



# The $\alpha$ -amylase and $\alpha$ -glucosidase inhibitory effects of Irish seaweed extracts



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## ARTICLE INFO

### Article history:

Received 28 January 2013

Received in revised form 11 March 2013

Accepted 26 April 2013

Available online 9 May 2013

### Keywords:

$\alpha$ -Amylase

$\alpha$ -Glucosidase

Antioxidant

Diabetes

Phenolics

Seaweed

## ABSTRACT

To date, numerous studies have reported on the antidiabetic properties of various plant extracts through inhibition of carbohydrate-hydrolysing enzymes. The objective of this research was to evaluate extracts of seaweeds for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effects. Cold water and ethanol extracts of 15 seaweeds were initially screened and from this, five brown seaweed species were chosen. The cold water and ethanol extracts of *Ascophyllum nodosum* had the strongest  $\alpha$ -amylase inhibitory effect with IC<sub>50</sub> values of 53.6 and 44.7  $\mu$ g/ml, respectively. Moreover, the extracts of *Fucus vesiculosus* Linnaeus were found to be potent inhibitors of  $\alpha$ -glucosidase with IC<sub>50</sub> values of 0.32 and 0.49  $\mu$ g/ml. The observed effects were associated with the phenolic content and antioxidant activity of the extracts, and the concentrations used were below cytotoxic levels. Overall, our findings suggest that brown seaweed extracts may limit the release of simple sugars from the gut and thereby alleviate postprandial hyperglycaemia.

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## 1. Introduction

Hydrolysis of dietary starch is the major source of glucose in the blood, with  $\alpha$ -amylase and  $\alpha$ -glucosidase being the key enzymes involved in starch breakdown and intestinal absorption, respectively. It is believed that inhibition of these enzymes can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet, and therefore can be an important strategy in the management of hyperglycaemia linked to type II diabetes (Kwon, Apostolidis, & Shetty, 2008).

Human  $\alpha$ -amylase is one of the major secretory products of the pancreas and salivary glands, playing a role in digestion of starch and glycogen (Kandra, 2003). A calcium metalloenzyme,  $\alpha$ -amylase catalyses the cleavage of  $\alpha$ -D-(1-4)glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins into shorter oligosaccharides (de Sales, de Souza, Simeoni, Magalhães, & Silveira, 2012; Etteberria, de la Garza, Campión, Martínez, & Milagro, 2012). Other amylolytic enzymes also participate in the process of starch breakdown, but the contribution of  $\alpha$ -amylase is a prerequisite for the initiation of this process (de Sales et al., 2012; Tangphatsornruang, Naconsie, Thammarongtham, & Narangajavana, 2005). A second enzyme,  $\alpha$ -glucosidase, which is also

located in the brush-border surface membrane of intestinal cells, activates the final step of the digestive process. This exo-type carbohydrase enzyme catalyses the hydrolysis of complex carbohydrates and disaccharides to absorbable monosaccharides (Kim, Nguyen, Kurihara, & Kim, 2010). Consequently, inhibitors of these hydrolytic enzymes suppress the influx of glucose from the intestinal tract to blood vessels resulting in a decrease in postprandial hyperglycaemia.

Currently there are some antidiabetic drugs, namely, acarbose, miglitol and voglibose, which act by inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. While efficient in attenuating the rise in blood glucose levels in many patients, the continuous use of these drugs is often associated with undesirable side effects, such as liver toxicity and adverse gastrointestinal symptoms (Etteberria et al., 2012; van de Laar, 2008). It is for this reason that there is a need for natural  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors which have no adverse or unwanted secondary effects.

Seaweeds and their organic extracts are known to contain a wide array of bioactive substances with diverse health benefits (Rindi, Soler-Vila, & Guiry, 2012). Moreover, they are considered to be good sources of polyphenols and have been shown to exhibit antidiabetic properties through inhibition of carbohydrate-hydrolysing enzymes (Apostolidis & Lee, 2010; Kandra, Gyémánt, Zajác, & Batta, 2004; Kim et al., 2010; Moon et al., 2011; Nwosu et al., 2011). Kawamura-Konishi et al. (2012) recently found that an algal phlorotannin, isolated from *Sargassum patens* C. Agardh, had a

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much lower (50%) inhibitory activity concentration ( $IC_{50}$ ) for  $\alpha$ -amylase inhibition than that of commercially available inhibitors. Accordingly, it has been acknowledged that seaweed extracts and their fractions could act as functional ingredients in foods used to control hyperglycaemia.

The objective of this study was to identify seaweed extracts that may be useful for diabetic care. To this end, cold water and ethanol based extracts of 15 Irish seaweeds were screened for potential  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity, with extracts of interest subsequently tested for their effect on Caco-2 cell function. The undifferentiated Caco-2 human colon cancer cell line was chosen as it is a reliable model for cultured colonocytes, is well characterised and is widely used for biochemical and nutritional studies (Schulz, 2011).

## 2. Material and methods

### 2.1. Chemicals

All chemicals and cell culture reagents were obtained from the Sigma Chemical Co. (Dublin, Republic of Ireland) unless otherwise stated. Tissue culture plastics were supplied by Sarstedt (Wexford, Republic of Ireland) and cell lines were from the European Collection of Animal Cell Cultures (Wiltshire, UK).

