JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Potential Antioxidant Capacity of Sulfated Polysaccharides from the Edible Marine Brown Seaweed *Fucus vesiculosus*

PILAR RUPÉREZ,*,† OUSSAMA AHRAZEM,‡ AND J. ANTONIO LEAL‡

Departamento de Metabolismo y Nutrición, Instituto del Frío, Consejo Superior de Investigaciones Científicas (CSIC), Ciudad Universitaria s/n, E 28040 Madrid, Spain, and Departamento de Microbiología Molecular, Centro de Investigaciones Biológicas (CSIC), Velázquez 144, E 28006 Madrid, Spain

Fucus vesiculosus was sequentially extracted with water at 22 °C (fraction 1 (F1)) and 60 °C (F2), and with 0.1 M HCl (F3) and 2 M KOH (F4) at 37 °C. Soluble fractions (42.3% yield) were composed of neutral sugars (18.9−48 g/100 g), uronic acids (8.8−52.8 g/100 g), sulfate (2.4−11.5 g/100 g), small amounts of protein (<1−6.1 g/100 g), and nondialyzable polyphenols (0.1−2.7 g/100 g). The main neutral sugars were fucose, glucose, galactose, and xylose. Infrared (IR) spectra of the fractions showed absorption bands at 820−850 and 1225−1250 cm^{-1} for sulfate. F1, F2, and F4 also exhibited an absorption band at 1425 cm^{-1}, due to uronic acids, and their IR spectra resembled that of alginate. F3 had an IR spectrum similar to that of fucoidan with an average molecular weight of 1.6×10^6 Da, calculated by molecular exclusion high-performance liquid chromatography. The presence of fucose in this polysaccharide was confirmed by ^1H NMR spectroscopy. This fraction showed the highest potential to be antioxidant by the ferric reducing antioxidant power (FRAP) assay, followed by the alkali- and water-soluble fractions. Sulfated polysaccharides from edible seaweeds potentially could be used as natural antioxidants by the food industry.

KEYWORDS: Edible seaweed; sulfated polysaccharide; antioxidant capacity; ferric reducing ability; *Fucus vesiculosus*

INTRODUCTION

Seaweeds have been consumed in Asia since ancient times, but to a much lesser extent in the rest of the world (*1*, *2*). Edible seaweeds are a rich source of dietary fiber, minerals, and proteins (*3*−*7*). Cell walls from marine algae characteristically contain sulfated polysaccharides, which are not found in land plants and which may have specific functions in ionic regulation (*8*). Seaweed dietary fibers are particularly rich in the soluble fractions, which in red seaweeds are mostly composed of sulfated galactans, such as agar or carrageenans (*5*, *8*, *9*). In brown seaweeds, soluble dietary fiber polysaccharides are alginates, fucans, and laminarans; the insoluble fibers are essentially made of cellulose (*5*, *8*, *10*).

Alginates are cell wall polyuronides, which consist of alternating units of mannuronic and guluronic acids; as polyelectrolytes, their solubility depends on pH. Their gelling characteristics are influenced by the mannuronic to guluronic acid ratio and the presence of divalent cations. The different thickening and gelling properties of alginates find wide applications in the food and pharmaceutical industry (*1*, *11*).

Fucans are cell wall polysaccharides composed of variable amounts of fucose, uronic acids, galactose, xylose, and sulfate. They have been classified into three families, according to their chemical composition: fucoidans (homofucans), ascophyllans, and glycuronofuco-galactans sulfate (*5*, *8*, *10*, *12*). Although the precise chemical structures of these polysaccharides are still unknown, two different structural models have been proposed for fucoidans (*10*, *13*). However, other studies have shown that the sulfated fucans of brown algae are present in a continuous spectrum of compounds, from high-uronic-acid-, low-sulfate-, fucose-containing polymers to highly sulfated fucoidans (*12*). Fucans are very soluble once extracted, but they do not develop highly viscous solutions. Fucans from brown seaweeds are byproducts in the preparation of alginates for the food and cosmetic industries (*14*).

Laminarans are not cell wall components but reserve β-glucans (*5*, *10*). The solubility of laminarans depends on the level of branching, but they are not thickening nor gelling agents, nor do they have current commercial use on an industrial scale (*5*, *11*).

Dietary fibers from edible seaweeds are little or non-digested by man (*2*, *4*, *5*, *9*, *15*). In addition, sulfated polysaccharides of soluble fibers from marine algae are not toxic for human, and, especially fucans and alginic acid derivatives, are known to exhibit different biological properties: anticoagulant, anti-inflamatory, antiviral, or antitumoral activities (*5*, *14*, *16*−*18*). The anticoagulant activity of sulfated polysaccharides from seaweeds has been the most investigated property as researchers attempt to find a substitute for heparin. In sulfated fucans, the anticoagulant capacity was shown to be related to sulfate content (*19*). The revised structure of fucoidan, a complex sulfated fucan from *Fucus vesiculosus*, may explain some of its biological activities (*13*).

Nevertheless, there are very few reports in the literature on the antioxidant capacity of algae. Alcoholic and aqueous extracts

* To whom correspondence should be addressed [telephone (34-91) 5445607, fax (34-91) 5493627, email pruperez@if.csic.es].
† Departamento de Metabolismo y Nutrición, Instituto del Frío.
‡ Departamento de Microbiología Molecular, Centro de Investigaciones Biológicas.

