

Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection

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Speciation of iodine in commercially available commonly consumed seaweed samples was performed using a multidimensional chromatographic approach coupled with inductively coupled plasma mass spectrometry (ICP-MS) for element specific detection. Analysis of alkaline extract (0.1 mol l⁻¹ NaOH) by size-exclusion chromatography coupled to ICP-MS (0.03 mol l⁻¹ Tris-HCl, pH 8.0) indicated the association of iodine with both high as well as low molecular weight fractions in Wakame, while in case of Kombu, only low molecular weight iodine species were found. Likely association of iodine with protein as well as polyphenolic species was indicated in the case of Wakame. Anion-exchange chromatography coupled to ICP-MS (0.005 mol l⁻¹ NaOH) confirmed that the most predominant inorganic iodine species present in both type of seaweeds is iodide. Protein bound iodinated species were hydrolyzed by enzymatic digestion using Proteinase K. Analysis of the hydrolysate using reversed-phase HPLC-ICP-MS (0.01 mol l⁻¹ Tris-HCl pH 7.3 : 0.01 mol l⁻¹ Tris-HCl pH 7.3 and 50% MeOH) revealed the presence of monoiodotyrosine and di-iodotyrosine in Wakame, which was later identified by matching the chromatographic retention time with the retention time of commercially available standards.

Introduction

Iodine is an essential micronutrient for human nutrition. Its role in the synthesis of thyroid hormones necessary for human growth and development, such as thyroxine, (tetraiodothyronine (T₄)) and triiodothyronine (T₃), is widely known.¹⁻³ Iodine deficiency leads to goiter and various disorders associated with growth and development, like dwarfism, mental retardation and neuromuscular defects, commonly referred to as iodine deficiency disorders (IDD).^{4,5} The recommended dietary allowance (RDA) of iodine is 150 µg d⁻¹ in the United States. In European and other countries, ranges between 150–200 µg d⁻¹ have been established.⁵ In order to prevent iodine deficiency disorders, supplementation of foodstuff with iodine is commonly practised.⁶ Milk, a source of iodine of animal origin, obtains most of it from iodine supplemented cattle feed. Usage of iodized salt has also become popular worldwide.⁶ Iodine is plentiful in oceans and marine animals, and sea plants such as some marine algae (seaweed) are naturally occurring sources of dietary iodine. Some seaweed can accumulate exceptionally high quantities of iodine available from the sea. *Hizikia* (Hiziki), *Undaria* (Wakame), *Laminaria* (Kombu) and *Porphyra* (Nori) are some of the commercially available seaweeds commonly consumed with very high iodine content.⁷ The total iodine content of seaweed depends on the species as well as the region in which they are found. The enrichment factor of *Laminaria japonica* for iodine reaches 10^{6,8}. The consumer preference for natural products over artificial ones provides the impetus for studying seaweed as source of iodine.⁹ Consumption of seaweed as a dietary source of iodine is not only restricted to Asian countries, but also increasingly in Europe and some African countries like Ghana.^{5,10} Recently, the possibility of using marine algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) as a food supplement has also been evaluated.¹¹

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The key to good thyroid function is adequate, but not excessive, iodine intake, which can not only cause high-iodine goiter, but also damage the nervous system, leading to retarded brain development and functional impediment.¹²⁻¹⁴ Another important fact associated with consumption of iodine is that, like other elements, bioavailability and toxicity is species dependent. Inorganic forms of iodine, such as iodide and iodate, are less toxic than molecular iodine and some organically bound iodine.⁸ Likewise, the bioavailability of organically bound iodine such as monoiodotyrosine (MIT) and di-iodotyrosine (DIT) is also less than that of mineral iodide.⁹ Considering the above, and the increasing use of seaweed as a dietary source of iodine, total analysis and characterization of iodine species in seaweed is an important pursuit.

Many different analytical techniques have been developed for iodine speciation in food, environmental and biological matrices. Interfacing various separation techniques, such as reversed-phase high-performance liquid chromatography (RP-HPLC),^{19,20} ion chromatography (IC),^{21,22} size-exclusion chromatography (SEC)²³ and capillary electrophoresis (CE)²⁴ with element selective ICP-MS detection has been employed in speciation studies of iodine to separate and characterize various iodine species. Although numerous papers have been published reporting total analysis of iodine in food samples,^{4,15-17} speciation studies of iodine in seaweed have only been recently described, in spite of the extensive consumption.¹⁸ Moreover, the well known capabilities of multidimensional chromatographic techniques, coupled with elemental specific detectors such as ICP-MS, for fast and reliable speciation analysis of iodine in seaweed, has never been reported.