### 2.2. Seaweed collection

Fifteen species were harvested between April 2009 and February 2011 from four different locations on the West coast of Ireland: Finnvara, Co. Clare (F), New Quay, Co. Clare (NQ), Spiddal, Co. Galway (S) and Mweenish, Co. Galway (M). The species collected were *Alaria esculenta* (Linnaeus) Greville (S) and *Laminaria digitata* (Hudson) J.V. Lamouroux (S) (May 2009); *Himanthalia elongata* (Linnaeus) S.F. Gray (F) (April 2010); *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druhl & G.W. Saunders (S) (September 2010); *Ulva intestinalis* Linnaeus (S), *Fucus serratus* Linnaeus (S), *Fucus vesiculosus* Linnaeus (S) and *Ascophyllum nodosum* (Linnaeus) Le Jolis (NQ) (October 2010); *Gracilaria gracilis* (Stackhouse) M.Steentoft, L.M.Irvine & W.F. Farnham (F), *Codium fragile* subsp. *fragile* (Surinagar) Hariot (F), *Pelvetia canaliculata* (Linnaeus) Decaisne & Thuret (S), *Chondrus crispus* Stackhouse (F), *Fucus spiralis* Linnaeus (S), *Laminaria hyperborea* (Gunnerus) Foslie (M), and *Palmaria palmata* (Linnaeus) Weber & Mohr (M) (February, 2011). Reference material for each sample was kept in NUI Galway as part of the Marine Functional Foods Research Initiative.

### 2.3. Extraction

Each of the collected seaweed samples was quickly washed in cold water to remove sand and other particulates and immediately frozen. All samples were freeze dried, ground to a fine powder and stored in vacuum-packed bags at  $-80^{\circ}\text{C}$  prior to extraction. Solid liquid extraction was employed to extract each of the seaweeds with two different solvent solutions, ethanol/water (80:20) and cold water. For the ethanol based extraction (referred to as ethanol extract from here on) dried seaweed material was mixed in ratio of 1:10 w/v with the extraction solvent (80:20, ethanol:water), and for the water-based extractions this ratio was changed to 1:20 w/v due to the viscosity of the material extracted. The ethanol and cold water extractions were performed in an orbital shaker (MaxQ 6000 Shaker, Thermo Fisher Scientific, Ireland) at 175 rpm. Following 3 h incubation, ethanol extracts were filtered through a Buchner funnel and the seaweed material was re-extracted with fresh solvents for a further 3 h. The process was repeated with a final overnight extraction to ensure exhaustive

extraction occurred, and the solvents were combined. The cold water extraction was filtered initially after 6 h and then after 24 h through glass wool filters due to the viscosity of the extracted material and combined. Ethanol was removed from the ethanol-based extractions using a rotary evaporator (Buchi Rotavapor R-220, Mason Technologies, Ireland) with the water bath set at  $60^{\circ}\text{C}$ . The cold water extractions were also concentrated using a rotary evaporator. Each extract was then freeze dried to remove the remaining water and stored as a fine powder at  $-80^{\circ}\text{C}$  prior to testing.

### 2.4. Sample preparation

Before each assay, stock solutions of each of the extracts were prepared. Cold water extracts were dissolved directly in distilled water, assay buffer (enzyme assays) or cell culture medium, and the ethanol extracts were dissolved in DMSO overnight, where the final concentration of the solvent in the stock solution was 2%. Equivalent quantities of DMSO were also added to control samples. Each extract was then filtered using a  $0.45\text{ }\mu\text{m}$  syringe filter. Finally each stock solution was diluted to give desired working concentration(s). For each independent experiment, all samples were tested in duplicate.

### 2.5. $\alpha$ -Glucosidase activity

For preliminary investigations,  $\alpha$ -glucosidase activity was measured in the presence of 10 mg/ml cold water and ethanol extracts from 15 seaweeds using the method described by Nampoothiri et al. (2011).

Extracts from five seaweeds were subsequently selected (*A. nodosum*, *F. serratus*, *F. spiralis*, *F. vesiculosus* and *P. canaliculata*) and their inhibitory effects measured at lower concentrations ( $1000\text{ }\mu\text{g/ml}$ – $0.1\text{ }\mu\text{g/ml}$ ). A volume of  $50\text{ }\mu\text{l}$  of extract solution in 100 mM sodium phosphate buffer (pH 6.9) and  $50\text{ }\mu\text{l}$  of 5 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside solution (in phosphate buffer) was mixed in a 96-well microplate and incubated at  $37^{\circ}\text{C}$  for 5 min. Phosphate buffer ( $100\text{ }\mu\text{l}$ ) containing  $0.1\text{ U/ml}$   $\alpha$ -glucosidase (from Baker's yeast) was then added to each well. Absorbance at 405 nm was recorded for 30 min using a microplate reader set to  $37^{\circ}\text{C}$  (BioTek Instruments, Inc., Winooski, Vermont, USA). Blank readings (no enzyme) were subtracted from each well and results were compared to the control. The pharmacological inhibitor, acarbose, was included as a positive control.

The activity of  $\alpha$ -glucosidase was calculated as follows:

$$\% \text{Activity} = \text{Absorbance of extract} / \text{Absorbance of control} \times 100$$

### 2.6. $\alpha$ -Amylase activity

Preliminary experiments on  $\alpha$ -amylase activity were performed according to Nampoothiri et al. (2011).

