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Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds

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ABSTRACT

Screening of potential antioxidant activities of water and 70% acetone extracts from ten species of Icelandic seaweeds was performed using three antioxidant assays. Significant differences were observed both in total phenolic contents (TPC) and antioxidant activities of extracts from the various species evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, oxygen radical absorbance capacity (ORAC) and ferrous ion-chelating ability assays. Acetone extracts from three Fucoid species had the highest TPC and consequently exhibited the strongest radical scavenging activities. High correlation was found between TPC of seaweed extracts and their scavenging capacity against DPPH and peroxyl radicals, indicating an important role of algal polyphenols as chain-breaking antioxidants. However, water extracts generally had higher ferrous ion-chelating activity than 70% acetone extracts and no correlation was found with their TPC, suggesting that other components such as polysaccharides, proteins or peptides in the extracts were more effective chelators of ferrous ions than phenolic compounds.

1. Introduction

Natural antioxidants with multifunctional potential are of high interest as alternatives for synthetic antioxidants to prevent oxidation in complex food systems like muscle food. Numerous studies have focused on natural antioxidants in terrestrial plants and their application in food systems to prevent oxidation. Aquatic plants are also gaining interest as a potential source of antioxidants. Results have shown that marine macroalgae are a rich source of various natural antioxidants such as polyphenols, which play an important role in preventing lipid peroxidation. A series of polyphenolic compounds such as catechins (e.g. gallocatechin, epicatechin and catechin gallate), flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae (Santoso, Yoshie, & Suzuki, 2002; Yoshie, Wang, Petillo, & Suzuki, 2000; Yoshie-Stark, Hsieh, & Suzuki, 2003). Phlorotannins, a group of phenolic compounds which are restricted to polymers of phloroglucinol, have been identified from several brown algal families such as Alariaceae, Fucaceae and Sargassaceae. Many studies have shown that phlorotannins are the only phenolic group detected in brown algae (Jormalainen & Honkanen, 2004; Koivikko, Loponen, Pihlaja, & Jormalainen, 2007). Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (Ahn et al., 2007). The multifunctional antioxidant activity of polyphenols is highly related to phenol rings which act as electron traps to scavenge peroxy, superoxide-anions and hydroxyl radicals. Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings (Hemat, 2007). In addition, sulphated polysaccharides, carotenoid pigments including astaxanthin and fucoxanthin have also been demonstrated to possess excellent antioxidant potential (Kobayashi & Sakamoto, 1999; Miyashita & Hosokawa, 2008; Rupérez, Ahrazem, & Leal, 2002; Yan, Chuda, Suzuki, & Nagata, 1999; Zhao, Xue, & Li, 2008).

Because of the reported multifunctional properties of seaweed extracts, their exploitation as a source of natural antioxidants for application in complex food system like fish muscle is of interest. Lipid oxidation is a principal cause of quality deterioration in muscle foods during processing and storage, resulting in the production of rancid odours and unpleasant flavours, changes of colour and texture as well as lowering nutritional value of foods (Hultin, 1994; Jittrepotch, Ushio, & Ohshima, 2006). Lipid oxidation of

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muscle phospholipids in fish may be induced by several catalysts, including iron from blood haemoglobin (Jónsdóttir, Bragadóttir, & Ólafsdóttir, 2007; Richards & Hultin, 2002). Many synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroxyquinone (TBHQ) and propyl gallate have been widely used in different food products. However, because of the potential health hazards, their use as food additives is under strict regulation in many countries. Moreover, BHT and α-tocopherol have been reported to be ineffective in retarding the oxidative deterioration in complex food system as fish muscle where both lipoxygenase (LOX) and haemeproteins are involved in the initiation of oxidation (He & Shahidi, 1997).

The first step in the search of a versatile antioxidant system based on seaweed is to characterise their antioxidant activity. When exploring the antioxidant potential of different seaweed species, the use of a single test is insufficient to identify the different mechanisms involved. Therefore, three antioxidant assays, DPPH radical scavenging activity, ferrous ion-chelating ability and ORAC, were chosen to evaluate the antioxidant activity of different seaweed species in this study. DPPH method measures the radical scavenging activity in organic systems and has been used extensively as a pre-screening method for new antioxidants from natural resources, due to its stability, simplicity, rapidity and reproducibility (Chen et al., 2008). Metal chelating capacity is claimed as one of the important mechanisms of antioxidant activity. The ferrous ions are the most powerful pro-oxidants among various species of transition metals present in food system (Hultin, 1994; Yomauchi, Tatsumi, Asano, Kato, & Ueno, 1988). However, oxidised haemeproteins such as met-haemoglobin and met-myoglobin have been reported to be more potent pro-oxidants than low-molecular-weight ferrous iron in fish muscle (Richards & Hultin, 2000; Undeland, Hultin, & Richards, 2003). In addition, ORAC assay was selected to measure the peroxyl radical absorption capacity of seaweed extracts. This methodology is regarded to be more biologically relevant than DPPH and other similar protocols and has been demonstrated to be especially useful for food samples and crude plant extracts when multiple constituents co-exist and complex reaction mechanisms are involved (Huang, Ou, & Prior, 2005). However, to our knowledge, no detailed studies have been performed on antioxidant potentials of seaweed extracts by using the ORAC assay.

The coastlines of Iceland are an abundant resource of seaweeds with broad species diversity, but little effort has been made to explore the antioxidant potential of seaweeds harvested in Iceland. Therefore, the aim of the present study was to screen for antioxidant activities in various types of edible Icelandic seaweeds by using three *in vitro* antioxidant activity assays and compare the effectiveness of water and solvent extraction. In addition, correlations between TPC and antioxidant activities were investigated to characterise the antioxidant properties. These pre-screening experiments reported herein will be a basis to selectively identify the most appropriate species for further characterisation and to evaluate suitability of active components from seaweed extracts as natural antioxidants for application in food muscle systems.

2. Materials and methods

2.1. Algal materials

Eight seaweed species, including six brown algae (Phaeophyta) (*Fucus vesiculosus* Linnaeus, *Fucus serratus* Linnaeus, *Laminaria hyperborea* (Gunnerus) Foslie, *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders (= *Laminaria saccharina* (Linnaeus) Lamouroux), *Laminaria digitata* (Hudson) Lamouroux, *Alaria esculenta* (Linnaeus) Greville) and two red algae (Rhodophyta) (*Palmaria palmata* (Linnaeus) Kuntze, *Chondrus crispus* Stackhouse) were collected in Hvassahraun coastal area nearby Hafnarfjordur, southwestern Iceland on March 19th, 2007. One brown algae (*Ascophyllum nodosum* (Linnaeus) LeJolis) and one green algae (Chlorophyta) (*Ulva lactuca* Linnaeus) were collected from the same area on May 16th, 2007. The freshly collected seaweeds were washed with clean seawater to remove salt, epiphytes and sand attached to the surfaces of the samples and transported to the laboratory. The samples were carefully rinsed with tap water, wiped with paper towel. For *L. hyperborea* and *S. latissima*, the stipes and hapteres were removed and the new and old parts of the blades were separated. The samples were lyophilised for 72 h, pulverised into powder and stored at −80 °C prior to extraction.