10.1021/jf010908o CCC: $22.00
Published on Web 01/08/2002

of seaweeds have been evaluated for antioxidant activity by lipoxygenase inhibition, 1,1-diphenyl-2-picrylhydrazyl (DPPH*) free radical scavenging activity and deoxyribose assays (*20*, *21*). The antioxidant activity of polyphenolic-rich extracts from edible seaweeds has also been reported (*22*).

In none of the above-mentioned reports was the antioxidant activity of sulfated polysaccharides from seaweeds tested. Recently, several marine alginate derivatives (*23*), sulfated fucoidans from the brown seaweed *Laminaria japonica* (*24*) and agar-like sulfated galactans from the red seaweed Nori (*25*), were evaluated for antioxidant activity by different in vitro methods.

As we presumed that *Fucus* fractions could possibly exhibit antioxidant power, the aim of this work was to obtain and analyze sulfated polysaccharides from *Fucus vesiculosus* and to test their potential as antioxidants by the FRAP assay. Additionally, a partial characterization of fraction F3, which gave the highest reducing power, was undertaken.

MATERIALS AND METHODS

Raw Material. The brown seaweed *Fucus* (*Fucus vesiculosus*) was obtained from a local supplier (Algamar C. B., Redondela, Pontevedra, Spain). Fresh marine seaweeds were collected in the spring of 1998, dried in the shade, and packed in polyethylene plastic bags for commercial distribution. Edible seaweeds were freeze-dried, milled to a particle size of less than 1.0 mm, and stored in airtight plastic flasks at 22 °C until analysis. Residual moisture content was determined by drying to constant weight at 105 °C in an oven. All analyses were performed in triplicate and results are reported on a dry matter basis.

Commercial standards of polysaccharides, laminaran (from *Laminaria digitata*), fucoidan (crude from *Fucus vesiculosus*), α-cellulose, and sodium alginate, were obtained from Sigma-Aldrich Chemicals (Alcobendas, Madrid, Spain).

Sequential Extraction of Seaweed Polysaccharides. The procedure was based on the different solubilities of the polysaccharides from brown seaweeds. Seaweeds (5 g) were extracted with distilled water (500 mL) with constant stirring at 22 °C for 1 h (fraction 1 (F1)) and, again, with water (500 mL) at 60 °C for 1 h (F2), to remove the bulk of the water-soluble polysaccharides. The water-insoluble residue was then sequentially extracted with 0.1 M HCl (500 mL) (F3) and with 2 M KOH (500 mL) (F4), each treatment at 37 °C for 16 h. The insoluble residue was washed with 2 M HCl (100 mL) and distilled water until neutral pH, and then dialyzed against running tap water (7 L/h) for 48 h and freeze-dried (F5, recovery 742 mg).

Each soluble fraction (F1 to F4) was filtered through a No. 3 sintered glass funnel (9.5 cm diameter) under reduced pressure. Then, the pH of the acid and alkali extracts was adjusted to 5.5, either with 2 M KOH or with concentrated HCl, as appropriate, and all of the extracts were dialyzed against water for 48 h (size 9/28.6 mm dialysis tubing, molecular weight cutoff 12−14 kDa, Medicell International Ltd., London). The dialyzed solutions were concentrated under reduced pressure at 40 °C and kept at −20 °C. An aliquot from each supernatant solution (F1 to F4) was freeze-dried for chemical analyses, and infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra. Recoveries of soluble fractions (mg): F1, 504.2; F2, 158.4; F3, 169.8; F4, 1283.6.

Chemical Analysis of Polysaccharide Fractions. Freeze-dried samples and commercial polysaccharide standards (10−15 mg) were hydrolyzed either with 0.05 M H_2SO_4 (10 mL) at 105 °C for 5 h (partial hydrolysis) or by the Saeman hydrolysis (*26*) with 12 M H_2SO_4 (1.5 mL, 30 °C, 1 h). Then concentrated sulfuric acid was diluted to 1 M H_2SO_4, and samples were hydrolyzed at 105 °C for 1.5 and 2.5 h (complete sugar hydrolysis).

Neutral sugars in *Fucus* fractions and commercial polysaccharide standards (laminaran, fucoidan, and cellulose) were quantified in the hydrolyzates colorimetrically by the anthrone method with d-glucose as a standard (*27*). Neutral sugars in hydrolyzates of commercial polysaccharides and fractions were identified and quantified by gas−liquid chromatography (GLC) as alditol acetates (*28*) with inositol as internal standard. A Shimadzu gas chromatograph model GC-14A equipped with a flame ionization detector (FID), an automatic injector (AOC-14), and a Hewlett-Packard HP-Chem Station with an HP-Deskjet 600 printer were used. The column was a Supelco SP-2330 capillary fused silica, 30 m × 0.32 mm i.d., 0.2 μm film thickness. The oven, injector, and detector temperatures were 240 °C (isothermal), 270 °C, and 270 °C, respectively. The split ratio was 1:5, and the carrier gas (nitrogen) head column pressure was 0.75 kg/cm^2.

Uronic acids were determined in 1 M H_2SO_4-hydrolyzates for 1.5 h of *Fucus* fractions and alginate with 3,5-dimethylphenol as the reagent by the method of Scott (*29*). Uronic acids were calculated with galacturonic acid as a standard and corrected for incomplete recovery of uronic acids (26.9 g/100 g) from alginate hydrolysis.