For subsequent assays, equal volumes ( $100\text{ }\mu\text{l}$ ) of extract and 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9 with 6 mM sodium chloride) were incubated in microtubes at  $25^{\circ}\text{C}$  for 10 min. A volume of  $100\text{ }\mu\text{l}$  of porcine pancreatic  $\alpha$ -amylase ( $0.5\text{ mg/ml}$ ) was added to each tube and samples were incubated at  $25^{\circ}\text{C}$  for a further 10 min. The reaction was stopped with  $200\text{ }\mu\text{l}$  of dinitrosalicylic acid colour reagent and tubes were incubated at  $100^{\circ}\text{C}$  for 5 min. Once samples had cooled to room temperature,  $50\text{ }\mu\text{l}$  was removed from each tube and transferred to the wells of 96-well microplate. The reaction mixture was diluted by adding  $200\text{ }\mu\text{l}$  of water to each well and absorbance was measured at 540 nm. Blank readings (no enzyme) were subtracted from each well and results were compared to the control. The

pharmacological inhibitor, acarbose, was included as a positive control.

The activity of  $\alpha$ -amylase was calculated as follows:

$$\% \text{Activity} = \text{Absorbance of extract} / \text{Absorbance of control} \times 100$$

## 2.7. Total polyphenol content

The concentration of total phenolic compounds in the extracts was determined using the method described by Zhang et al. (2006) with minor modification. Each standard and sample (10  $\mu$ l) was mixed with 50  $\mu$ l of Folin–Ciocalteu's reagent in a 96-well microplate and incubated at room temperature for 5 min. Following this, 40  $\mu$ l of 7.5% sodium carbonate solution was added to each well and the plate was incubated in the dark for 2 h. Absorbance was recorded at 750 nm and results expressed as  $\mu$ g gallic acid equivalents per mg of extract ( $\mu$ g GAE/mg).

## 2.8. Total antioxidant capacity

Total antioxidant capacity of extracts was determined using the method described by Prieto, Pineda, and Aguilar (1999). Briefly, 100  $\mu$ l of each standard and sample was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the mixture was incubated at 95 °C for 90 min. Once samples had cooled to room temperature, absorbance was measured at 695 nm using a microplate reader. The antioxidant capacity of each sample was expressed as  $\mu$ g ascorbic acid equivalents per mg of extract ( $\mu$ g AAE/mg).

## 2.9. Maintenance of cell line

Human colonic carcinoma Caco-2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS), 2 mM L-glutamine and 1% non-essential amino acids. The cells were grown at 37 °C/5% CO<sub>2</sub> in a humidified incubator. The cells were screened for mycoplasma contamination by the Hoechst staining method (Mowles, 1990) and were cultured with 0.5% Penicillin–Streptomycin (5000 U/ml). Exponentially growing cells were used throughout.

## 2.10. Treatment of cells

For each cell culture assay, the Caco-2 cells were seeded at  $5 \times 10^4$  cells/ml and were allowed to attach for 24 h prior to treatment. On the day of the experiment the cells were treated in DMEM, supplemented with 2.5% heat-inactivated FBS. Cells were then exposed to various concentrations of cold water or ethanol extracts (1000–0.1  $\mu$ g/ml) based on extract stock solutions of 2000 and 4  $\mu$ g/ml in DMEM. Samples were incubated for up to 48 h at 37 °C/5% CO<sub>2</sub>.

## 2.11. Assessment of cell viability

Cytotoxicity of the extracts was measured by MTS assay using the CellTiter 96® AQueous One Solution Assay (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions. This assay requires cells that are actively able to metabolise 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to an insoluble formazan precipitate. Briefly, cells were treated with cold water or ethanol extracts in the wells of a 96-well plate for 48 h. At the

end of the treatment, 20  $\mu$ l of MTS reagent was added. Following 4 h incubation at 37 °C/5% CO<sub>2</sub> the absorbance at 490 nm, with reference wavelength of 610 nm, was recorded using a microplate reader.

## 2.12. Statistics

All data points are mean values  $\pm$  standard error (SE) of at least three independent experiments. Where appropriate, data were analysed by one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test or Tukey's Multiple Comparison test. The software employed for statistical analysis was Graph-Pad Prism, Version 4.

## 3. Results and discussion

### 3.1. Identification of extracts with $\alpha$ -amylase and $\alpha$ -glucosidase inhibitory effects

According to numerous *in vivo* studies, inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is believed to be one of the most effective approaches for diabetes care (Etxeberria et al., 2012; van de Laar, 2008). Cold water and ethanol extracts of seaweeds were initially screened for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effects using a pre-incubation method (Table 1 and 2). Whilst most studies to date have focused on one species or class of seaweed, this study evaluated the inhibitory effects of 15 seaweed species from three different phyla; Ochrophyta (*A. esculenta*, *A. nodosum*, *F. serratus*, *F. spiralis*, *F. vesiculosus*, *H. elongata*, *L. digitata*, *L. hyperborea*, *P. canaliculata* and *S. latissima*), Rhodophyta (*C. crispus*, *G. gracilis* and *P. palmata*) and Chlorophyta (*C. fragile* and *U. intestinalis*). At 10 mg/ml, extracts of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* significantly ( $P < 0.05$ ) reduced  $\alpha$ -amylase and  $\alpha$ -glucosidase activity to <20%. Moreover, the ethanol extract of *F. spiralis* also had significant ( $P < 0.01$ ) inhibitory effects on both enzymes, decreasing  $\alpha$ -amylase and  $\alpha$ -glucosidase activity to 11% and 0% respectively of the control. Hence, these five brown seaweeds, *A. nodosum*, *F. serratus*, *F. spiralis*, *F. vesiculosus* and *P. canaliculata*, were selected for further analyses. The remaining 10 seaweeds were not examined further.