2.2. Chemicals

2,2́-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Iron (II) chloride and GC-grade acetone were purchased from Sigma–Aldrich (Steinheim, Germany). Fluorescein sodium salt (FL), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid monosodium salt (Ferrozine), Folin–Ciocalteu's phenol reagent, phloroglucinol, α-tocopherol, citric acid (anhydrous) were obtained from Fluka (Buchs, Switzerland). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT) and L-ascorbic acid were from Sigma–Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2{\cdot}2H_2O$) was from ICN Biomedical Inc. (Aurora, OH, USA). All other reagents were of analytical grade.

2.3. Preparation of sample extract

Five grams of the algal powder was mixed with 100 ml of distilled water or 70% aqueous acetone (v/v), incubated in a platform shaker (Innova™ 2300, New Brunswick Scientific, Edison, NJ) for 24 h at 200 rpm and at room temperature. The mixture was centrifuged at 3500 rpm for 10 min at 4 °C and filtered with Whatman no. 4 filter paper. Acetone in the solvent extract was removed by rotary evaporation. The concentrate and the supernatant of water extract were freeze-dried and weighed. Each extraction was conducted in duplicate. The extraction yield was expressed as g dried extract/100 g dried algal powder. The dried duplicate extracts were pooled and stored at −80 °C until analysed. Each dried extract was then re-dissolved in distilled water at a concentration of 5 mg/ml as a stock solution. The stock solution was used both for the determination of TPC and antioxidant activities.

2.4. Determination of total phenolic content

The TPC of seaweed extract was determined in accordance with a protocol described by Turkmen, Sari, and Velioglu (2005) with minor modifications. One millilitre aliquot of each diluted sample (the extract stock solutions further diluted with distilled water, concentration ranged from 0.25 (*F. vesiculosus*) to 5 mg/ml (*L. digitata*)) was mixed with 5 ml of Folin–Ciocalteu reagent (10% in distilled water) in a test tube. After 5 min, 4 ml of sodium carbonate (7.5% in distilled water) were added to each tube, the test tubes were cap-screwed and vortexed. The samples were incubated for 2 h at room temperature in the darkness. The absorbance was measured at 725 nm with a UV–vis spectrophotometer (Ultrospec 3000 pro, Amersham Pharmacia Biotech, Ltd., Cambridge, UK). A standard curve with serial phloroglucinol solutions (ranging from 20 μg/ml to 100 μg/ml) was used for calibration. The analyses were done in triplicate. Results were expressed as gram of phloroglucinol equivalents (PGE) per 100 g of extract.

2.5. DPPH radical scavenging activity assay

The method of Brand-Williams, Cuvelier, and Berset (1995) as modified by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998) was used for measuring the DPPH radical scavenging ability of seaweed extracts.

The extract stock solutions were further diluted with distilled water at various ratios (at least 5 different dilutions were prepared for each extract) based on their free radical scavenging activities. An aliquot of each dilution (0.1 ml) was added to 3.9 ml of DPPH· (64 μM or 2.5×10^{-2} g/l in methanol prepared daily). The mixtures were allowed to stand for 2 h at room temperature. The absorbance was measured at 515 nm with the Ultrospec 3000 pro UV–vis spectrophotometer. L-ascorbic acid, BHT and α-tocopherol were used as reference standards. All measurements were performed in duplicate.

The calibration curve made with DPPH· between 4.15×10^{-3} and 4.15×10^{-2} g/l was used to calculate the remaining concentration of DPPH· in the reaction medium.

The percentage of remaining DPPH· (%$DPPH^{\cdot}_R$) was calculated as follows:

$$\%[DPPH^{\cdot}_R] = [(DPPH^{\cdot})_T/(DPPH^{\cdot})_{T=0}] \times 100$$

where $(DPPH^{\cdot})_{T=0}$ was the concentration of DPPH· at time zero (initial concentration) and $(DPPH^{\cdot})_T$ was the concentration of DPPH· after 2 h.

The percentage of remaining DPPH· was plotted against the sample/standard concentration to obtain EC_{50} value, which represents the concentration of the extract or standard antioxidant (mg/ml) required to scavenge 50% of the DPPH· in the reaction mixture. Its reciprocal, the antiradical power (ARP, $ARP = 1/EC_{50}$) was also calculated for each of the extracts.

2.6. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed according to Davalos, Gomez-Cordoves, and Bartolome (2004) with slight modifications. Mx300 real-time PCR System (Stratagene Inc., La Jolla, CA) was used for the fluorescence measurements controlled by MxPro computer program. All reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4). The extract stock solutions were further diluted appropriately with 75 mM, pH 7.4 sodium phosphate buffer (concentration range from 0.005 (*F. vesiculosus*) to 1 mg/ml (*L. digitata*)). Ten microlitres of each diluted sample were placed in the well (200 μl) of a microplate. Afterwards 60 μl fluorescein (70 nM) were added and the mixtures were pre-incubated at 37 °C for 15 min. Subsequently, 30 μl AAPH (12 mM) were added rapidly using a multichannel pipette to initiate the oxidation reaction. The microplate was placed immediately in the reader and the fluorescence (FAM) was recorded every 0.1 min for the first 20 cycles and every minute thereafter until the fluorescence of the last reading declined to <5% of the initial value (around 80 min). Excitation and emission filter wavelengths were set at 484 nm and 520 nm, respectively. 75 mM phosphate buffer (pH 7.4) was used as a blank. At least three independent assays were performed for each sample.

The antioxidant curves (fluorescence versus time) were normalised. The data from the curves were multiplied by the factor:

$$\frac{\text{fluorescence}_{\text{blank},t=0}}{\text{fluorescence}_{\text{sample},t=0}}$$

The area under the fluorescence decay curve (AUC) was calculated by the normalised curves with the following equation:

$$AUC = (0.5 + f_1/f_0 + f_2/f_0 + \cdots + f_{20}/f_0) \times 0.1 + (f_{21}/f_0 + f_{22}/f_0 + \cdots + f_{79}/f_0) \times 1 + 0.5 \times (f_{80}/f_0)$$

where f_0 was the fluorescence reading at the initiation of the reaction and f_{80} was the last measurement.

The net AUC was obtained by subtracting the AUC of the blank from that of a sample or standard. The ORAC value was calculated and expressed as micromoles of Trolox equivalents per gram extract (μmol of TE/g extract) using the calibration curve of Trolox.