Sulfate was determined spectrophotometrically by the gelatin−barium chloride turbidimetric method (*30*). In brief, polysaccharidic fractions (5 mg) were first hydrolyzed with 1 M HCl (1 mL) at 105 °C for 16 h. Then, samples were passed through a Whatman GF/A glass microfiber filter (7 cm diameter) and their volume was made up to 2 mL with distilled water. Aliquots (0.2 mL) of the filtrate were taken and evaporated to dryness in SVL tubes at 40 °C under reduced pressure to remove HCl. Then, distilled water (0.2 mL), trichloroacetic acid reagent (3.8 mL), and gelatin−barium chloride reagent (1 mL) were added to each tube and mixed. After 15 min, absorbance was measured at 500 nm. Sulfate content in the samples was calculated with a calibration curve of K_2SO_4 (50−200 μg SO_4^{2-}).

Soluble polyphenols were determined spectrophotometrically in aqueous solutions of polysaccharide fractions F1 to F4 from *Fucus*, by the Folin−Ciocalteau method, using gallic acid as a standard (*31*), and they were expressed as gallic acid equivalents (GAE).

Protein. Total nitrogen in freeze-dried polysaccharide samples was determined with a Leco FP-2000 protein/nitrogen analyzer. In brief, powdered samples (50−100 mg) were weighed into ceramic boats and loaded into the FP-2000 analyzer, where they were combusted in the pure oxygen environment of the furnace. After passing through a thermo-electric cooler to drop out water, an aliquot from combustion gases was taken. Gases were scrubbed, and all nitrogen was reduced to N_2 and detected by a thermal-conductivity cell. A blank without sample was run, and the instrument was calibrated with EDTA. Protein was calculated as nitrogen × 6.25.

Infrared Spectra. Infrared spectra (IR) of freeze-dried polysaccharidic fractions from *Fucus* and commercial polysaccharides for comparison (alginate, laminaran, fucoidan, and cellulose) were obtained as KBr disks, phase resolution 128, and averaging 25 scans/min, using a Bruker IFS 28 Equinox infrared spectrophotometer, equipped with an OPUS-2.52 software for data acquisition.

Potential Antioxidant Activity of Soluble Polysaccharide Fractions by the Ferric Reducing/Antioxidant Power (FRAP) Assay. This method allows determination of the ferric reducing ability (μmol $Fe^{(III)}$ converted into $Fe^{(II)}$) in aqueous solutions of the samples, as a measurement of their antioxidant power. The reducing power of soluble polysaccharidic fractions (F1 to F4) from *Fucus* was determined according to Benzie and Strain (*32*), as modified by Pulido et al. (*33*). The FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ [2,4,6-tri(2-pyridyl-5-triazine) Fluka Chemicals, Madrid, Spain] solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6. In brief, 900 μL of freshly prepared FRAP reagent at 37 °C was mixed with 90 μL of distilled water and 30 μL of test sample (or water for the reagent blank). Absorbance readings at 595 nm were taken every 15 s from 0 to 30 min using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) equipped with an automatic 6-cell holder thermostated at 37 °C. Aqueous solutions of known $Fe^{(II)}$ concentrations (100−2000 μmol/L $FeSO_4 \cdot 7H_2O$) were used for calibration. Results were expressed as μmol $Fe^{(II)}$/g sample dry weight. For comparison of potencies, values were also calculated as μmol Trolox/g sample dry weight from regression equations previously published (*33*) of Trolox at 4 and 30 min of reaction with the FRAP reagent.

Molecular Weight Determination by HPLC. Average molecular weight of polysaccharide F3 from *Fucus* was determined by molecular exclusion HPLC on a TSK-Gel G 5000 PW stainless steel column (300

Table 1. Chemical Composition of Polysaccharide Fractions from *Fucus vesiculosus* (g/100 g dry weight)[a]

fraction	yield (%)	total carbohydrate		sulfate	protein	polyphenols (GAE)
		neutral sugar	uronic acids[b]			
F1	10.1	33.3 ± 2.6	39.1 ± 1.2	6.4 ± 0.7	1.4 ± 0.2	1.5 ± 0.0
F2	3.2	25.3 ± 1.8	36.8 ± 1.5	5.1 ± 0.9	4.2 ± 0.0	2.7 ± 0.8
F3	3.4	48.4 ± 1.2	8.8 ± 0.5	11.5 ± 0.1	trace	0.1 ± 0.0
F4	25.7	18.9 ± 0.3	52.8 ± 1.8	2.4 ± 0.0	6.1 ± 0.4	1.1 ± 0.0
F5	14.8	7.1 ± 0.0	5.1 ± 0.2	1.7 ± 0.8	2.6 ± 0.1	nd

[a] Data are mean value of triplicate determinations ± standard deviation; F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue. Neutral sugar and uronic acids in sulfuric acid hydrolysates by gas chromatographic and colorimetric methods, respectively. GAE, gallic acid equivalents; trace is less than 1 g/100 g; nd, not determined. [b] Values calculated with galacturonic acid as standard and corrected with alginate values.