The extracts of interest were then examined at lower concentrations (1000–10  $\mu$ g/ml for  $\alpha$ -amylase inhibition, 1000–1  $\mu$ g/ml for  $\alpha$ -glucosidase inhibition) using a modified protocol, whereby

**Table 1**

Activity (% of control) of  $\alpha$ -amylase and  $\alpha$ -glucosidase following pre-incubation with 10 mg/ml cold water seaweed extracts.

Seaweeds	$\alpha$ -amylase	$\alpha$ -glucosidase
<i>Alaria esculenta</i>	117 $\pm$ 6	132 $\pm$ 17
<i>Ascophyllum nodosum</i>	10 $\pm$ 1 <sup>a</sup>	8 $\pm$ 4 <sup>b</sup>
<i>Chondrus crispus</i>	140 $\pm$ 18	146 $\pm$ 23
<i>Codium fragile</i>	131 $\pm$ 18	159 $\pm$ 29
<i>Fucus serratus</i>	16 $\pm$ 3 <sup>a</sup>	18 $\pm$ 4
<i>Fucus spiralis</i>	139 $\pm$ 19	67 $\pm$ 12
<i>Fucus vesiculosus</i>	14 $\pm$ 3 <sup>a</sup>	6 $\pm$ 6 <sup>b</sup>
<i>Gracilaria gracilis</i>	139 $\pm$ 14	171 $\pm$ 28
<i>Himanthalia elongata</i>	110 $\pm$ 5	7 $\pm$ 1 <sup>b</sup>
<i>Laminaria digitata</i>	113 $\pm$ 4	3 $\pm$ 1 <sup>b</sup>
<i>Laminaria hyperborea</i>	96 $\pm$ 22	135 $\pm$ 46
<i>Palmaria palmata</i>	119 $\pm$ 4	183 $\pm$ 13
<i>Pelvetia canaliculata</i>	17 $\pm$ 2 <sup>a</sup>	18 $\pm$ 2
<i>Saccharina latissima</i>	115 $\pm$ 7	88 $\pm$ 25
<i>Ulva intestinalis</i>	132 $\pm$ 19	158 $\pm$ 24

Data represent the mean  $\pm$  SE ( $n = 4$ ).

<sup>a</sup>  $P < 0.01$ .

<sup>b</sup>  $P < 0.05$  relative to control (100% activity), ANOVA followed by Dunnett's Multiple Comparison test.

**Table 2**

Activity (% of control) of  $\alpha$ -amylase and  $\alpha$ -glucosidase following pre-incubation with 10 mg/ml ethanol seaweed extracts.

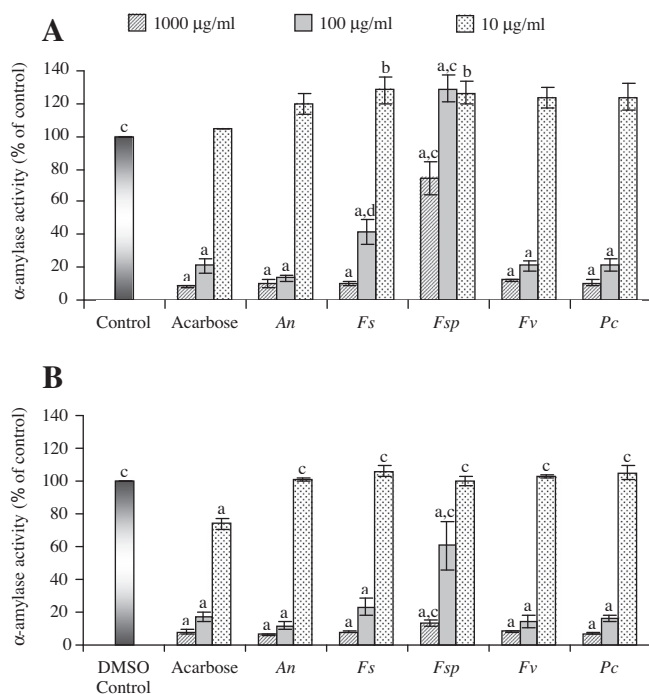
Seaweed	$\alpha$ -amylase	$\alpha$ -glucosidase
<i>Alaria esculenta</i>	123 $\pm$ 17	168 $\pm$ 3
<i>Ascophyllum nodosum</i>	11 $\pm$ 3 <sup>a</sup>	1 $\pm$ 1 <sup>a</sup>
<i>Chondrus crispus</i>	128 $\pm$ 15	205 $\pm$ 13 <sup>a</sup>
<i>Codium fragile</i>	113 $\pm$ 4	173 $\pm$ 6
<i>Fucus serratus</i>	14 $\pm$ 3 <sup>a</sup>	8 $\pm$ 2 <sup>b</sup>
<i>Fucus spiralis</i>	11 $\pm$ 1 <sup>a</sup>	0 $\pm$ 9 <sup>a</sup>
<i>Fucus vesiculosus</i>	15 $\pm$ 3 <sup>a</sup>	0 $\pm$ 14 <sup>b</sup>
<i>Gracilaria gracilis</i>	119 $\pm$ 5	164 $\pm$ 5
<i>Himantalia elongata</i>	111 $\pm$ 6	131 $\pm$ 42
<i>Laminaria digitata</i>	128 $\pm$ 16	198 $\pm$ 10 <sup>a</sup>
<i>Laminaria hyperborea</i>	111 $\pm$ 33	163 $\pm$ 54
<i>Palmaria palmata</i>	126 $\pm$ 14	201 $\pm$ 16 <sup>a</sup>
<i>Pelvetia canaliculata</i>	13 $\pm$ 3 <sup>a</sup>	0 $\pm$ 5 <sup>a</sup>
<i>Saccharina latissima</i>	118 $\pm$ 20	186 $\pm$ 11 <sup>b</sup>
<i>Ulva intestinalis</i>	126 $\pm$ 17	195 $\pm$ 16 <sup>b</sup>

