metabolic syndrome (Abidov *et al.*, 2010; Oryza,
 2011).

With an aim of reducing or eliminating acetone
 usage in the present study, mechanical processing in
 the form of fine blending was applied as a pretreat-
 ment before extraction. Dehydration prior to mechani-
 cal blending produces a smaller particle size compared
 to blending of raw, wet seaweed, due to the brittle na-
 ture of the dried thalli. This reduction of particle size
 further aids in the extraction of fucoxanthin by ruptur-
 ing the membrane-bound thylakoids within the algal
 cells. As lyophilisation via freeze-drying is a high-
 energy consumption process, the aim was to dehydrate
 the seaweed using a low-energy-consuming method,
 such as oven incubation at 40 °C. Predrying of brown
 macroalgae has been incorporated as part of other
 optimisation methods to increase enzymatic extraction
 yields. For example, Puspita *et al.* (2017) dried three
 species of Sargassum away from direct sunlight (tem-
 perature not specified) for 7 days before extraction of
 phenolic compounds with a combination of proteases
 and carbohydrases. Olivares-Molina & Fernández
 (2016) air-dried *Lessonia nigrescens*, *Macrocystis pyri-
 fera* and *Durvillaea antarctica* at room temperature for
 5 days prior to α -amylase and cellulase extraction of
 phlorotannins; Adalbjörnsson & Jónsdóttir (2015) used
 freeze-drying (number of hours not specified) before
 multienzymatic extraction of polyphenols. However,
 the shorter drying conditions of only 12 h (at 40 °C)
 optimised in the present study are more suitable for
 fucoxanthin-specific extraction as this xanthophyll is
 degraded via oxidation in prolonged exposure to air
 (Zhao *et al.*, 2014). In addition, oven incubation at
 40 °C only consumes approximately 1.6 MJ h⁻¹ kg⁻¹
 in energy, compared to 4.1 MJ h⁻¹ kg⁻¹ for freeze-
 drying (Rudy, 2009), which is a 61% reduction in
 energy usage. An approximate cost analysis of the pro-
 posed extraction technique, at laboratory scale, for the
 model species is as follows. One kilogram of raw
F. vesiculosus (€1.75 kg⁻¹) has a mean dry mass of
 288 g of which 0.0657% (0.657 mg g⁻¹) was quantified
 by HPLC to be fucoxanthin. This is 0.189 g fucoxan-
 thin per raw kilogram. The volume of Viscozyme
 (€0.05 cent mL⁻¹) required to treat 288 g of dried sea-
 weed according to the optimised parameters is
 28.15 mL, costing €1.41. Therefore, a total raw mate-
 rial cost of €3.16 kg⁻¹ (seaweed plus Viscozyme)
 would produce 0.189 g fucoxanthin or €16.72 to pro-
 duce one gram of fucoxanthin. Current market prices
 for fucoxanthin vary widely. For example, analytical
 grade fucoxanthin ($\geq 95\%$ purity) retails at
 €4450 g⁻¹ (Sigma-Aldrich, 2018). Online seaweed pro-
 ducers based in Asia offer organic solvent-extracted

1 fucoxanthin for a very low average price of \$200 kg⁻¹
 2 (\$0.20 g⁻¹), with product specifications claiming puri-
 3 ties ranging from 5% to 80% fucoxanthin (Alibaba,
 4 2018). However, the chemical solvents used to extract
 5 fucoxanthin are not specified and may not be

considered safe for human consumption under the
 international Food Chemicals Codex, European Food
 Safety Authority Commission Directive 2010/67/EU or
 Sections 201 and 409 of the U.S. Federal Food, Drug,
 and Cosmetic Act (FDA, 2002; EU, 2010; USP, 2018).

POOR QUALITY FIG



9 **Figure 3** LC-MS spectra of fucoxanthin standard and two enzymatic extracts of *Fucus vesiculosus*.


1 Viscozyme is suitable for the preparation of extracts
2 for human consumption as it is compliant with purity
3 specifications set out by the FAO and WHO (FAO/
4 WHO, 2010) and is derived from the non-toxic fungus
5 *A. aculeatus*.