2.7. Ferrous ion-chelating ability assay

The ferrous ion-chelating ability was determined according to the method of Decker and Welch (1990) with minor modifications. One hundred microlitres of each extract stock solution (5 mg/ml) were mixed with 135 μl of distilled water and 5 μl of 2 mM $FeCl_2$ in a microplate. The reaction was initiated by the addition of 10 μl of 5 mM ferrozine. The solutions were well mixed and allowed to stand for 10 min at room temperature. After incubation, the absorbance was measured at 562 nm with a Tecan Sunrise microplate reader (Tecan Austria Gesellschaft, Salzburg, Austria). Distilled water (100 μl) instead of sample solution was used as a control. Distilled water (10 μl) instead of ferrozine solution was used as a blank, which is used for error correction because of unequal colour of the sample solutions. L-ascorbic acid, citric acid and EDTA-Na_2 were used as reference standards. All measurements were performed in triplicate. The ferrous ion-chelating ability was calculated as follows:

$$\text{Ferrous ion} - \text{chelating ability}(\%) = [(A_0 - (A_1 - A_2)]/A_0 \times 100.$$

where A_0 was the absorbance of the control, A_1 was the absorbance of the sample or standard and A_2 was the absorbance of the blank.

2.8. Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's test was carried out to test for differences between species and extractants (water and 70% acetone) in the statistical program NCSS 2000 (NCSS, Kaysville, Utah, USA). Significance of differences was defined at the 5% level ($p < 0.05$). Multivariate analysis was performed by the Unscrambler 9.7 software package (CAMO AS, Trondheim, Norway). The main variance in the data set was detected using principal component analysis (PCA). All data were mean centred and scaled to equal unit variance prior to PCA. Full cross-validation was used in the validation models. Partial least square regression (PLSR) was used to test the quantitative correlation between TPC and antioxidant properties of all the water and 70% acetone extracts. DPPH, ORAC and chelating activity were used as *X* predictors and TPC as *Y* response factor.

3. Results and discussion

3.1. Extraction yield

Considerable variations in extraction yield were found among different seaweed species (data not shown). The highest extraction yield was recorded for the water extract of *U. lactuca* (44.7 g of dried extract/100 g dried algal powder) whereas the lowest for 70% acetone extract of *C. crispus* (10.5 g of dried extract/100 g dried algal powder). Extractants also have an impact on the yield. For most seaweed species, the extraction yields of water extracts were higher than those of 70% acetone extracts which indicated that most of the soluble components in seaweeds were high in polarity. It should also be noted that the water extracts of *L. hyperborea* (both old and new blades) and *S. latissima* (new blades) were very viscous and difficult to filtrate through the filter paper due to the high content of alginate in the extracts, resulting in low extraction yield. Interestingly, large differences were observed between water

extracts and 70% acetone extracts for some species such as *U. lactuca*, *P. palmata* and *C. crispus*. These seaweeds are known to contain high levels of water-soluble components, such as soluble polysaccharides, protein and peptides (Galland-Irmouli et al., 1999) which were poorly extracted by 70% acetone.

3.2. Total phenolic content

Significant differences were found in TPC among different seaweed species, ranging from 0.4 to 24.2 g PGE/100 g extract (Fig. 1). Brown algae generally contained higher amounts of polyphenols than red and green algae. Intra-thallus variation in TPC was also observed both in *L. hyperborea* and *S. latissima*. Old blades had higher TPC values compared to new blades. This is in agreement with earlier studies by Connan, Delisle, Deslandes, and Ar Gall (2006) who reported that there was a high correlation between the age of the tissues and TPC for *L. hyperborea*. High levels of TPC were found in fucoid seaweed species. The TPC in 70% acetone extracts of *A. nodosum*, *F. serratus* and *F. vesiculosus* were 15.9, 24.0 and 24.2 g PGE/ 100 g extract, respectively while the TPC of the water extracts of *A. nodosum*, *F. serratus* and *F. vesiculosus* were 13.8, 16.9 and 17.6 g PGE/100 g extract, respectively. The highest amount of TPC was observed for 70% acetone extract of *F. vesiculosus*. Similar levels of TPC were reported for 70% acetone extract (30 g PGE /100 g extract on dry weight basis) and methanol extract (23.21 g PGE /100 g extract) of *F. vesiculosus* from the Finnish Archipelago Sea and Atlantic coast of Canada (Jormalainen, Honkanen, Vesakoski, & Koivikko, 2005; Zhang et al., 2006).

For most seaweed species, 70% aqueous acetone was more efficient to extract polyphenolic compounds compared to water (Fig. 1). Phenolic compounds are generally more soluble in polar organic solvents than in water. The effective extractants recommended are aqueous mixtures of methanol, ethanol and acetone (Waterman & Mole, 1994). Koivikko, Loponen, Honkanen, and Jormalainen (2005) compared the extraction efficacy of soluble polyphenols (mostly phlorotannins) from *F. vesiculosus* by using eight extractants with different polarities. 70% aqueous acetone (v/v) was found to be the most efficient solvent. It has been postulated that acetone has the ability to inhibit protein–polyphenol complex formation during extraction (Hagerman, 1988) or even break down hydrogen bonds formed between phenolic group and protein carboxyl group (Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995). On the contrary, other compounds such as water-soluble polysaccharides, proteins and organic acids were simultaneously extracted when using water as only extractant (Chirinos, Rogez, Campos, Pedreschi, & Larondelle, 2007). Because of safety concerns regarding the use of some organic solvent extracts in food, the optimization of extraction of polyphenols from *F. vesiculosus* by using approved food-grade solvent such as food-grade alcohol and ethyl acetate is now in progress in our laboratory.

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

The DPPH radical scavenging activity of water and 70% acetone seaweed extracts increased in a concentration-dependent manner and also increased with the increment of the incubation time (data not shown).

The comparison of DPPH radical scavenging activity of water and 70% aqueous acetone extracts of seaweeds are shown in Fig. 2. Generally, 70% aqueous acetone extracted more compounds with scavenging abilities on DPPH radicals than water. As pointed out by some researchers, change in extractant polarity alters its efficacy to extract a specific group of antioxidant compounds and influences the antioxidant properties of the extracts. (Zhou & Yu, 2004). Among all of the 70% acetone extracts, *F. vesiculosus* exhibited the most effective scavenging ability on DPPH radicals (ARP = 93.9, $EC_{50} = 10.7\times10^{-3}$ mg/ml), followed by *F. serratus* (ARP = 90.8, $EC_{50} = 11.0\times10^{-3}$ mg/ml), *A. nodosum* (ARP = 54.1, $EC_{50} = 18.5\times10^{-3}$ mg/ml), the old blades of *L. hyperborea* (ARP = 38.8, $EC_{50} = 25.8\times10^{-3}$ mg/ml) and *S. latissima* (ARP = 33.6, $EC_{50} = 29.8\times10^{-3}$ mg/ml). The other extracts showed relatively weak scavenging potentials. The lowest ARP value (0.4, EC_{50} = 2.5 mg/ml) was found in 70% acetone extract of *C. crispus*, which was approximately 230-fold lower than that of *F. vesiculosus* (Fig. 2). It was observed that the extracts containing high levels of TPC were also potent DPPH radical scavenger, suggesting that algal polyphenols may be the principal constituents responsible for the antiradical properties of the extracts. Nevertheless, it should be pointed out that because of the non-specifity of 70% acetone extraction, other classes of antioxidant compounds such as fucoxanthin and sterols could be partially and simultaneously extracted and thus may have contributed to the overall activities.