Data represent the mean  $\pm$  SE ( $n = 4$ ).

<sup>a</sup>  $P < 0.01$ .

<sup>b</sup>  $P < 0.05$  relative to control (100% activity), ANOVA followed by Dunnett's Multiple Comparison test.

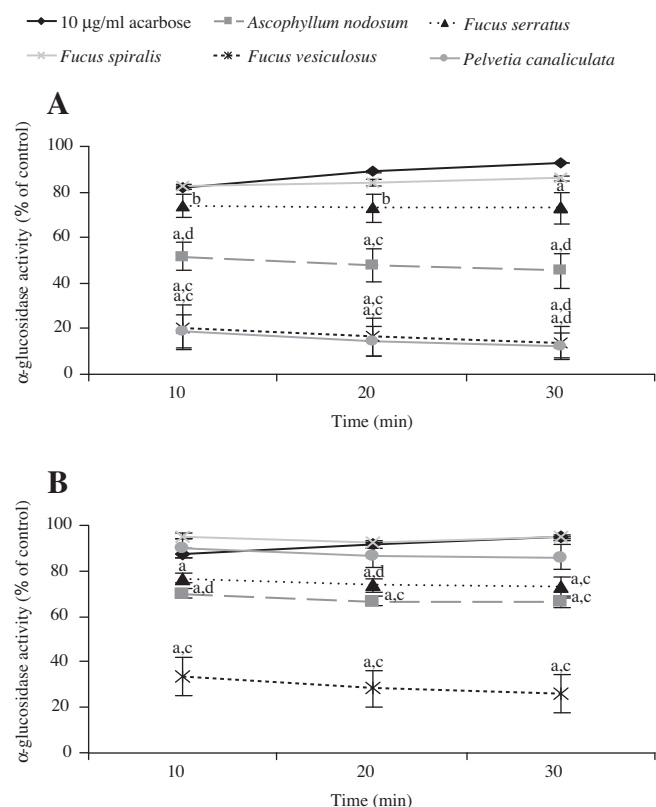
the enzyme was added at the final stages of the experiment. For  $\alpha$ -amylase inhibition, the  $IC_{50}$  values for cold water extracts of *A. nodosum*, *F. serratus*, *F. spiralis*, *F. vesiculosus* and *P. canaliculata* were 53.6, 86.1, 282.7, 63.5 and 66.1  $\mu$ g/ml, respectively. Four of the cold water extracts had a similar inhibitory effect as the positive control, with concentrations of 1000 and 100  $\mu$ g/ml significantly ( $P < 0.01$ ) inhibiting  $\alpha$ -amylase activity (Fig. 1A). However, as demonstrated in our previous findings, the cold water extract of *F. spiralis* was not an effective inhibitor. Using a comparable extract of *A. nodosum*, Apostolidis and Lee (2010) observed similar levels of  $\alpha$ -amylase inhibitory activity. Moreover, the authors reported that the  $IC_{50}$  values were inversely proportional to the temperature of extraction and the total phenolic content of the



**Fig. 1.** Inhibitory effect of cold water (A) and ethanol (B) extracts on  $\alpha$ -amylase activity. Data represent the mean ( $\pm$ SE) of at least three independent experiments (<sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$  relative to control, <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.05$  relative to acarbose, ANOVA followed by Dunnett's Multiple Comparison test). An, *Ascophyllum nodosum*; Fs, *Fucus serratus*; Fsp, *Fucus spiralis*; Fv, *Fucus vesiculosus*; Pc, *Pelvetia canaliculata*.

seaweed. As shown in Fig. 1B, ethanol extracts of *A. nodosum* ( $IC_{50}$  44.7  $\mu$ g/ml), *F. serratus* ( $IC_{50}$  70.6  $\mu$ g/ml), *F. vesiculosus* ( $IC_{50}$  59.1  $\mu$ g/ml) and *P. canaliculata* ( $IC_{50}$  51.0  $\mu$ g/ml) also had the same inhibitory profile as acarbose. The  $IC_{50}$  for  $\alpha$ -amylase inhibition of the ethanol *F. spiralis* extract was 109.0  $\mu$ g/ml. Like the current findings, ethanol extracts from four brown seaweeds were recently identified as  $\alpha$ -amylase inhibitors, while the green algae tested had no inhibitory effect (Kawamura-Konishi et al., 2012). These studies, along with numerous others (Apostolidis & Lee, 2010; Nwosu et al., 2011), add to the increasing body of evidence, that members of the class Phaeophyceae are an excellent source of  $\alpha$ -amylase inhibitors. While the active doses reported may be out of physiological range to affect  $\alpha$ -amylase at blood level, the  $IC_{50}$  values for cold water and ethanol extracts of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* have strong implications for the activity of salivary  $\alpha$ -amylase. Consumption of these extracts could tentatively reduce the activity of  $\alpha$ -amylase in the saliva and, in turn, decrease postprandial hyperglycaemia.