6 Although enzymatic extraction is a green chemical
7 technique, it can have some limitations such as lower
8 yields, low selectivity and longer treatment times in
9 comparison with traditional organic solvent extraction.
10 Other non-conventional technologies have shown effi-
11 cacy in the extraction of bioactive compounds from
12 algae (Barba *et al.*, 2015). These include ultrafiltration
13 by molecular weight cut-off membrane filters (Zhu
14 *et al.*, 2017), pulsed electric fields, supercritical fluids,
15 microwave, ultrasound and accelerated solvent extrac-
16 tion. For example, Parniakov *et al.* (2015) observed a
17 ninefold increase in total chlorophyll yield from the
18 alga *Nannochloropsis* using ultrasound-assisted water,
19 ethanol and dimethyl sulphoxide extraction. Ultra-
20 sound, using solvents such as food-grade ethanol, can
21 also be coupled with technologies like supercritical car-
22 bon dioxide extraction. Food-grade ethanol and car-
23 bon dioxide are less expensive than some enzyme
24 preparations and are suitable for extracting algal
25 bioactives for nutritional and shelf-life enhancement in
26 food products (Roohinejad *et al.*, 2017). However, the
27 initial instrumental set-up costs of these techniques
28 may be cost-prohibitive for small-scale seaweed pro-
29 ducers, compared to enzymatic flask incubation.

30 Moisture content determination

31 The moisture content of the blade, stipe and holdfast
32 of each species before and after oven incubation (12 h,
33 40 °C) is detailed in Table S1.

34 Identification of fucoxanthin by LC-MS

35 To confirm the presence of fucoxanthin in enzymatic
36 extracts, LC-MS characterisation was used after initial
37 HPLC-guided identification. The HPLC peak from
38 the purified enzymatic extract of *F. vesiculosus*
39 (14.937 min) was compared to the corresponding
40 commercial fucoxanthin standard peak and further char-
41 acterised by its positive ions. Figure 3 shows the LC-
42 ESI-MS spectra of  IC chromatogram of fucoxanthin
43 standard overlaid with two P-TLC enzymatic extracts of
44 *F. vesiculosus* in full-scan mode from m/z 100 to 1000
45 atomic mass units (amu), (ii) ESI-MS spectrum of the
46 three peaks depicted in A showing the selected ion 658.91
47 and its major fragments due to the loss of 18 and 78 amu
48 and (iii) fucoxanthin molecular structure. Fucoxanthin is
49 known to have a molecular mass of 658.91 g. A proto-
50 nated parent ion (molecular mass (M)+H) was observed
51 in the fucoxanthin standard at m/z 659. An identical ion
52 was observed in the *F. vesiculosus* enzymatic extracts.

Two daughter ions were also present as major ESI-MS
fragments. These can be seen in all three samples at m/z
641 and m/z 581. The ion at m/z 641 corresponds to the
loss of water $[M+H-18]^+$. This is characteristic for a
compound such as fucoxanthin which contains a hydro-
xyl group. The second most abundant ion was at m/z
581, which is due to the loss of acetic acid and water
 $[M+H-18-60]^+$ from the parent fucoxanthin ion. These
findings are in full agreement with reported mass frag-
mentation data for fucoxanthin (Avula *et al.*, 2015; Zhu
et al., 2016; Rajauria *et al.*, 2017), thus confirming its
presence in the seaweed extract.

Conclusion

In this work, an effective and sustainable process to
extract fucoxanthin from brown seaweeds was opti-
mised. The cellulose content and structure in the cell
walls of brown seaweeds limit the efficiency of organic
solvent extraction. The application of enzymes could
provide a solution by achieving a considerable level
of hydrolysis of cellulose in the macroalgal cell walls
as presented in this study. In addition, the application
of enzyme-based fucoxanthin extraction has advan-
tages over organic solvent extraction. It is a green
chemical process with no toxic waste; it converts
water-insoluble raw materials into partially water-
soluble materials and is a relatively low-cost technol-
ogy considering the cost-effectiveness of the enzymes
utilised. From an application perspective, based on
reported clinical trials, the fucoxanthin yields
achieved in this study offer potential for complemen-
tary treatments of global epidemic nutrition-related
disorders such as type 2 diabetes, obesity, cancer and
metabolic syndrome. These findings may be applied
to the development of value-added nutraceutical
products from seaweed biomass.