The aim of the present study is an initial characterization and identification of iodine species in commercially available seaweed samples. An approach involving multidimensional

chromatographic techniques coupled to ICP-MS is utilized for the separation and determination of iodine species in seaweed. Size exclusion chromatography was used to investigate the association of iodine with various molecular weight fractions extracted from different media and to separate inorganic iodine from organically bound iodine. Anion-exchange chromatography was used for separating inorganic forms of iodine. Reversed-phase high-performance liquid chromatography was used for the separation and identification of low molecular weight iodine species in seaweed samples. Identification of iodine species was performed by matching the peak retention times with those of standards.

Experimental

Instrumentation

Chromatographic separations were performed using an Agilent 1100 liquid chromatographic system (Agilent Technologies, Palo Alto, CA, USA) equipped with a HPLC binary pump, an autosampler, a vacuum degasser, a thermostated column compartment and a diode array detector. The chromatographic columns used were a Superdex 75 HR (10 mm × 300 mm × 13 µm particle size) column (Amersham Pharmacia Biotech AB, Uppsala, Sweden) for size-exclusion chromatography, an Ion pac AS-11 (Dionex, Sunnyvale, CA, USA) column (2.0 mm id × 250 mm length × 13 µm particle size) for ion exchange chromatography and a C₁₈ Alltima (Alltech, Deerfield, IL, USA) column (4.6 mm id × 150 mm length × 5 µm particle size) for RP-HPLC. Chromatographic conditions are summarized in Table 1.

An Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with a micromist nebulizer and a Peltier cooled spray chamber (2 °C) and a shielded torch system was used for iodine specific detection. The outlet of the UV detector

was connected online to the liquid sample inlet of the ICP-MS nebulizer using 300 mm long by 0.25 mm PEEK tubing. For RP-HPLC, online dilution of the chromatographic eluent containing organic solvent was performed as follows (before its nebulization into the plasma): the outlet carrying the chromatographic eluent at a flow rate of 0.5 ml min⁻¹ was connected to the one arm of PTFE tee-piece, while through the other arm 2% nitric acid was introduced at a flow rate of 0.5 ml min⁻¹ in order to provide a dilution factor of 1 : 2. Dilution was performed to reduce the organic solvent (methanol) load introduced into the plasma. The instrumental operating conditions are summarized in Table 1.

Reagents and standards

All reagents used were analytical grade reagents and the presence of iodine was not detected in the working range. All solutions were prepared in 18 MΩ cm doubly deionized water generated by a NanoPure treatment system (Barnstead, Boston, MA, USA). The following reagents were purchased from Sigma (Sigma-Aldrich Co., St. Louis, MO, USA): tris (hydroxymethyl)aminomethane (TRIS), ammonium bicarbonate and sodium dodecyl sulfate (SDS). Hydrochloric acid, sodium hydroxide, and potassium iodate were obtained from Fisher (Fairlawn, NJ, USA). HPLC grade methanol, ethanol, and acetone (Fisher Scientific, Pittsburgh, PA, USA) were used throughout. Individual standard solutions of 3-iodotyrosine (MP Biomedicals, Irvine, CA, USA) and 3,5-diiodotyrosine (Cambridge Corporation, San Diego, CA, USA) were prepared by the dissolution of chemicals in methanol. For the determination of total iodine, working solutions were prepared daily by appropriate dilution of the 10 µg ml⁻¹ iodide standard solution obtained from High-Purity Standards (Charleston, SC, USA). Nitric acid (Suprapure) 68% from Pharmaco and hydrogen peroxide 30% from Fisher Scientific were used for sample digestion.

Procedures

Sample collection and preparation. Commercially available dried seaweed samples [marine algae Kombu (*Laminaria japonica*) and Wakame (*Undaria pinnatifida*)] were obtained from local Asian stores in USA for total iodine analysis and speciation studies. The above mentioned marine algae were selected for speciation studies because they are commonly consumed, especially in various Asian recipes, and they represent sources of high iodine concentration. The dried algae samples were ground in a household coffee grinder.