In the  $\alpha$ -glucosidase inhibition experiments, the  $IC_{50}$  of acarbose was  $\sim$ 150  $\mu$ g/ml which corroborates the value reported by Moon et al. (2011). In that study, the authors examined the effect of extracts from two brown seaweeds, *Ecklonia stolonifera* Okamura and *Eisenia bicyclis* (Kjellman) Setchell, on  $\alpha$ -glucosidase activity and found that their inhibitory effect was substantially stronger than that of acarbose. Similarly, our investigations found that all extracts abolished  $\alpha$ -glucosidase activity at 1000, 100 and 10  $\mu$ g/ml (data not shown). Even at 1  $\mu$ g/ml, cold water extracts of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* significantly ( $P < 0.01$ ) inhibited  $\alpha$ -glucosidase activity over 30 min relative to



**Fig. 2.** Inhibitory effect of 1  $\mu$ g/ml cold water (A) and ethanol (B) extracts on  $\alpha$ -glucosidase activity. Data represent the mean ( $\pm$ SE) of at least three independent experiments (<sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$  relative to control, <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.05$  relative to 10  $\mu$ g/ml acarbose, ANOVA followed by Dunnett's Multiple Comparison test). An, *Ascophyllum nodosum*; Fs, *Fucus serratus*; Fsp, *Fucus spiralis*; Fv, *Fucus vesiculosus*; Pc, *Pelvetia canaliculata*.

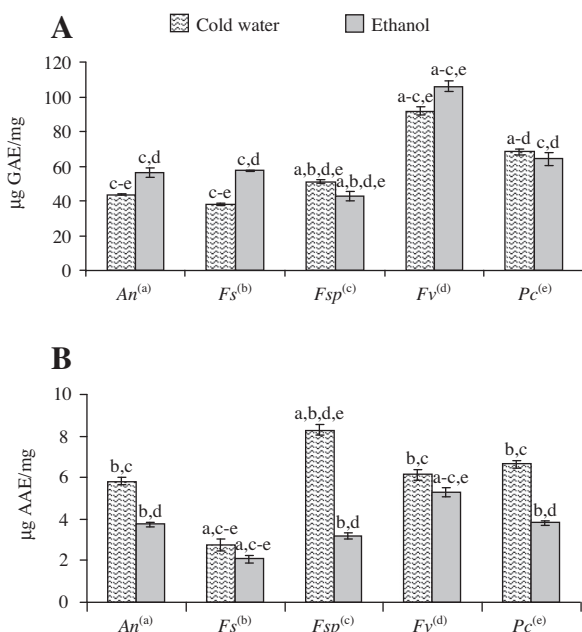


the control (Fig. 2A). With the exception of *P. canaliculata*, comparable levels of inhibition were also found with the ethanol extracts (Fig. 2B). These results support the findings of Apostolidis and Lee (2010) and Nwosu et al. (2011), who found that extracts of *A. nodosum* had strong inhibitory effects on both  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. Moreover, we have identified the *F. vesiculosus* extract as the strongest  $\alpha$ -glucosidase inhibitor regardless of the extraction method used. The  $IC_{50}$  values for  $\alpha$ -glucosidase inhibition of the cold water and ethanol extracts of *F. vesiculosus* were 0.32 and 0.49  $\mu$ g/ml, respectively, making it amongst the most potent seaweed extract studied to date. Indeed, with  $IC_{50}$  values for  $\alpha$ -glucosidase inhibition at <2  $\mu$ g/ml, the physiological relevance of all the cold water and ethanol extracts is quite strong. The presence of the extracts in the blood at these concentrations is attainable, and so makes their  $\alpha$ -glucosidase inhibitory capabilities an attractive and realistic approach to diabetes management.

Only mild  $\alpha$ -amylase inhibition is recommended in order to prevent the abnormal bacterial fermentation of undigested carbohydrates in the colon, which results in flatulence and diarrhoea (Ettxeberria et al., 2012). Given that extracts of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* have a lower inhibitory effect against  $\alpha$ -amylase and stronger inhibitory activity against  $\alpha$ -glucosidase, they may form the basis of a particularly effective therapy for postprandial hyperglycemia with minimal side effects.

### 3.2. Total phenol content and total antioxidant capacity

The ability of naturally occurring polyphenols from plants to inhibit enzymes including  $\alpha$ -amylase and  $\alpha$ -glucosidase has been widely reported (Apostolidis, Karayannakidis, Kwon, Lee, & Seeram, 2011; Apostolidis & Lee, 2010; Honma, Koyama, & Yazawa, 2011; Moon et al., 2011; Roy et al., 2011). Furthermore, a number of these studies have demonstrated a strong correlation between phenolic content, enzyme inhibition and antioxidant properties.