References

- Abidov, M., Ramazanov, Z., Seifulla, R. & Grachev, S. (2010). The effects of Xanthigen™ in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. *Diabetes, Obesity and Metabolism*, **12**, 72–81. <https://doi.org/10.1111/j.1463-1326.2009.01132.x>.
- Abu-Ghannam, N. & Shannon, E. (2017). Seaweed carotenoid, fucoxanthin, as functional food. In: *Microbial Functional Foods and Nutraceuticals* (edited by V. Gupta, H. Treichel, V. Shapaval, L.A. de Oliveira & M.G. Tuohy). Pp. 39–64 Hoboken: John Wiley & Sons.
- Adalbjörnsson, B. & Jónsdóttir, R. (2015). Chapter 8: enzyme-enhanced extraction of antioxidant ingredients from algae. In: *Natural Products From Marine Algae – Methods and Protocols* (edited by D.B. Stengel & S. Connan) New York, NY: Humana Press.
- Alibaba. (2018). Fucoxanthin. URL https://www.alibaba.com/trade/search?fsb=y&IndexArea=product_en&SearchText=fucoxanthin&viewtype=G. Accessed 14/03/18.
- Avula, B., Wang, Y.-H. & Khan, I.A. (2015). Arsenic speciation and fucoxanthin analysis from seaweed dietary supplements using LC-