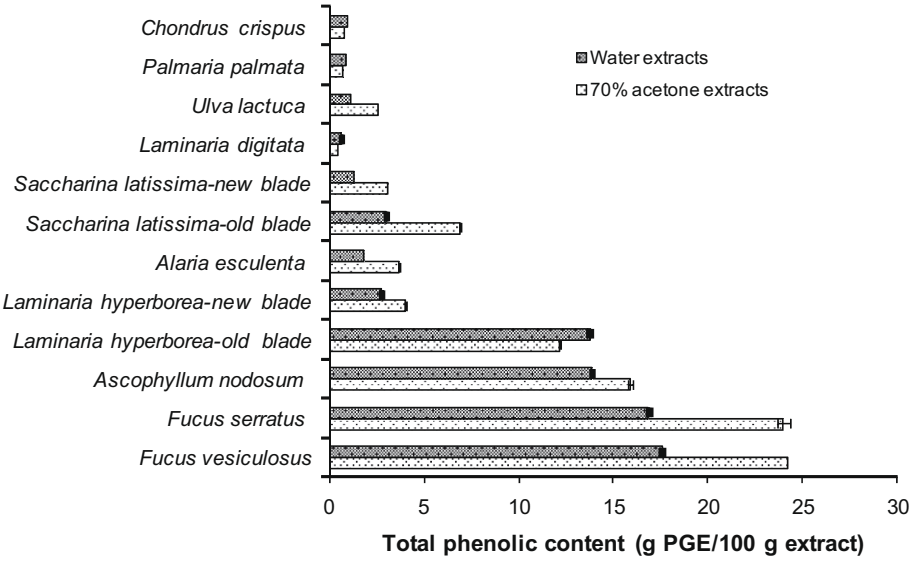

Fig. 1. Total phenolic content (TPC) in water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds. Values are means ± S.D. (n = 3).

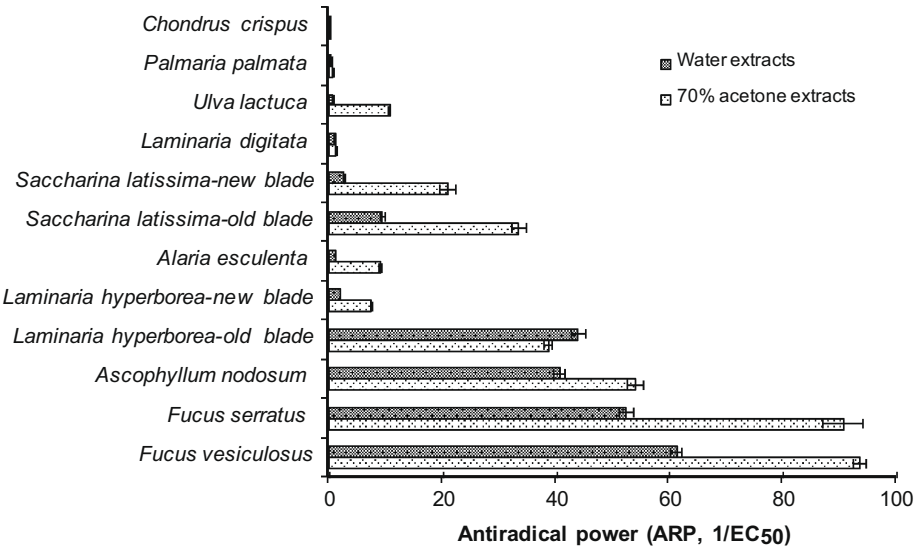

Fig. 2. DPPH radical scavenging activity (antiradical power (ARP); ARP = $1/EC_{50}$) of water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds ($n = 2$, bars are mean dev.).

All the reference compounds, ascorbic acid (ARP = 400.9, $EC_{50} = 2.5\times10^{-3}$ mg/ml), BHT (ARP = 305.2, $EC_{50} = 3.3\times10^{-3}$ mg/ml) and α-tocopherol (ARP = 168.6, $EC_{50} = 5.9\times10^{-3}$ mg/ml), exhibited higher DPPH radical scavenging effect when compared with all the seaweed extracts tested.

Because of different extraction, measurement methods and units used in the various antioxidant activity studies on seaweed reported in the literature, direct comparison of our results on radical scavenging activity of seaweed extracts with other studies is not feasible. However, similar tendency was observed by Jiménez-Escrig, Jiménez-Jiménez, Pulido, and Saura-Calixto (2001) who reported that brown seaweeds generally showed better DPPH scavenging capacity than red seaweeds. The highest scavenging activity was recorded in *Fucus* species and no activity was detected for *C. crispus*.

3.3.2. ORAC

Further evaluation of the chain-breaking antioxidant activities of different Icelandic seaweed extracts was conducted by ORAC-FL assay. The peroxyl radical scavenging activities of seaweed extracts varied considerably from 4 to 2567 μmol of TE/ g extract, depending on seaweed species and extractant used (Fig. 3). In general, 70% aqueous acetone extracts showed higher scavenging activities against peroxyl radicals than water extracts. The highest ORAC value was determined in the 70% acetone extract from *F. vesiculosus* (2567 μmol TE/g extract), followed by *F. serratus* (2545 μmol TE/g extract), *A. nodosum* (1417 μmol TE/g extract) and old blades of *L. hyperborea* (975 μmol TE/g extract). *L. digitata* showed very low peroxyl radical scavenging activity, which was around 640 times weaker than that of *F. vesiculosus*.

Because of lack of ORAC data on seaweeds, our results cannot be evaluated by comparing with peroxyl radical scavenging activities of other seaweed species. However, the ORAC values of three fucoid species in the present study were comparable to or even higher than those reported in the literature for various high antioxidant fruit and spice extracts, including 50% acetone extract of strawberry (540 μmol of TE/g extract) (Huang, Ou, Woodill, Flanagan, & Prior, 2002), 80% ethanol extract of blackberry (674.2 μmol of TE/g extract), (Elisia, Hu, Popovich, & Kitts, 2007), 50% acetone extract of cinnamon (1256 μmol of TE/g extract), (Su et al., 2007), but still lower than methanol extract of rosemary (2800–4360 μmol of TE/g extract) (Ho, Tsai, Tsai, & Lin, 2008; Tsai, Tsai, Chien, Lee, & Tsai, 2008) and Nutmeg (3570 μmol of TE/g extract) (Ho et al., 2008).