Total iodine determination. Both types of seaweeds were analyzed for total iodine content by ICP-MS after complete digestion using an MES 1000 closed vessel microwave digestion system (CEM Corp., Matthews, NC, USA). Sample amounts of 0.1 g were weighed and 5 ml of 65% concentrated nitric acid and 1 ml of 30% hydrogen peroxide were added in microwave vessels. The following mineralization program was applied for complete digestion: the microwave power was increased over three steps with 5 min intervals from 250 W to 500 W and finally to 1000 W and held at 1000 W for another 25 min. Temperature limits of 120, 150, 160 and 200 °C were set for each of four steps. The mixture was cooled for 20 min at room temperature. The digested samples were adjusted to a pH between 9 and 10, by addition of 3% NH₃ at ratio of 1 : 1. The addition of NH₃ was performed to give a stable iodide signal⁴. Finally, the samples were diluted to 100 ml with doubly deionized water. Reagent blank was digested in the same way. Tellurium was added as an internal standard. Appropriate dilution was performed when needed. The method of standard additions (at µg l⁻¹ of iodine) was applied for quantification of the iodine present in the samples.

Table 1 ICP-MS and chromatographic instrumental parameters

ICP-MS parameters	
Forward power	1500 W
Plasma gas flow rate	15.0 l min ⁻¹
Auxiliary gas flow rate	0.87 l min ⁻¹
Carrier gas flow rate	1.20 l min ⁻¹
Dwell time	0.1 s per isotope
Isotopes monitored	¹²⁷ I
SEC chromatography parameters	
Column	Superdex 75 HR 10/30
Mobile phase	0.03 mol l ⁻¹ Tris-HCl buffer, pH 8.0
Flow rate	0.6 ml min ⁻¹
Injection volume	100 µl
Ion chromatography parameters	
Column	Ion Pac AS-11 anion exchange column (250 mm × 2.0 mm i.d. × 13 µm)
Mobile phase	0.005 mol l ⁻¹ sodium hydroxide
Flow rate	0.3 ml min ⁻¹
Injection volume	20 µl
RP-HPLC parameters	
Column	Alltima C ₁₈ (150 mm × 4.6 mm, 5 µm)
Mobile phase	(A) 0.01 mol l ⁻¹ Tris-HCl (pH 7.3); (B) 0.01 mol l ⁻¹ Tris-HCl (pH 7.3) and 50% MeOH
Flow rate	0.5 ml min ⁻¹
Injection volume	50 µl
Make up solution	2% (v/v) HNO ₃ ; 0.5 ml min ⁻¹
Gradient	0–5 min—100% A to 45% B; 5–8 min—45% B to 85% B; 8–10 min—85% B to 100% B; and 10–40 min—100% B

Extraction of iodine from seaweed in various media. For SEC studies, iodine was extracted from samples with each of the following solutions: 0.1 mol l⁻¹ NaOH for extracting high as well as low molecular weight fractions,²⁵ 0.1 mol l⁻¹ HCl for extraction of a low molecular weight fraction²⁶ and aqueous buffer 0.03 mol l⁻¹ Tris-HCl (pH 8.0). Approximately 0.1 g of both types of seaweed sample were weighed in different glass vials and treated separately with 10 ml of the above mentioned solutions. Extraction was carried out for 30 min at room temperature with constant stirring using a magnetic stirrer. The extracts were centrifuged for 10 min at 5000 rpm and the supernatant was separated from the residue. Dilutions of the above extracts were made 1 : 10 and 1 : 50 for Wakame and Kombu samples, respectively. After previous filtration with 0.45 µm PVDF filters, 100 µl of the resulting solution was introduced into SEC-UV-ICP-MS system.

Extraction of high MW iodine species from Wakame. Investigation of the association of iodine with high molecular weight fractions was performed by extracting possible proteins from the Wakame sample according to Hou *et al.*¹⁸ and analyzing the extract by SEC-UV-ICP-MS. About 1.0 g of sample was weighed in a glass vial and leached three times with acetone to remove pigments from the plant matrix. Centrifugation was performed each time in order to separate residue from the supernatant (acetone). The residue obtained from the above step was treated with a solution containing 1% CaCl₂ and 0.5% caffeine to remove carbohydrates and polyphenols, respectively, from the plant matrix. Centrifugation was performed at 5000 rpm for 10 min and supernatant was discarded and the residue collected. Finally, the residue was sonicated for 48 h in 10 ml of a 0.03 mol l⁻¹ Tris-HCl (pH 8.0) solution containing 1% SDS, 0.05% NaN₃ and 100 mg of PVPP (polyvinyl polypyrrolidone). PVPP is also used for absorbing polyphenolic compounds from the plant matrix. After centrifugation (10 min, 5000 rpm), proteins were precipitated by adding acetone up to a final concentration of 80% and the solution was kept at -20 °C for 12 h. Finally, the precipitate was collected and solubilized in 3 ml of the above Tris-HCl solution containing 1% SDS. Before introduction to the SEC-UV-ICP-MS system, the above solution was diluted 1 : 10 with Tris-HCl buffer used in the mobile phase.