- MS. *Journal of AOAC International*, **98**, 321–329. <https://doi.org/10.5740/jaoacint.14-166>.
- Barba, F.J. (2017). Microalgae and seaweeds for food applications: challenges and perspectives. *Food Research International*, **99**, 969–970.
- Barba, F.J., Grimi, N. & Vorobiev, E. (2015). New approaches for the use of non-conventional cell disruption technologies to extract potential food additives and nutraceuticals from microalgae. *Food Engineering Reviews*, **7**, 45–62. <https://doi.org/10.1007/s12393-12014-19095-12396>.
- Billakanti, J.M., Catchpole, O.J., Fenton, T.A., Mitchell, K.A. & MacKenzie, A.D. (2013). Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from *Udaxia pinatifida* using dimethyl ether and ethanol. *Process Biochemistry*, **48**, 1999–2008. <https://doi.org/10.1016/j.procbio.2013.09.015>
- Charoensiddhi, S., Conlon, M.A., Vuaran, M.S., Franco, C.M. & Zhang, W. (2016a). Impact of extraction processes on prebiotic potential of the brown seaweed *Ecklonia radiata* by *in vitro* human gut bacteria fermentation. *Journal of Functional Foods*, **24**, 221–230. <https://doi.org/10.1016/j.jff.2016.04.016>.
- Charoensiddhi, S., Lorbeer, A.J., Lahnstein, J., Bulone, V., Franco, C.M. & Zhang, W. (2016b). Enzyme-assisted extraction of carbohydrates from the brown alga *Ecklonia radiata*: effect of enzyme type, pH and buffer on sugar yield and molecular weight profiles. *Process Biochemistry*, **51**, 1503–1510. <https://doi.org/10.1016/j.procbio.2016.07.014>.
- Chumsri, P., Sirichote, A. & Itharat, A. (2008). Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract. *Songklanakar Journal of Science and Technology*, **30**, 133–139. <http://rdo.psu.ac.th/sjstweb/journal/130-Suppl-131/0125-3395-0130-S0121-0133-0139.pdf>.
- Deniaud-Bouët, E., Kervarec, N., Michel, G., Tonton, T., Kloreg, B. & Hervé, C. (2014). Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae. *Annals of Botany*, **114**, 1203–1216. <https://doi.org/10.1093/aob/mcu096>.
- D’Orazio, N., Gemello, E., Giamme, M.A., de Girolamo, M., Ficoneri, C. & Riccioni, G. (2012). Fucoxanthin: a treasure from the sea. *Marine Drugs*, **10**, 604–616. <https://doi.org/10.3390/md10030604>.
- Durnford, D.G. (2003). Structure and regulation of algal light-harvesting complex genes. *Photosynthesis in Algae*. Pp. 63–82 Dordrecht: Springer.
- EU. (2010). Commission Directive (2010) 2010/67/EU of 20 October 2010 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. *The Official Journal of the European Union (L Series)*, **277**, 17–26.
- FAO/WHO. (2010). *Evaluation of Certain Food Additives: Seventy-first Report of the Joint FAO/WHO Expert Committee on Food Additives* (vol. 71). Geneva, Switzerland: World Health Organization & Food and Agriculture Organization of the United Nations. <https://www.ncbi.nlm.nih.gov/pubmed/20942228>.
- FDA. (2002). Federal Food, Drug, and Cosmetic Act 2002. United States Code, Title 21, The Food and Drug Administration. URL <http://www.fda.gov/regulatoryinformation/legislation/federalfooddrugandcosmeticactfdca/>. Accessed 13/03/18.
- Gupta, V.G. (2016). *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Cellulase System Properties and Applications*. Amsterdam: Elsevier Science.
- Gupta, S., Cox, S. & Abu-Ghannam, N. (2011). Effect of different drying temperatures on the moisture and phytochemical constituents of edible Irish brown seaweed. *LWT – Food Science and Technology*, **44**, 1266–1272. <https://doi.org/10.1016/j.lwt.2010.12.022>.
- Heo, S.-J., Jeon, Y.-J., Lee, J., Kim, H.-T. & Lee, K.-W. (2003). Antioxidant effect of enzymatic hydrolyzate from a kelp, *Ecklonia cava*. *Algae*, **18**, 341–347. <https://doi.org/10.4490/algae.2003.18.4.341>.
- Heo, S.-J., Park, E.-J., Lee, K.-W. & Jeon, Y.-J. (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology*, **96**, 1613–1623. <https://doi.org/10.1016/j.biortech.2004.07.013>.