Table 2. Neutral Sugars Determined by GLC (g/100 g dry weight) in Hydrolyzed Fractions of *Fucus vesiculosus*[a]

neutral sugars	sulfuric acid hydrolysis	F1	F2	F3	F4	F5
fucose	0.05 M, 5 h	10.5 ± 0.4	7.2 ± 1.2	23.3 ± 3.4	4.9 ± 0.1	1.4 ± 0.1
	1 M, 1.5 h	14.1 ± 1.1	8.1 ± 0.8	33.5 ± 2.8	6.5 ± 0.1	2.8 ± 0.3
	1 M, 2.5 h	13.8 ± 0.1	8.5 ± 0.5	39.1 ± 2.2	7.6 ± 0.2	3.0 ± 0.5
xylose	0.05 M, 5 h	0.9 ± 0.1	1.2 ± 0.3	2.0 ± 0.3	1.0 ± 0.1	0
	1 M, 1.5 h	2.8 ± 0.6	2.4 ± 0.2	2.3 ± 0.1	1.7 ± 0.2	0
	1 M, 2.5 h	1.9 ± 0.4	2.3 ± 0.3	2.8 ± 0.2	1.8 ± 0.3	0
galactose	0.05 M, 5 h	0	0	0	0	0
	1 M, 1.5 h	2.3 ± 0.4	2.5 ± 0.0	1.9 ± 0.0	2.1 ± 0.3	0
	1 M, 2.5 h	4.4 ± 1.5	3.4 ± 1.0	2.2 ± 0.3	2.3 ± 0.3	0
glucose	0.05 M, 5 h	1.3 ± 0.4	0	0	0	0
	1 M, 1.5 h	6.5 ± 1.7	4.8 ± 0.3	0	2.2 ± 0.1	2.1 ± 0.1
	1 M, 2.5 h	7.8 ± 0.8	4.6 ± 0.0	0	2.8 ± 0.3	3.0 ± 1.2
total sugar	0.05 M, 5 h	12.8 ± 0.8	8.5 ± 1.4	25.3 ± 3.5	5.9 ± 0.2	1.4 ± 0.1
	1 M, 1.5 h	25.7 ± 3.9	17.7 ± 1.3	37.8 ± 2.6	12.5 ± 0.5	4.9 ± 0.4
	1 M, 2.5 h	27.9 ± 3.0	18.8 ± 0.2	44.1 ± 1.6	14.5 ± 0.7	6.0 ± 1.8

[a] Data are mean value of triplicate determinations ± standard deviation; F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue.

× 7.5 mm i.d.) with a TSK-Gel PWH guard column (75 × 7.5 mm i.d.) from TosoHaas (Teknokroma, Barcelona, Spain). The column was eluted isocratically with Milli-Q filtered and degassed water at 40 °C with a flow rate of 0.8 mL/min. A blue dextran-2000 standard, average molecular weight 2,000 kDa (Pharmacia Biotech Europe GmbH, Barcelona, Spain), and a Shodex pullulan standard P-82 kit (range of molecular weights in kDa: P-800 = 788, P-400 = 404, P-200 = 212, P-100 = 112, P-50 = 47.3, P-20 = 22.8, P-10 = 11.8, P-5 = 5.9; Showa-Denko, Japan) obtained from Waters Chromatography, S. A. (Madrid, Spain) were used for calibration of the molecular size. A portion of an aqueous solution of the sample (F3) and standards (2 mg/mL) were filtered through 0.22-μm filters, and 50 μL was injected into the HPLC. Average molecular weight of polysaccharide peaks in F3 was calculated by comparison of their retention times with the calibration curve. The following HPLC instruments were used: Kontron autosampler 360, Kontron pump system 325, Waters differential refractometer, Jones chromatography thermostatic oven, Kontron Data System 450-MT2, and Hewlett-Packard Deskjet 600 printer.

^{1}H NMR Analysis. An aliquot of freeze-dried F3 (8 mg) from *Fucus* was dissolved in 2 mL of D_2O and filtered through 0.45-μm filters to remove insoluble material. ^{1}H NMR spectra were obtained in D_2O solutions at 70 °C, acquisition time 3 s and 128 repetitions, in an INOVA-400 spectrophotometer (^{1}H, 400 MHz). Chemical shifts in ppm (δ) are related to HOD residual peak (4.31 ppm at 70 °C).

RESULTS AND DISCUSSION

Sequential Extraction and Chemical Analyses of Polysaccharide Fractions. The sequential extraction procedure was based on the different solubilities of polysaccharides from brown seaweeds. Laminarans are soluble, but their solubility in water depends on the branching level: the higher the branching degree, the higher the solubility; thus, low-branched laminarans are soluble only in warm water (60−80 °C) (*5*). Fucans were extracted with diluted hydrochloric acid, meanwhile alginates were extracted with 2 M KOH. Alginates form insoluble precipitates at acid pH, but they are stable in solution between pH 6 and 9. Finally, the acid- and alkali-insoluble material contained residual polysaccharides plus cellulose.

The yield and composition of polysaccharide fractions from *Fucus* are shown in **Table 1**. Total recovery (57.2%) corresponded to nondialyzable compounds, as free minerals and low-molecular-weight substances were removed during exhaustive dialysis of the fractions. Recovery of soluble fractions (F1−F4) amounted to 42.3% of the seaweed dry weight. Main components were neutral sugars (18.9−48.4 g/100 g), uronic acids (8.8−52.8 g/100 g), and sulfate (2.4−11.5 g/100 g). F1, F2, and F4 contained a high proportion of uronic acids from alginates. F3 showed the highest neutral sugar content. The insoluble residue (F5) represented 14.8% of the seaweed and its carbohydrate content, which was quite low, could be related to the high values of Klason lignin (31.1 g/100 g) associated to the insoluble dietary fiber of *Fucus vesiculosus* (*7*). Minor components in all fractions were protein and nondialyzable polyphenols (**Table 1**).