**Fig. 3.** Total polyphenol content (A) and total antioxidant capacity (B) per mg of seaweed extract. Data represent the mean ( $\pm$ SE) of three independent experiments. <sup>a-e</sup>Denote a significant difference between mean values, where <sup>a</sup>denotes a significant difference from *Ascophyllum nodosum* and <sup>d</sup>denotes a significant difference from *Fucus serratus*, etc. ANOVA followed by Tukey's Multiple Comparison test. *An*, *Ascophyllum nodosum*; *Fs*, *Fucus serratus*; *Fsp*, *Fucus spiralis*; *Fv*, *Fucus vesiculosus*; *Pc*, *Pelvetia canaliculata*.

In the current study, the cold water and ethanol extracts contained similar levels of phenolic compounds (Fig. 3A). Ethanol extracts of *F. vesiculosus* had the highest content of total polyphenols (106.3  $\pm$  2.9  $\mu$ g GAE/mg) followed in decreasing order by cold water extracts of *F. vesiculosus* (91.9  $\pm$  2.4  $\mu$ g GAE/mg) and *P. canaliculata* (68.4  $\pm$  1.9  $\mu$ g GAE/mg), ethanol extracts of *P. canaliculata* (64.3  $\pm$  3.6  $\mu$ g GAE/mg), *F. serratus* (57.6  $\pm$  0.4  $\mu$ g GAE/mg) and *A. nodosum* (56.3  $\pm$  2.5  $\mu$ g GAE/mg), cold water extracts of *F. spiralis* (51.2  $\pm$  1.0  $\mu$ g GAE/mg) and *A. nodosum* (43.6  $\pm$  0.4  $\mu$ g GAE/mg), ethanol extracts of *F. spiralis* (42.9  $\pm$  2.5  $\mu$ g GAE/mg) and cold water extracts of *F. serratus* (38.1  $\pm$  0.8  $\mu$ g GAE/mg). Hence, the high levels of phenolic compounds contained in the extracts of *F. vesiculosus* are most likely responsible for the strong  $\alpha$ -glucosidase inhibitory activity observed in Fig. 2. A distinct inverse correlation between phenolic contents of *A. nodosum* and  $IC_{70}$  for  $\alpha$ -glucosidase inhibition was also observed by Apostolidis et al. (2011).

Antioxidants may be defined as proteins, enzymes or other small molecules that, when present at low concentrations compared to those of the oxidizable substrate, significantly delay or inhibit oxidation of that substrate. In addition, they can be classified into preventing antioxidants, scavenging antioxidants, and repair and *de novo* antioxidants (Halliwell, 1996; Niki, 2010). Scavenging antioxidants play their roles by scavenging reactive free radicals to protect biologically essential molecules from oxidative modification, acting as the second line defence *in vivo*. Moreover, the beneficial effects of these antioxidants have been supported by epidemiological studies (Gey, 1998; Niki, 2010; Niki, 2012). Hence, assessment of total antioxidant capacity for scavenging free radicals was performed on the five seaweed species by competition method which is widely used (Niki, 2010; Niki, 2011). As demonstrated in Fig. 3B, the cold water extracts had a higher antioxidant capacity than their ethanol equivalents. In decreasing order, the total antioxidant capacity of the cold water extracts was *F. spiralis* > *P. canaliculata* > *F. vesiculosus* > *A. nodosum* > *F. serratus*, while the ethanol extract with the highest antioxidant capacity was *F. vesiculosus* followed by *P. canaliculata* > *A. nodosum* > *F. spiralis* > *F. serratus*. These results show that the total polyphenol content of *F. vesiculosus* and *P. canaliculata* extracts did correlate to their antioxidant capacity. This is in agreement with an earlier study by Wang et al. (2009), whereby three fucoid species with the highest total polyphenol content displayed the greatest scavenging activities. Interestingly, the extracts of *F. vesiculosus* and *P. canaliculata* also exhibited the strongest inhibitory effect on  $\alpha$ -amylase and  $\alpha$ -glucosidase. Thus, it appears that the antidiabetic properties of these two extracts are associated with phenolic content and/or antioxidant activity.

In contrast to its total polyphenol content, the antioxidant capacity of cold water *F. spiralis* extract was found to be much greater than expected. However, other antioxidant compounds have been identified in seaweeds, including tocopherols, carotenoids and sterols, which may have contributed to the overall scavenging effect (Lordan, Ross, & Stanton, 2011; Pioveti, Deffo, Valls, & Peiffer, 1991; Takaichi, 2011).

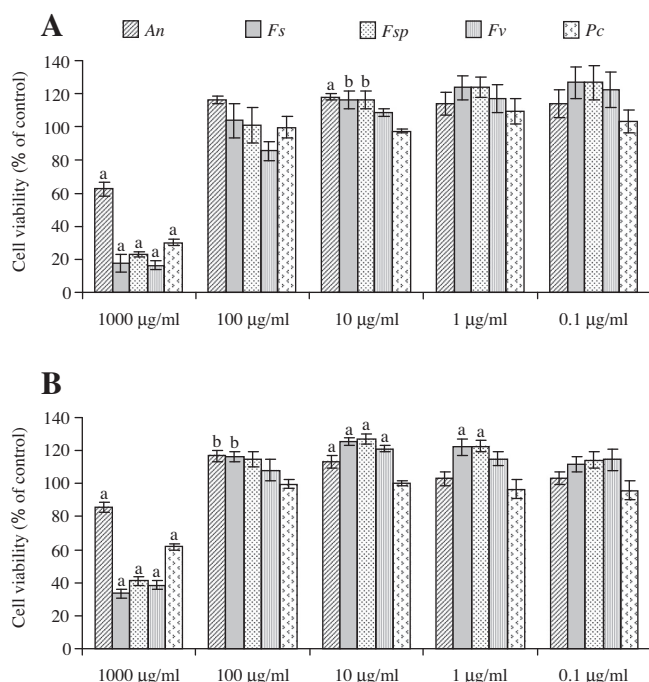
It is important to note, however, that antioxidant capacity *in vivo*, including bioavailability, cannot be assessed by a simple extrapolation of *in vitro* results. Accordingly, and as recently highlighted by Chiva-Blanch and Visioli (2012), future research should focus on identifying actions of antioxidants that are biologically more important, such as anti-inflammatory activity, activation of Phase II enzymes and modulation of the gut microbiota.