- Hurd, C.L., Harrison, P.J., Bischof, K. & Lobban, C.S. (2014). *Seaweed Ecology and Physiology*. Cambridge: Cambridge University Press.
- Hussain, E., Wang, L.-J., Jiang, B., Riaz, S., Butt, G.Y. & Shi, D.-Y. (2016). A review of the components of brown seaweeds as potential candidates in cancer therapy. *Royal Society of Chemistry Advances*, **6**, 12592–12610. <https://doi.org/10.1039/C5RA23995H>.
- Inggrid, H. & Santoso, H. (2016). Natural red dyes extraction on roselle petals. In: *IOP Conference Series: Materials Science and Engineering*. vol. **162**. Pp. 012029. Bristol: IOP Publishing.
- Jose, S. & Archana, S. (2017). Environmental and economic sustainability of algal lipid extractions: an essential approach for the commercialization of algal biofuels. In: *Algal Biofuels: Recent Advances and Future Prospects* (edited by S.K. Gupta, A. Malik & F. Bux). Pp. 281–313 Cham: Springer International Publishing.
- Kerton, F.M. & Marriott, R. (2013). *Alternative Solvents for Green Chemistry, Issue 20 of RSC Green Chemistry Series*. Cambridge: Royal Society of Chemistry.
- Kim, S.-K. (2011). *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*. Hoboken: John Wiley & Sons.
- Kim, S.-K. & Chojnacka, K. (2015). *Marine Algae Extracts: Processes, Products, and Applications*, 2, vol. **Set**. Hoboken: John Wiley & Sons.
- Kong, Z.-L., Kao, N.-J., Hu, J.-Y. & Wu, C.-S. (2016). Fucoxanthin-rich brown algae extract decreases inflammation and attenuates colitis-associated colon cancer in mice. *Journal of Food and Nutrition Research*, **4**, 137–147. <https://doi.org/10.3934/biophy.2017.1.152>.
- Landrum, J.T. (2009). *Carotenoids: Physical, Chemical, and Biological Functions and Properties*. Boca Raton: CRC Press.
- Larkum, A., Douglas, S. & Raven, J.A. (2012). *Photosynthesis in Algae*, vol. **14**. Dordrecht: Springer Science & Business Media.
- Lin, J., Huang, L., Yu, J. *et al.* (2016). Fucoxanthin, a marine carotenoid, reverses scopolamine-induced cognitive impairments in mice and inhibits acetylcholinesterase *in vitro*. *Marine Drugs*, **14**, 67. <https://doi.org/10.3390/md1404067>.
- Loureiro, R., Gachon, C.M.M. & Rebours, C. (2015). Seaweed cultivation: potential and challenges of crop domestication at an unprecedented pace. *New Phytologist*, **206**, 489–492. <https://doi.org/10.1111/nph.13278>.
- Manns, D., Andersen, S.K., Saake, B. & Meyer, A.S. (2016). Brown seaweed processing: enzymatic saccharification of *Laminaria digitata* requires no pre-treatment. *Journal of Applied Phycology*, **28**, 1287–1294. <https://doi.org/10.1007/s10811-015-0663-9>.
- Matsui, M., Tanaka, K., Higashiguchi, N. *et al.* (2016). Protective and therapeutic effects of fucoxanthin against sunburn caused by UV irradiation. *Journal of Pharmacological Sciences*, **132**, 55–64. <https://doi.org/10.1016/j.jphs.2016.1008.1004>.
- Mei, C., Zhou, S., Zhu, L., Ming, J., Zeng, F. & Xu, R. (2017). Antitumor effects of Laminaria extract fucoxanthin on lung cancer. *Marine Drugs*, **15**, 39. <https://doi.org/10.3390/md15020039>.
- Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M. & Hosokawa, M. (2011). The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. *Journal of the Science of Food and Agriculture*, **91**, 1166–1174. <https://doi.org/10.1002/jsfa.4353>.
- Nishikawa, S., Hosokawa, M. & Miyashita, K. (2012). Fucoxanthin promotes translocation and induction of glucose transporter 4 in skeletal muscles of diabetic/obese KK-A y mice. *Phytomedicine*, **19**, 389–394. <https://doi.org/10.1016/j.phymed.2011.11.001>.
- Olivares-Molina, A. & Fernández, K. (2016). Comparison of different extraction techniques for obtaining extracts from brown seaweeds and their potential effects as angiotensin I-converting enzyme (ACE) inhibitors. *Journal of Applied Phycology*, **28**, 1295–1302. <https://doi.org/10.1007/s10811-015-0665-7>.

