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

Tao Wang [a,b,c,*], Rósa Jónsdóttir [b], Guðrún Ólafsdóttir [a,d]

[a] Department of Food Science and Nutrition, Faculty of Science, University of Iceland, Hjardarhagi 2-6, 107 Reykjavik, Iceland
[b] Matís ohf., Food Research, Innovation and Safety, Biotechnology and New Products, Skulagata 4, 101 Reykjavik, Iceland
[c] Faculty of Food Science and Engineering, Dalian Fisheries University, Heishijiao 52, 116023 Dalian, China
[d] Syni Laboratory Service, Lynghâls 3, 110 Reykjavík, Iceland

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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

* Corresponding author. Address: Faculty of Food Science and Engineering, Dalian Fisheries University, Heishijiao 52, 116023 Dalian, China. Tel.: +86 411 84763557; fax: +86 411 84763508.
*E-mail address:* wang_tao633@yahoo.com (T. Wang).

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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.