3.3.3. Ferrous ion-chelating activity

Metal chelating activities of all algal extracts were tested at a concentration of 5 mg/ml. In general, water extracts showed significantly higher ferrous ion-chelating capacity than 70% acetone extracts (Fig. 4). In accordance with DPPH and ORAC results, potent chelation abilities were again detected in the water extracts from three fucoid species, near or above 95% at 5 mg/ml. Interestingly, however, water extracts from *P. palmata* and *C. crispus* with significantly lower polyphenol levels as well as weaker scavenging activities against DPPH and peroxyl radicals, also exhibited equivalent chelating effects. The high binding capacities to different heavy metals of algal dietary fibres such as alginate, fucoidan from brown algae and carrageenan, agar from red algae are well documented. However, there are contradictory reports in the literature regarding metal chelating capacities of polyphenols. Some studies have demonstrated that polyphenols derived from brown seaweeds are potent ferrous ion chelators (Chew, Lim, Omar, & Khoo, 2008; Senevirathne et al., 2006) and metal chelating potency of phenolic compounds are dependent upon their unique phenolic structure and the number and location of the hydroxyl groups (Santoso, Yoshie-Stark, & Suzuki, 2004). In contrast, other authors have claimed that metal chelation play a minor role in the overall antioxidant activities of some polyphenols (Rice-Evans, Miller, & Paganga, 1996). The study conducted by Toth and Pavia (2000) showed that other compounds such as polysaccharides (e.g. alginates and fucoidan) and/or phytochelatins were more effective than phlorotannins for the detoxification and resistance to copper accumulation in *A. nodosum*. Andjelkovic et al. (2006) reported that the ability of phenolic compounds to chelate iron were far lower than that of EDTA. In addition, some peptides as well as proteins have also been reported to possess the abilities to chelate metal ions (Saiga, Tanabe, & Nishimura, 2003).

For comparison, several standards were also tested in this experiment. EDTA-Na_2 was excellent chelator for ferrous ions and its chelating capacity was 99.8% at a concentration as low as 0.05 mg/ml, much higher than all the seaweed extracts. Citric acid showed considerably lower effect of 21.5% at 5 mg/ml. Ascorbic acid had rather weak chelating capacity, which was only 8.2% at 20 mg/ml.

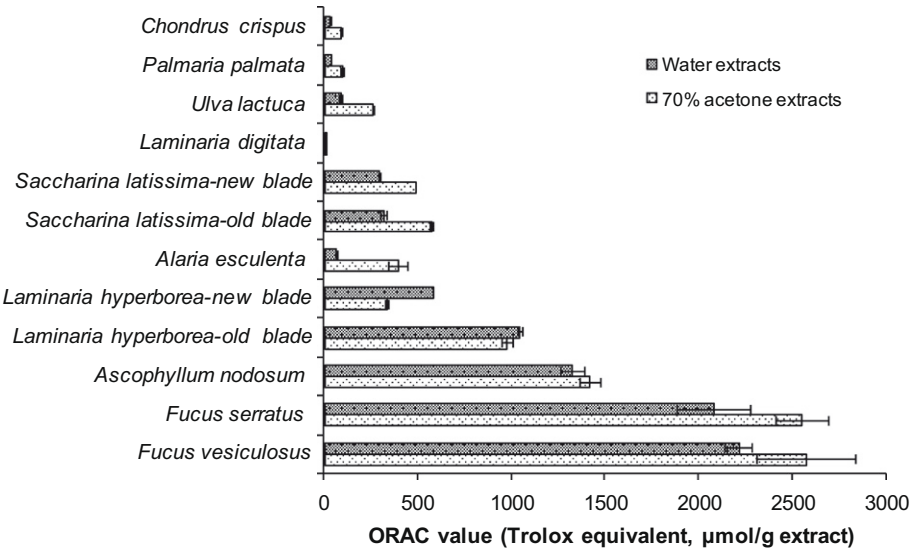

Fig. 3. Oxygen radical absorbance capacity (ORAC) of water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds. Values are means ± S.D. (n = 3).

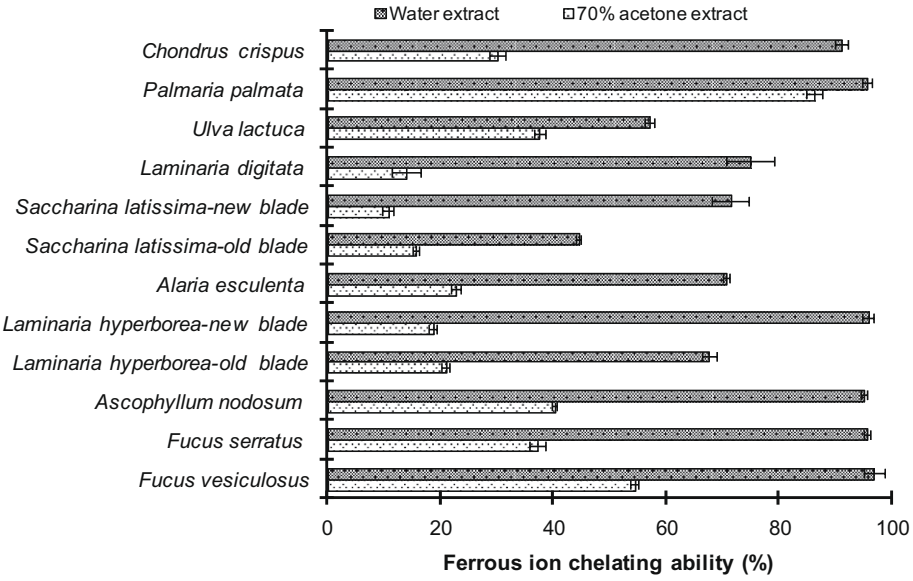

Fig. 4. Ferrous ion-chelating activities of water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds. Values are means ± S.D. (n = 3).

3.4. Correlations between TPC and different antioxidant activity assay

PCA was carried out to gain an overview of the similarities and differences among the 10 algal species and to investigate the relationships among TPC and different antioxidant activity assays. The first two principal components explained 74% and 25% of the total variance in the data set, respectively (Figs. 5 and 6). PC1 showed high correlation with TPC, ORAC and DPPH radical scavenging activity. Accordingly, three fucoid seaweeds (*F. vesiculosus, F. serratus* and *A. nodosum*) with the highest TPC, DPPH radical scavenging activities and ORAC values were located furthest to the right along PC1 (Fig. 5). On the other hand, *L. digitata, P. palmata* and *C. crispus* with rather low levels of TPC as well as weak scavenging activities against DPPH and peroxyl radicals were situated on the opposite side of PC1. It is noted that TPC, ORAC and DPPH radical scavenging activity were closely loaded on PC1 (Fig. 6), indicating that TPC and these two antioxidant properties were highly correlated with each other. PC2 mainly explained variation between samples with regard to ferrous ion-chelating ability. Almost all the water extracts appeared in the positive part of the PC2 due to their relatively high ferrous ion-chelating capacity whereas 70% acetone extracts, with the exception of *P. palmata*, were all located in the negative part of the PC2. In addition, ferrous ion-chelating capacity loaded heavily on the second component while TPC has low loading, which illustrates well that no clear correlation exists between TPC and chelating capacity and thus phenolic compounds do not appear to be effective metal chelators (Fig. 6).