### 3.3. Cell viability

While the antidiabetic effects of the seaweed extracts have been established, this study also aims to validate the efficacy of the extracts as prospective functional food ingredients. The

demonstration of safety for a functional ingredient is critical and determination of the toxicological potential is important to predict the consequences of exposure at different dose levels (Kruger & Mann, 2003). Following 48 h exposure, 1000 µg/ml of cold water and ethanol extracts significantly ( $P < 0.01$ ) decreased viability of the Caco-2 cells (Fig. 4). At lower concentrations, however, the cells remained viable for all seaweed extracts. Indeed, some of the extracts significantly ( $P < 0.05$ ) increased cell proliferation, which can be attributed to the supplementation of the cell culture media with nutrients from the extracts. These results are somewhat in contrast to those reported by O'Sullivan et al. (2011), whereby up to 2 mg/ml of seaweed extracts had no effect on Caco-2 cell viability. However, the authors only document the cytotoxic effects at 24 h. It is probable that this incubation time is too short and potential toxic effects did not develop in this time. Hence, it is likely that, following longer exposure, 2 mg/ml of the extracts would have a harmful effect on cells. Also, it is worth noting that very long exposure times, 72 h and longer, can result in an increased number of dead cells in control samples. It is for this reason that 48 h was selected as the most suitable incubation time for this set of experiments.

Inhibition of Caco-2 cell proliferation by seaweed extracts has been found to be related to phenol content (Nwosu et al., 2011). It has emerged that the higher the phenol content of the extract, the higher the induced level of toxicity (Galati & O'Brien, 2004; Nemeikaitė-Čėnienė, Imbrasaitė, Sergedienė, & Čėnas, 2005; Selassie, Kapur, Verma, & Rosario, 2005). As previously noted, these seaweed extracts has high phenol content which may explain the cytotoxic effects caused by them at 1000 µg/ml. Overall, these findings demonstrate that the  $IC_{50}$  of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* extracts for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition are greatly below cytotoxic levels.



**Fig. 4.** Measurement of cytotoxicity using the MTS assay as an indicator of mitochondrial function. Caco-2 cells were treated with 1000, 100, 10, 1 and 0.1 µg/ml cold water (A) or ethanol (B) extracts for 48 h. Results are expressed as a percentage of the control. Data represent the mean ( $\pm$ SE) of at least three independent experiments ( $^aP < 0.01$ ,  $^bP < 0.05$  relative to control, ANOVA followed by Dunnett's Multiple Comparison test). *An*, *Ascophyllum nodosum*; *Fs*, *Fucus serratus*; *Fsp*, *Fucus spiralis*; *Fv*, *Fucus vesiculosus*; *Pc*, *Pelvetia canaliculata*.

Although this study focuses on the antidiabetic potential of seaweed extracts, and the Caco-2 cell line which was used as a model for cultured colonocytes, the findings observed in these cell viability assays could also be looked at from a chemotherapeutic perspective. Owing to their cytotoxic effects, 1000 µg/ml of the seaweed extracts could potentially be employed in the inhibition of cancer development and/or progression. Conversely, the increase in cell growth observed following exposure to lower doses of the extracts should be given some consideration as they may support the proliferation of carcinogenic disease.

#### 4. Conclusions

With the prevalence of type II diabetes on the increase, efforts are being made to identify natural therapies that can control hyperglycaemia and its knock-on effects. The results of this study demonstrate the efficacy of brown seaweed extracts, in particular *F. vesiculosus* and *P. canaliculata*, to inhibit enzymes involved in intestinal carbohydrate digestion and assimilation. Due to their availability and strong inhibitory properties, these algal extracts have potential for use in functional food applications aimed at lowering glycaemic response. Additionally, the extracts are capable of inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase at non-toxic levels, and cold water and ethanol extraction are desirable for food products because of the absence of solvent residues. While it appears that polyphenols, and more specifically phlorotannins, are responsible for the inhibition and scavenging activities of these seaweed extracts, this needs to be confirmed. Thus, future studies will include fractionation of extracts, isolation and characterisation of bioactive components, and further examination of biological activities.

#### Acknowledgements

This project (Grant-Aid Agreement No. MFFRI/07/01) was carried out under the *Sea Change* Strategy with the support of the Marine Institute and the Department of Agriculture, Food and the Marine, funded under the National Development Plan 2007–2013.

The authors thank Dr. Pádraig Harnedy of the University of Limerick for her valuable help and advice, and Ms. Natalie Hefferan of Teagasc Food Research Centre, Ashtown, for her assistance with the extractions.

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