Neutral sugars determined by GLC in partially and completely hydrolyzed fractions of *Fucus* are shown in **Table 2**. Main sugar released from fractions with 0.05 M sulfuric acid for 5 h corresponded to fuco-furanose. Regarding complete sugar hydrolysis, except for F5 (insoluble residue), sugar recoveries as determined by GLC were slighty higher in 2.5 h-hydrolyzates. F1 and F2 contained fucose, glucose, galactose, and xylose. Glucose is the main constituent of laminarans. The acid-soluble fraction (F3) was mainly composed of fucose (39.1 g/100 g) and small amounts of xylose and galactose. A great proportion must be fuco-furanose (23.3 g/100 g), considering it was released after partial sugar hydrolysis; fuco-furanose has been reported in fucoidan from *Fucus vesiculosus*. This fraction would mainly contain fucoidan, a polysaccharide composed of sulfated fucose (*10*, *13*). This polysaccharide was composed of fucose (97%), with trace amounts of galactose and xylose (<3%), uronic acids

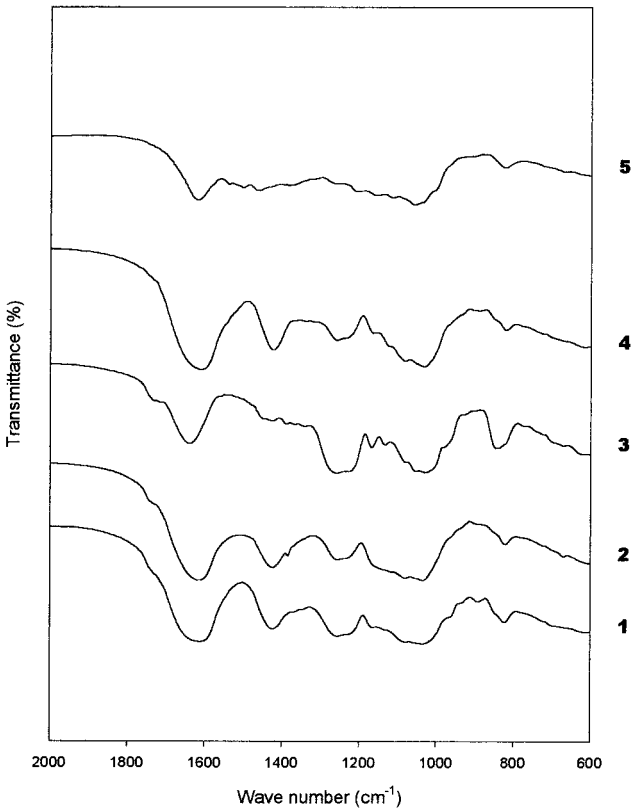

Figure 1. Infrared spectra of polysaccharide fractions from *Fucus vesiculosus.* F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue.

(3−4%), and differential sulfate content of fucan chains (*13*). Fucans obtained from *Fucus vesiculosus* and some other brown algae by sequential chemical extraction were in a continuous spectrum of compounds from highly sulfated homofucans, to high-uronic acid, low-sulfate, and fucose-containing polymers. The general composition of the fucans depended on their extraction sequence (*12*). F4 (alkali-soluble) was composed of fucose, glucose, galactose, and xylose. This fraction would contain the bulk of the alginates (52.8 g/100 g, see **Table 1**). The insoluble residue (F5) contained small amounts of residual fucose and glucose from cellulose.

Infrared Spectra. IR spectra of polysaccharide fractions from *Fucus vesiculosus* are shown in **Figure 1**. Fractions F1, F2, F4, and F5 showed a small absorption band at 820 cm^{-1} of sulfate groups at an equatorial position (*13*, *16*). F3 had a broad absorption band at 850 cm^{-1}; absorption at 844 cm^{-1} was reported for fucoidan and it was suggested to be due to sulfate groups at the axial C-4 position of fucose (*13*). Absorption in all fractions at 1225−1255 cm^{-1} was also due to sulfate. The band was larger in F3 in accordance with its higher sulfate content. This band is common to all sulfated polysaccharides and it is an index of the degree of sulfation: its intensity increasing with the sulfate content. A broad absorption band at 1240 cm^{-1} was also found in sulfated polysaccharides from the brown seaweed Wakame, indicating the presence of ester sulfate (*16*). A small shoulder at 1730 cm^{-1} (F1, F2, and F3) and a large absorption band at 1425 cm^{-1} in F1, F2, and F4 were due to uronic acids, in agreement with a higher uronic acid content in these fractions. The IR spectra of F1 and F2 were quite similar and resembled that of sulfate-containing laminaran and alginate standards. F3 had an IR spectrum similar to that of commercial fucoidan and fucoidan preparation previously reported (*13*); meanwhile, the IR spectrum of F4 resembled that of an alginate plus sulfate, possibly from residual fucoidan.

Table 3. Ferric Reducing Ability/Antioxidant Power Values[a] of Soluble Polysaccharide Fractions from *Fucus vesiculosus*

	4 min		30 min	
fraction	μmol Fe(II)/g	μmol Trolox/g	μmol Fe(II)/g	μmol Trolox/g
F1	78.2 ± 1.3	39.1 ± 0.6	113.9 ± 20.8	54.5 ± 9.2
F2	92.3 ± 10.4	54.9 ± 4.6	191.0 ± 17.7	97.5 ± 7.8
F3	209.8 ± 4.5	99.7 ± 2.0	263.9 ± 4.8	123.0 ± 2.1
F4	101.1 ± 1.6	51.1 ± 0.7	167.6 ± 6.7	80.0 ± 3.0

[a] Results are expressed as μmol equivalent of Fe(II) or Trolox per gram dry weight of fractions in aqueous solution. Data are mean value of triplicate determinations ± standard deviation; F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C.