High and significant correlation between TPC and DPPH radical scavenging activity of seaweed extracts was further demonstrated by Pearson correlation analysis (WE: r^2 = 0.99; AE: r^2 = 0.99). Similarly, a high correlation was also obtained between TPC and ORAC value (WE: r^2 = 0.96; AE: r^2= 0.99). Previous studies have shown that phenolic compounds are the main contributors to the antioxidant activity of various seaweeds. A positive correlation has been documented between TPC and antioxidant activity of different seaweed extracts by many researchers (Athukorala, Kim, & Jeon, 2006;

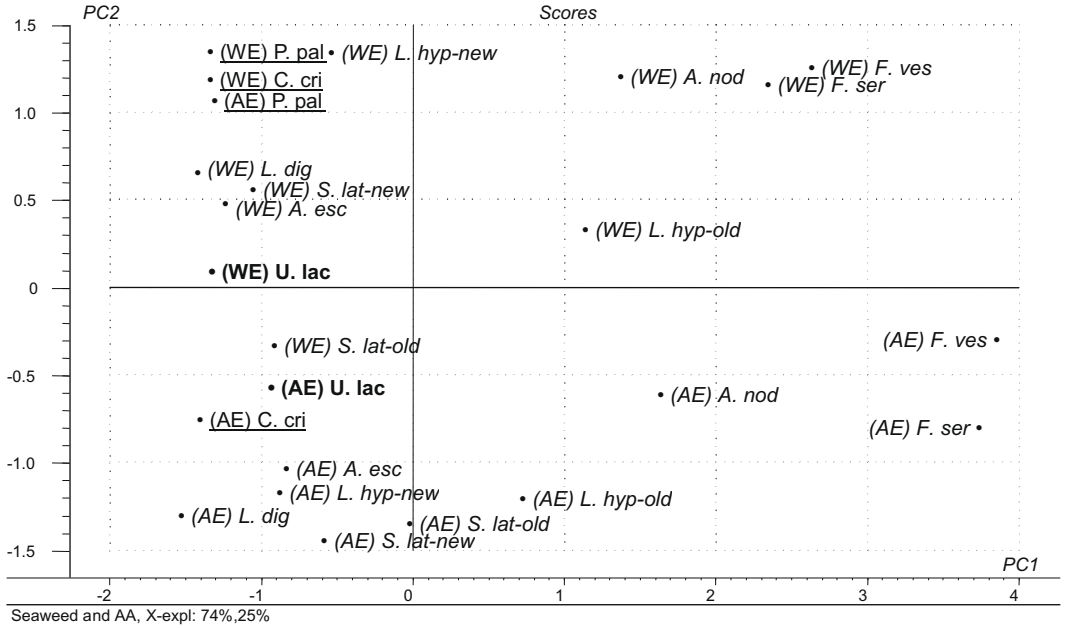

Fig. 5. PCA score plot of total phenolic content (TPC) and antioxidant activities (AA) (DPPH, ORAC and ferrous ion-chelating activity) of water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds (F. ves, *F. vesiculosus*; F. ser, *F. serratus*; A. nod, *A. nodosum*; L. hyp, *L. hyperborea*; A. esc, *A. esculenta*; S. lat, *S. latissima*; L. dig, *L. digitata*; U. lac, *U. lactuca*; P. pal, *P. palmata*; C. cri, *C. crispus*). Brown seaweeds are indicated by italics; red seaweeds by underline; green seaweeds in bold font.

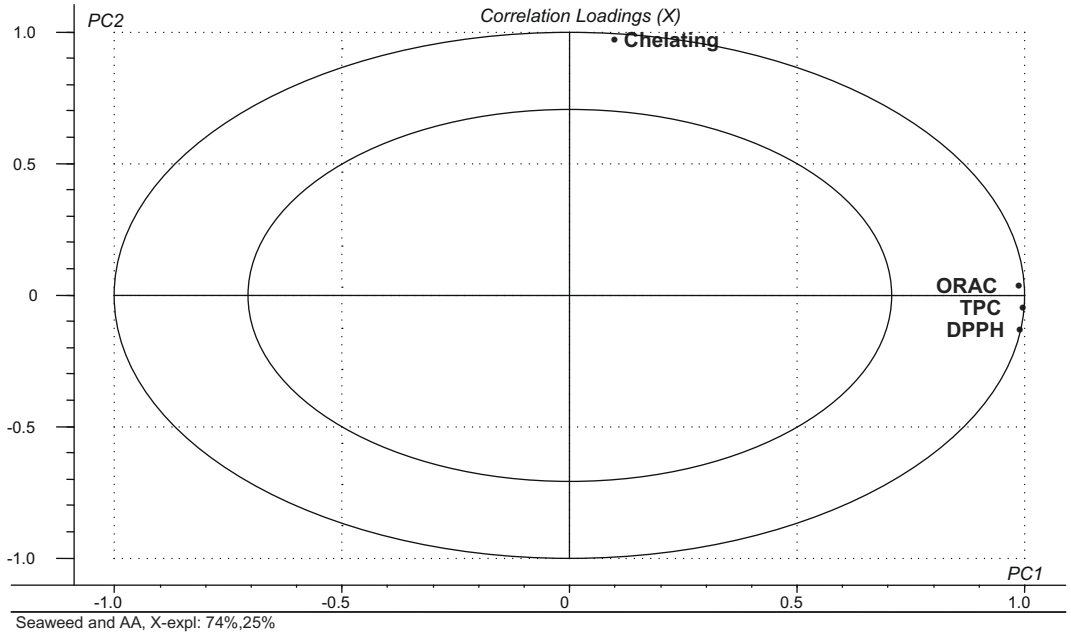

Fig. 6. PCA loading plot of total phenolic content (TPC) and antioxidant activities (AA) (DPPH, ORAC and ferrous ion-chelating activity) of water (WE) and 70% acetone extracts (AE) from selected Icelandic seaweeds.