- Oryza. (2011). *Fucoxanthin: Dietary Ingredient for Prevention of Metabolic Syndrome and Beauty Enhancement*. Tokyo, Japan: Oryza Oil & Fat Chemical Co. URL <http://www.oryza.co.jp/pdf/english/Fucoxanthin1.0.pdf>. Accessed 02/01/18.
- Park, P.J., Shahidi, F. & Jeon, Y.J. (2004). Antioxidant activities of enzymatic extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry. *Journal of Food Lipids*, **11**, 15–27. <https://doi.org/10.1111/j.1745-4522.2004.tb00257.x>.
- Parniakov, O., Apicella, E., Koubaa, M. *et al.* (2015). Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* spp. *Bioresource Technology*, **198**, 262–267. <https://doi.org/10.1016/j.biortech.2015.09.020>.
- Poojary, M., Barba, F., Aliakbarian, B. *et al.* (2016). Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. *Marine Drugs*, **14**, 214. <https://doi.org/10.3390/md14110214>.
- Puspita, M., Deniel, M., Widowati, I. *et al.* (2017). Antioxidant and antibacterial activity of solid-liquid and enzyme-assisted extraction of phenolic compound from three species of tropical *Sargassum*. In: *IOP Conference Series: Earth and Environmental Science*, vol. **55**. Pp. 012057. Bali, Indonesia: IOP Publishing.
- Qin, Y., Meng, L. & Wang, F. (2013). Extraction and antioxidant activity of fucoxanthin from *Laminaria japonica*. *Food Science*, **34**, 279–283. <https://doi.org/10.7506/spkx1002-6630-201316057>.
- Rajauria, G. & Abu-Ghannam, N. (2013). Isolation and partial characterization of bioactive fucoxanthin from *Himanthalia elongata* brown seaweed: a TLC-based approach. *International Journal of Analytical Chemistry*, **2013**, 6. <https://doi.org/10.1155/2013/802573>.
- Rajauria, G., Foley, B. & Abu-Ghannam, N. (2017). Characterization of dietary fucoxanthin from *Himanthalia elongata* brown seaweed. *Food Research International*, **99**, 995–1001. <https://doi.org/10.1016/j.foodres.2016.09.023>.
- Ramus, J., Lemons, F. & Zimmerman, C. (1977). Adaptation of light-harvesting pigments to downwelling light and the consequent photosynthetic performance of the eulittoral rockweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. *Marine Biology*, **42**, 293–303. <https://doi.org/10.1007/BF00402191>.
- Roohinejad, S., Koubaa, M., Barba, F.J., Saljoughian, S., Amid, M. & Greiner, R. (2017). Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Research International*, **99**, 1066–1083. <https://doi.org/10.1016/j.foodres.2016.08.016>.
- Roy, S., Llewellyn, C.A., Egeland, E.S. & Johnsen, G. (2011). *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography*. Cambridge: Cambridge University Press.
- Rudy, S. (2009). Energy consumption in the freeze-and convection-drying of garlic. *TEKA Commission of Motorization and Power Industry in Agriculture*, **9**, 259–266. URL <http://www.pan-ol.lublin.pl/wydawnictwa/TMot9/Rudy.pdf>. Accessed 15/03/18.
- Schmid, M. & Stengel, D.B. (2015). Intra-thallus differentiation of fatty acid and pigment profiles in some temperate Fucales and Laminariales. *Journal of Phycology*, **51**, 25–36. <https://doi.org/10.1111/jpy.12268>.
- Shannon, E. & Abu-Ghannam, N. (2016). Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. *Marine Drugs*, **14**, 81. <https://doi.org/10.3390/md1404081>.
- Shannon, E. & Abu-Ghannam, N. (2017). Optimisation of fucoxanthin extraction from Irish seaweeds by response surface methodology. *Journal of Applied Phycology*, **29**, 1027–1036. <https://doi.org/10.1007/s10811-016-0983-4>.
- Sigma-Aldrich. (2018). Fucoxanthin analytical standard. URL <https://www.sigmaaldrich.com/catalog/product/sial/16337?lang=en®ion=IE> Accessed 13/03/2018.
- Stengel, D.B. & Dring, M.J. (1998). Seasonal variation in the pigment content and photosynthesis of different thallus regions of *Ascophyllum nodosum* (Fucales, Phaeophyta) in relation to position in the canopy. *Phycologia*, **37**, 259–268. <https://doi.org/10.2216/i0031-8884-37-4-259.1>.
- Sugawara, T., Baskaran, V., Tsuzuki, W. & Nagao, A. (2002). Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mice. *The Journal of Nutrition*, **132**, 946–951. <https://doi.org/10.1093/jn/132.5.946>.
- USP. (2018). United States Pharmacopeia Food Chemicals Codex. URL http://www.foodchemicalscode.org/?_ga=2.71906579.1217754048.1521054781-90202469.1521054781. Accessed 13/03/18.
- Wang, T., Jónsdóttir, R., Kristinsson, H.G. *et al.* (2010). Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmata*. *LWT – Food Science and Technology*, **43**, 1387–1393. <https://doi.org/10.1016/j.lwt.2010.05.010>.
- Wells, M.L., Potin, P., Craigie, J.S. *et al.* (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, **29**, 949–982. <https://doi.org/10.1007/s10811-016-0974-5>.
- Zhao, D., Kim, S.-M., Pan, C.-H. & Chung, D. (2014). Effects of heating, aerial exposure and illumination on stability of fucoxanthin in canola oil. *Food Chemistry*, **145**, 505–513. <https://doi.org/10.1016/j.foodchem.2013.08.045>.
- Zhu, J., Sun, X., Chen, X., Wang, S. & Wang, D. (2016). Chemical cleavage of fucoxanthin from *Undaria pinnatifida* and formation of apo-fucoxanthinones and apo-fucoxanthinols identified using LC-DAD-APCI-MS/MS. *Food Chemistry*, **211**, 365–373. <https://doi.org/10.1016/j.foodchem.2016.05.064>.
- Zhu, Z., Wu, Q., Di, X. *et al.* (2017). Multistage recovery process of seaweed pigments: investigation of ultrasound assisted extraction and ultra-filtration performances. *Food and Bioprocess Technology*, **104**, 40–47. <https://doi.org/10.1016/j.fbp.2017.1004.1008>.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Moisture content before and after oven incubation for 12 h at 40 °C.

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main.