Potential Antioxidant Activity of Soluble Polysaccharide Fractions by the FRAP Assay. The current literature reports that many different in vitro methods are being used to evaluate antioxidants of interest in food and biological systems (*34*). In some of these protocols, samples were extracted with organic solvents and antioxidant assays were performed in alcoholic solutions (*20*−*22*); however, on these conditions polysaccharides would precipitate. Thus, the antioxidant power of sulfated polysaccharides from *Fucus* in aqueous solution (**Table 3**) was estimated from their ability to reduce TPTZ-Fe(III) to TPTZ-Fe(II) complex, as determined by the FRAP assay (*32*, *33*). FRAP values increased considerably from 4 to 30 min, as it has been described for other vegetable and seaweed samples (*22*). F3, which contained fucoidan, showed the highest reducing power at 4 and 30 min (209.8 and 263.9 μmol Fe(II)/g sample dry weight, respectively). When FRAP values for F3 were expressed as μmol Trolox/g sample dry weight for comparison (**Table 3**), they were 3.8 times higher at 4 min than a polyphenolic-rich extract from *Fucus* (*22*). Of the seaweeds tested, organic extracts from *F. vesiculosus* showed the highest antioxidant activity with both DPPH* and FRAP assays, and good efficiency in the in vitro inhibition of LDL oxidation (*22*). The antioxidant activity of *Fucus* extracts was attributed to their phloroglucinol content, because carbohydrates had been removed in the preparation of the samples. In our study, polyphenol content in F3 (**Table 1**) was the lowest of all the soluble fractions tested for antioxidant power.

F1 and F2, rich in alginates and laminaran, and F4 which contained the bulk of alginates, exhibited lower antioxidant potentials than F3 (**Table 3**). Nevertheless, except for F1 at 30 min, FRAP values were higher than those obtained previously for organic extracts from *Fucus* (*22*).

The antioxidant potential of sulfated polysaccharides from the brown seaweed *Fucus vesiculosus* by the FRAP assay was higher than that of agar-like sulfated galactans from the red seaweed Nori (*25*). This was in agreement with Matsukawa et al. (*20*), who found that the antioxidant activity of brown algae was superior to that of red or green groups.

Although the antioxidant capacity of organic or aqueous extracts from seaweeds has been screened by different in vitro methods (*20*, *21*), their composition was generally not ascertained. Thus, there are very few reports in the literature on the antioxidant capacity of sulfated polysaccharides from brown seaweeds. Antioxidant properties of alginate derivatives in a phosphatidylcholine−liposomal suspension (*23*) and of crude and modified sulfated fucoidans from *Laminaria japonica* in LDL oxidation systems (*24*) have been studied. However, the relationships between structure of seaweed polysaccharides

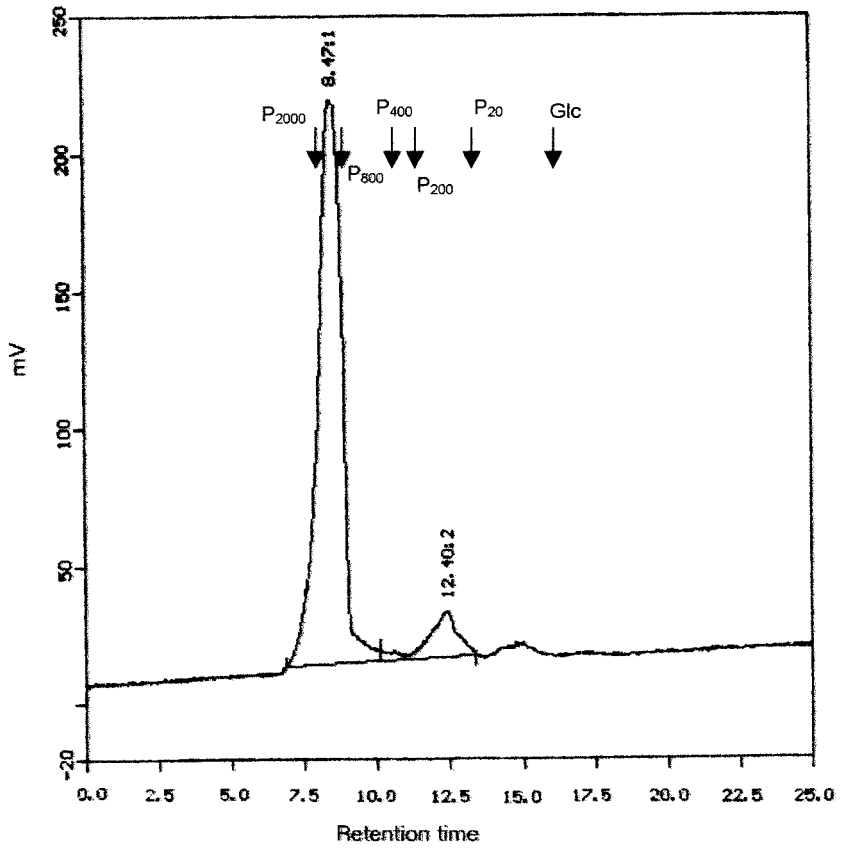

Figure 2. Molecular weight determination of polysaccharide fraction F3 (acid-soluble) from *Fucus vesiculosus* by HPLC. Range of molecular weight in kDa: P2000 = 2000; P800 = 788; P400 = 404; P200 = 212; P20 = 22.8; Glc, glucose.