Jiménez-Escrig et al., 2001; Karawita et al., 2005). A study on the phenolic composition of brown algae *Eisenia bicyclis* by HPLC analyses revealed that the crude polyphenol fraction (ethyl acetate subfraction of methanol extract) was mainly composed of phlorotannin compounds (more than 82%) as well as small amounts of other unknown compounds (Shibata et al., 2004). Preliminary results from a study in our laboratory using similar procedures for the fractionation of *F. vesiculosus* extract showed that the polyphenol-enriched fraction (ethyl acetate fraction) possessed higher DPPH radical scavenging activity than any other fractions as well as the original crude extract (unpublished data). Therefore, phlorotannins, the most abundant group of polyphenols in these seaweeds, appear to be the major contributors to the high scavenging activities. However, this needs to be verified by further characterisation of the extracts. Other active components in different seaweed extracts may have synergistic effects on the scavenging activities, which can give contradictory results. Therefore the assumed role of polyphenols as being mainly responsible for scavenging abilities may be overestimated. Heo, Park, Lee, and Jeon (2005) reported that some enzymatic extracts of *E. cava* and *S. coreanum* exhibited only weak DPPH radical scavenging activities, even though they contained the same level of TPC as other extracts

with higher antiradical activities. Other unknown compounds such as low-molecular-weight polysaccharides, proteins or peptides could also contribute to the scavenging effect.

Interestingly, ferrous ion-chelating ability of water and 70% acetone extracts correlated neither with TPC, nor with DPPH or ORAC (r^2 ranging from 0.17 to 0.46). A similar result was reported by Zhao et al. (2008) who stated that the ferrous ion-chelating ability of malting barley extracts exhibited poor correlations with both TPC and other antioxidant activities (DPPH radical scavenging activity, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical scavenging activity and reducing power).

PLSR modelling of the antioxidant properties vs. TPC of seaweed extracts yielded high multivariate correlation coefficient of 0.98 with low RMSEP (1.11). The first two PLSR components explained 99% of the *x*-variables (antioxidant properties) and 98% of the *y*-variables (TPC), respectively. The analysis of the β-coefficients of the variables showed that both DPPH radical scavenging activity and ORAC had high regression coefficient (0.500 and 0.496, respectively) whereas coefficient for ferrous ion-chelating was low (0.033).

Based on these analyses, it can be speculated that the major role of algal polyphenols is as potent radical scavengers and primary, chain-breaking antioxidants. In contrast to what has been reported by Chew et al. (2008), our results showed that algal polyphenols are probably not strong chelators of transition metals. Other components such as polysaccharides, proteins or peptides might be more important for the observed chelating effects of the extracts. However, further study is needed to elucidate the mechanism of antioxidant action of different compounds in the seaweed extracts.

It should also be noted that the reaction conditions of the different antioxidant assays may have a great influence on the effectiveness of various antioxidants. Therefore, to provide further evidence for feasible application of algal polyphenols as natural antioxidants in food for example seafood, the antioxidant potentials of algal polyphenols measured by DPPH, ORAC and metal chelating assays need to be confirmed under appropriate conditions (temperature and pH) of fish muscle system (Decker, Warner, Richards, & Shahidi, 2005).

4. Conclusions

The results of this screening experiment demonstrated that the different Icelandic seaweed species contained different levels of TPC and possessed diverse antioxidant properties. The type of extractant had great impact both on TPC and antioxidant activity of seaweed extracts. 70% acetone was more efficient for polyphenol extraction than water. In general, high TPC correlated with high DPPH and ORAC values, indicating that algal polyphenols were mainly responsible for the free radical scavenging activities of the extracts. However, other co-extracted active compounds such as fucoxanthin and sterols in 70% acetone extracts and sulphated polysaccharides, proteins or peptides in water extracts may also contribute to the overall scavenging effect. Three fucoid species (*F. vesiculosus, F. serratus* and *A. nodosum*) with the highest TPC exhibited the greatest scavenging activities and have therefore be selected for further studies in our laboratory. Interestingly, no clear correlation was found between TPC and chelating ability and thus algal polyphenols did not appear to be effective metal chelators in this experiment. Although the antioxidant activities of the crude seaweed extracts have not been demonstrated herein to be superior to standard compounds (BHT, α-tocopherol and EDTA-Na_2), further fractionation and purification of active components will most likely improve the activity and other potential health benefits may promote their use as natural sources of antioxidants. Future studies will include optimization of extraction, fractionation, purification and characterisation of the active components of these fucoid species. Antioxidant studies in model systems will also be performed to gain a better understanding of their relative antioxidant contribution and mechanisms of action to support the development of seaweed based natural antioxidants for application in functional foods and nutraceuticals.

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References

Ahn, G. N., Kim, K. N., Cha, S. H., Song, C. B., Lee, J., Heo, M. S., et al. (2007). Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. *European Food Research and Technology, 226*(1–2), 71–79.

Andjelkovic, M., Camp, J. V., Meulenaer, B. D., Depaemelaere, G., Socaciu, C., Verloo, M., et al. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chemistry, 98*(1), 23–31.

Athukorala, Y., Kim, K. N., & Jeon, Y. J. (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food and Chemical Toxicology, 44*(7), 1065–1074.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology Lebensmittel-Wissenschaft und Technologie, 28*, 25–30.

Chen, Y., Cai, L., Zhao, C., Xu, H. C., Cao, C. Y., Liu, Y., et al. (2008). Spectroscopic, stability and radical-scavenging properties of a novel pigment from gardenia. *Food Chemistry, 109*(2), 269–277.

Chew, Y. L., Lim, Y. Y., Omar, M., & Khoo, K. S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT – Food Science and Technology, 41*(6), 1067–1072.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. *Separation and Purification Technology, 55*(2), 217–225.

Connan, S., Delisle, F., Deslandes, E., & Ar Gall, E. (2006). Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. *Botanica Marina, 49*(1), 39–46.

Davalos, A., Gomez-Cordoves, C., & Bartolome, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*(1), 48–54.

Decker, E. A., Warner, K., Richards, M. P., & Shahidi, F. (2005). Measuring Antioxidant Effectiveness in Food. *Journal of Agricultural and Food Chemistry, 53*(10), 4303–4310.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry, 38*(3), 674–677.

Elisia, I., Hu, C., Popovich, D. G., & Kitts, D. D. (2007). Antioxidant assessment of an anthocyanin-enriched blackberry extract. *Food Chemistry, 101*(3), 1052–1058.

Galland-Irmouli, A. V., Fleurence, J., Lamghari, R., Luçon, M., Rouxel, C., Barbaroux, O., et al. (1999). Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *Journal of Nutritional Biochemistry, 10*(6), 353–359.

Hagerman, A. E. (1988). Extraction of tannin from fresh and preserved leaves. *Journal of Chemical Ecology, 14*(2), 453–461.

He, Y. H., & Shahidi, F. (1997). Antioxidant activity of green tea and its catechins in a fish meat model system. *Journal of Agricultural and Food Chemistry, 45*(11), 4262–4266.

Hemat, R. A. S. (2007). Fat and muscle dysfunction. In R. A. S. Hemat (Ed.), *Andropathy* (pp. 83–85). Dublin, Ireland: Urotext.

Heo, S. J., Park, E. J., Lee, K. W., & Jeon, Y. J. (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology, 96*(14), 1613–1623.

Ho, S. C., Tsai, T. H., Tsai, P. J., & Lin, C. C. (2008). Protective capacities of certain spices against peroxynitrite-mediated biomolecular damage. *Food and Chemical Toxicology, 46*(3), 920–928.

Huang, D. J., Ou, B. X., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856.

Huang, D. J., Ou, B. X., Woodill, M. H., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural of Food Chemistry, 50*(16), 4437–4444.

Hultin, H. O. (1994). Oxidation of lipids in seafoods. In F. Shahidi & J. R. Botta (Eds.), *Seafoods: Chemistry, Processing Technology and Quality* (pp. 49–74). Glasgow, UK: Blackie Academic and Professional.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R., & Saura-Calixto, F. (2001). Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture, 81*(5), 530–534.

Jittrepotch, N., Ushio, H., & Ohshima, T. (2006). Effects of EDTA and a combined use of nitrite and ascorbate on lipid oxidation in cooked Japanese sardine (*Sardinops melanostictus*) during refrigerated storage. *Food Chemistry, 99*(1), 70–82.

Jónsdóttir, R., Bragadóttir, M., & Ólafsdóttir, G. (2007). The role of volatile compounds in odor development during hemoglobin-mediated oxidation of cod muscle membrane lipids. *Journal of Aquatic Food Products Technology, 16*(4), 67–86.

Jormalainen, V., & Honkanen, T. (2004). Variation in natural selection for growth and phlorotannins in the brown alga *Fucus vesiculosus*. *Journal of Evolutionary Biology, 17*(4), 807–820.

Jormalainen, V., Honkanen, T., Vesakoski, O., & Koivikko, R. (2005). Polar extracts of the brown alga *Fucus vesiculosus* (L.) reduce assimilation efficiency but do not deter the herbivorous isopod Idotea baltica (Pallas). *Journal of Experimental Marine Biology and Ecology, 317*(2), 143–157.

Kallithraka, S., Garcia-Viguera, C., Bridle, P., & Bakker, J. (1995). Survey of solvents for the extraction of grape seed phenolics. *Phytochemical Analysis, 6*(5), 265–267.

Karawita, R., Siriwardhana, N., Lee, K. W., Heo, M. S., Yeo, I. K., Lee, Y. D., et al. (2005). Reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition properties of different solvent fractions from *Hizikia fusiformis*. *European Food Research and Technology, 220*(3–4), 363–371.

Kobayashi, M., & Sakamoto, Y. (1999). Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. *Biotechnology Letters, 21*(4), 265–269.

Koivikko, R., Loponen, J., Honkanen, T., & Jormalainen, V. (2005). Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. *Journal of Chemical Ecology, 31*(1), 195–212.

Koivikko, R., Loponen, J., Pihlaja, K., & Jormalainen, V. (2007). High-performance liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus*. *Phytochemical Analysis, 18*(4), 326–332.

Miyashita, K., & Hosokawa, M. (2008). Beneficial health effects of seaweed carotenoid, fucoxanthin. In C. Barrow & F. Shahidi (Eds.), *Marine nutraceuticals and functional foods* (pp. 297–319). Boca Raton, FL: CRC Press/Taylor & Francis Group.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933–956.

Richards, M. P., & Hultin, H. O. (2000). Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. *Journal of Agricultural and Food Chemistry, 48*(8), 3141–3147.

Richards, M. P., & Hultin, H. O. (2002). Contributions of blood and blood components to lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry, 50*(3), 550–564.

Rupérez, P., Ahrazem, O., & Leal, J. A. (2002). Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry, 50*(4), 840–845.

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry, 51*(12), 3661–3667.

Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*(2), 270–276.

Santoso, J., Yoshie, Y., & Suzuki, T. (2002). The distribution and profile of nutrients and catechins of some Indonesian seaweeds. *Fisheries Science, 68*(supplement), 1647–1648.

Santoso, J., Yoshie-Stark, Y., & Suzuki, T. (2004). Anti-oxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. *Fisheries Science, 70*(1), 183–188.

Senevirathne, M., Kim, S. H., Siriwardhana, N., Ha, J. H., Lee, K. W., & Jeon, Y. J. (2006). Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Science and Technology International, 12*(1), 27–38.

Shibata, T., Kawaguchi, S., Hama, Y., Inagaki, M., Yamaguchi, K., & Nakamura, T. (2004). Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology, 16*(4), 291–296.

Su, L., Yin, J. J., Charles, D., Zhou, K. Q., Moore, J., & Yu, L. L. (2007). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry, 100*(3), 990–997.

Toth, G., & Pavia, H. (2000). Lack of phlorotannin induction in the brown seaweed *Ascophyllum nodosum* in response to increased copper concentrations. *Marine Ecology Progress Series, 192*, 119–126.

Tsai, T. H., Tsai, T. H., Chien, Y. C., Lee, C. W., & Tsai, P. J. (2008). *In vitro* antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chemistry, 110*(4), 859–864.

Turkmen, N., Sari, F., & Velioglu, Y. S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry, 93*(4), 713–718.

Undeland, I., Hultin, H. O., & Richards, M. P. (2003). Aqueous extracts from some muscles inhibit hemoglobin-mediated oxidation of cod muscle membrane lipids. *Journal of Agricultural and Food Chemistry, 51*(10), 3111–3119.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites (Methods in ecology)*. Oxford, UK: Blackwell Scientific Publications.

Yan, X. J., Chuda, Y., Suzuki, M., & Nagata, T. (1999). Fucoxanthin as the major antioxidant in *Hizikia fusiformis*, a common edible seaweed. *Bioscience, Biotechnology and Biochemistry, 63*(3), 605–607.

Yomauchi, R., Tatsumi, Y., Asano, M., Kato, K., & Ueno, Y. (1988). Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agricultural and Biological Chemistry, 52*(3), 849–850.

Yoshie-Stark, Y., Hsieh, Y. P., & Suzuki, T. (2003). Distribution of flavonoids and related compounds from seaweeds in Japan. *Journal of Tokyo University of Fisheries, 89*, 1–6.

Yoshie, Y., Wang, W., Petillo, D., & Suzuki, T. (2000). Distribution of catechins in Japanese seaweeds. *Fisheries Science, 66*(5), 998–1000.

Zhang, Q., Zhang, J. Z., Shen, J. K., Silva, A., Denis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology, 18*(3–5), 445–450.

Zhao, H. F., Fan, W., Dong, J. J., Lu, J., Chen, J., Shan, L. J., et al. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry, 107*(1), 296–304.

Zhao, X., Xue, C. H., & Li, B. F. (2008). Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*. Journal of Applied Phycology, DOI: 10.1007/s10811-007-9282-4.

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebennsmittel-Wissenschaft und-Technologie, 37*(7), 717–721.