(alginate, fucoidan and its derivatives) and antioxidative mechanisms have not yet been elucidated. According to Xue et al. (*23*), differences in antioxidant abilities of alginates might be related to sulfate content and molecular weight. Moreover, anionic groups in alginates would display a significant antioxidant effect (*23*). Different molecular weight and neutral sugar composition of fucoidans from *L. japonica* would also play a role inhibiting human LDL oxidation in vitro (*24*).

Molecular Weight Determination by HPLC. Further work focused on F3, which contained a fucoidan-like polysaccharide and exhibited the highest antioxidant potential. Thus, **Figure 2** shows its molecular weight determination by HPLC. Main peak in the chromatogram amounted to 92% of the fraction, and its average molecular weight was 1.6×10^6 Da. Minor peak average molecular weight was 43,000 Da. The main sulfated polysaccharide was larger than the average size of 680,000 Da, which was previously reported for high-molecular-weight fucoidan (*13*, *19*). Differences could be due to the extraction method or to seasonal or geographical variations.

^{1}H NMR analysis. The ^{1}H NMR spectrum of F3 from *Fucus* (**Figure 3**), showed an intense band at 1.4 ppm due to 6-deoxisugar protons, corresponding to the main neutral sugar in this fraction, fucose. The intensity of the peaks in the spectrum was proportional to the number of protons in the fraction. Apparently, there was a mixture of α- and β-glycosidic linkages in the polysaccharide, as inferred from the lower intensity bands at about 5.5 and 4.8 ppm due to anomeric protons (α- and β-glycosidic configuration, respectively). The ratio of the intense peak at 1.4 ppm and the peaks corresponding to anomeric protons was approximately 2.8.

In summary, sulfated polysaccharides from *Fucus vesiculosus*, and especially F3 which contained fucoidan, exhibited antioxidant potential. Nevertheless, at present, the mechanisms by which sulfated polysaccharides from marine algae exert their antioxidant power are still unknown. In this sense, it is of great interest to have available highly purified and well characterized sulfated polysaccharides with which to elucidate their mode of action. Antioxidant activity may arise not only from polyphenols (22), but from some other components of polysaccharides from edible seaweeds. Sulfated polysaccharides, and especially fucans, byproducts in the preparation of alginates from edible brown seaweeds, could be used as sources of natural antioxidants with potential applications in the food industry.

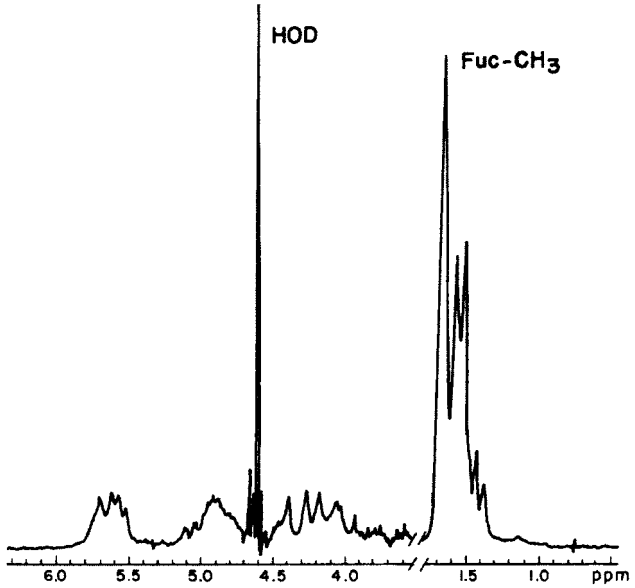

Figure 3. ^{1}H NMR spectra of polysaccharide fraction F3 (acid-soluble) from *Fucus vesiculosus* obtained in D_2O at 70 °C.

ACKNOWLEDGMENT

Thanks are given to Mrs. I. Fernández-Conde and G. Toledano for technical assistance and to Dr. M. Bernabé (Instituto de Química Orgánica, C.S.I.C.) for helpful discussion of the ^{1}H-NMR spectra.

ABBREVIATIONS USED

DPPH*, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalents; GLC, gas−liquid chromatography; ^{1}H NMR, proton nuclear magnetic resonance; HPLC, high-performance liquid chromatography; IR, infrared spectra.

LITERATURE CITED

(1) Chapman, V. J.; Chapman, D. J. *Seaweeds and Their Uses*. Chapman and Hall: London, 1980.

(2) Indergaard, M.; Minsaas, J. Animal and human nutrition. In *Seaweed Resources in Europe: Uses and Potential*; Guiry, M. D., Blunden, G., Eds.; John Wiley & Sons: New York, 1991; pp 21−64.

(3) Lahaye, M. Marine algae as sources of fibres: determination of soluble and insoluble dietary fibre contents in some "sea vegetables". *J. Sci. Food Agric.* **1991**, *54*, 587−594.

(4) Mabeau, S.; Fleurence, J. Seaweed in food products: biochemical and nutritional aspects. *Trends Food Sci. Technol.* **1993**, *4*, 103−107.

(5) Lahaye, M.; Kaeffer, B. Seaweed dietary fibres: structure, physicochemical and biological properties relevant to intestinal physiology. *Sci. Aliments* **1997**, *17*, 563−584.

(6) Fleurence, J. Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.* **1999**, *10*, 25−28.

(7) Rupérez, P.; Saura-Calixto, F. Dietary fibre and physicochemical properties of edible seaweeds. *Eur. Food Res. Technol.* **2001**, *212*, 349−354.

(8) Kloareg, B.; Quatrano, R. S. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr. Mar. Biol. Annu. Rev.* **1988**, *26*, 259−315.

(9) Bobin-Dubigeon, C.; Hoebler, C.; Lognoné, V.; Dagorn-Scaviner, C.; Mabeau, S.; Barry, J.-L.; Lahaye, M. Chemical composition, physicochemical properties, enzymatic inhibition and fermentative characteristics of dietary fibres from edible seaweeds. *Sci. Aliments* **1997**, *17*, 619−639.

(10) Percival, E.; McDowell, R. H. Chemistry and Enzymology of Marine Algal Polysaccharides. Academic Press: London, 1967.

(11) Indergaard, M.; Ostgaard, K. Polysaccharides for food and pharmaceutical uses. In *Seaweed Resources in Europe: Uses and Potential;* Guiry, M. D., Blunden, G., Eds.; John Wiley & Sons: New York, 1991; pp 169−183.

(12) Mabeau, S.; Kloareg, B.; Joseleau, J.-P. Fractionation and analysis of fucans from brown algae. *Phytochemistry* **1990**, *29*, 2441−2445.

(13) Pantakar, M. S.; Oehninger, S.; Barnett, T.; Williams, R. L.; Clark, G. F. A revised structure for fucoidan may explain some of its biological activities. *J. Biol. Chem.* **1993**, *268*, 21770−21776.

(14) Boisson-Vidal, C.; Haroun, F.; Ellouali, M.; Blondin, C.; Fischer, A. M.; de Agostini, A.; Josefonvicz, J. Biological activities of polysaccharides from marine algae. *Drugs Future* **1995**, *20*, 1237−1249.

(15) Michel, C.; Lahaye, M.; Bonnet, C.; Mabeau, S.; Barry, J.-L. *In vitro* fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *Br. J. Nutr.* **1996**, *75*, 263−280.

(16) Mori, H.; Kamei, H.; Nishide, E.; Nisizawa, K. Sugar constituents of some sulfated polysaccharides from the sporophylls of Wakame (*Undaria pinnatifida*) and their biological activities. In *Marine Algae in Pharmaceutical Science*; Hoppe, H. A., Levring, T., Tanaka, Y., Eds.; Walter de Gruyter & Co.: Berlin, 1982; Vol. 2, pp 109−121.

(17) Fujihara, M.; Iizima, N.; Yamamoto, I.; Nagumo, T. Purification and chemical and physical characterization of an antitumor polysaccharide from the brown seaweed *Sargassum fulvellum*. *Carbohydr. Res.* **1984**, *125*, 97−106.

(18) Noda, H.; Amano, H.; Arashima, K.; Nisizawa, K. Antitumor activity of marine algae. *Hydrobiologia* **1990**, *204/205*, 577−584.

(19) Nishino, T.; Nishioka, C.; Ura, H.; Nagumo, T. Isolation and partial characterization of a novel amino-sugar containing fucan sulfate from commercial *Fucus vesiculosus* fucoidan. *Carbohydr. Res.* **1994**, *255*, 213−224.

(20) Matsukawa, R.; Dubinsky, Z.; Kishimoto, E.; Masaki, K.; Masuda, Y.; Takeuchi, T.; Chihara, M.; Yamamoto, Y.; Niki, E.; Karube, I. A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol.* **1997**, *9*, 29−35.

(21) Yan, X.; Nagata, T.; Fan, X. Antioxidative activities in some common seaweeds. *Plant Foods Hum. Nutr.* (*Dordrecht, Neth*) **1998**, *52*, 253−262.

(22) Jiménez-Escrig, A.; Jiménez-Jiménez, I.; Pulido, R.; Saura-Calixto, F. Antioxidant activity of fresh and processed edible seaweeds. *J. Sci. Food Agric.* **2001**, *81*, 530−534.

(23) Xue, C.; Yu, G.; Hirata, T.; Terao, J.; Lin, H. Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 206−209.

(24) Xue, C.; Fang, Y.; Lin, H.; Chen, L.; Li, Z.; Deng, D.; Lu, C. Chemical characters and antioxidative properties of sulfated polysaccharides from *Laminaria japonica*. *J. Appl. Phycol.* **2001**, *13*, 67−70.

(25) Rupérez, P. Antioxidant activity of sulphated polysaccharides from the Spanish marine seaweed Nori. Proceedings of the COST 916 European Conference on *Bioactive compounds in plant foods. Health effects and perspectives for the food industry*; Tenerife, Canary Islands, Spain, April 2001; p 114.

(26) Adams, G. A. Complete acid hydrolysis. *Meth. Carbohydr. Chem.* **1965**, *5*, 269−276.

(27) Loewus, F. A. Improvement in the anthrone method for determination of carbohydrates. *Anal. Chem.* **1952**, *24*, 219.

(28) Englyst, H. N.; Cummings, J. H. Improved method for measurement of dietary fibers as nonstarch polysaccharides in plant foods. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 808−814.

(29) Scott, R. W. Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936−941.

(30) Chaplin, M. E.; Kennedy, J. F. *Carbohydrate Analysis*. IRL Press: Oxford, 1986; pp 136−137.

(31) Montreau, F. R. Sur le dossage des composés phénoliques totaux dans les vins par la méthode Folin-Ciocalteau. *Conn. Vigne Vin.* **1972**, *24*, 397−404.

(32) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(33) Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396−3402.

(34) Frankel, E. N.; Meyer, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925−1941.

Received for review July 12, 2001. Revised manuscript received November 7, 2001. Accepted November 7, 2001. This work was supported by Comisión Interministerial de Ciencia y Tecnología, CICYT Project no. ALI98-0830.

JF010908O