Iodine-containing high MW species (most likely proteins) were digested by employing enzymatic hydrolysis with Proteinase K.²⁷ About 10 ml of a Tris-HCl (pH 8.0) buffer solution containing 1 mM CaCl₂ was added to 1 ml of protein extract with 0.03 g of Proteinase K enzyme. The solution was kept at a constant temperature of 50 °C with continuous stirring for 12 h. Addition of Proteinase K and stirring were repeated. The final mixture was filtered with 0.45 µm filters. A volume of 100 µl of the resulting solution was injected into the SEC-ICP-MS system and 20 µl was injected to RP-HPLC-ICP-MS system. Prior to injection into the RP-HPLC-ICP-MS system, the hydrolysate was filtered through 10 kDa molecular weight cut-off membrane filters (Centricon) to remove partially digested proteins and enzyme.

Extraction of polyphenol-bound iodine from Wakame. Iodine bound to polyphenols was extracted by treating about 0.5 g of

sample with 10 ml of 75% ethanol three times at room temperature. Supernatants from the above step were combined and concentrated to a small volume under reduced pressure at 50 °C. The final solution was filtered and 100 µl was introduced into the SEC-ICP-MS system.

Chromatographic conditions. Calibration of size-exclusion column was performed by following set of protein standards: albumin (66 kDa); myoglobin (17.6 kDa); aprotinin (6.5 kDa); and Vitamin B₁₂ (1.35 kDa).

In the case of RP-HPLC-ICP-MS, optimization of chromatographic conditions was performed so that the final flow introduced into the ICP-MS system does not interfere with the normal nebulization process and the stability of the plasma was maintained throughout the chromatographic run. A final flow of 1.0 ml min⁻¹ was introduced into the sample introduction system of the ICP-MS and was obtained by combining the flow from the chromatographic column with an external flow of 2% nitric acid solution (both 0.5 ml min⁻¹) using a T-junction after the column.

Results and discussion

Total iodine concentration in samples and extracts

Initially, total iodine determination was performed on the ground sample. Total iodine was also determined in the three extraction media for both seaweed samples.

Results of the total iodine determination are summarized in Table 2. For validation purposes, standard reference material (Citrus Leaves—NIST 1572) was also analyzed for total iodine, as described in the experimental section. The result obtained (2.0 ± 0.05 µg g⁻¹) was in reasonable agreement with the certified value. (1.84 ± 0.03 µg g⁻¹). For the HCl and Tris-HCl extracts of both the samples, the pH was adjusted in the range 9–11 with diluted ammonia solution for stabilizing the iodine signal.

Optimization of SEC-ICP-MS

Size exclusion chromatography was utilized as a preliminary method to understand the distribution of iodine in different molecular weight fractions in the seaweed extracts. Chromatographic conditions were optimized to obtain separation between various forms of iodine in the shortest time possible. Buffers including 0.03 mol l⁻¹ ammonium bicarbonate (pH 8.0), 0.03 mol l⁻¹ ammonium carbonate (pH 9.0) and 0.03 mol l⁻¹ Tris-HCl (pH 8.0) were assayed as mobile phases for SEC. Tris-HCl was selected as it provided the least amount of non-specific interaction between the inorganic iodine and the stationary phase of the column, resulting in faster elution of inorganic iodine species from the column as compared to above two buffers.

Fractionation studies of iodine in seaweed extracts

SEC-ICP-MS of Wakame extracts. As a first approach to iodine speciation, seaweed was treated with the 0.1 mol l⁻¹ sodium hydroxide solution. Alkaline solutions are known to solubilize both high as well as low molecular weight fractions.

Table 2 Comparison of different media for the extraction of iodine from seaweed samples and inorganic iodine species present (95% confidence interval, *n* = 6)

	Total content/µg g ⁻¹ (% RSD)	Extractability (%)			Iodide/µg g ⁻¹	Iodate/µg g ⁻¹
		NaOH	HCl	Tris-HCL		
Kombu	4170 (5.6)	93 ± 2.5	42 ± 0.5	79 ± 1.2	3940	Not detectable
Wakame	226 (4.8)	98 ± 2.8	20 ± 0.8	30 ± 0.3	140	4.16

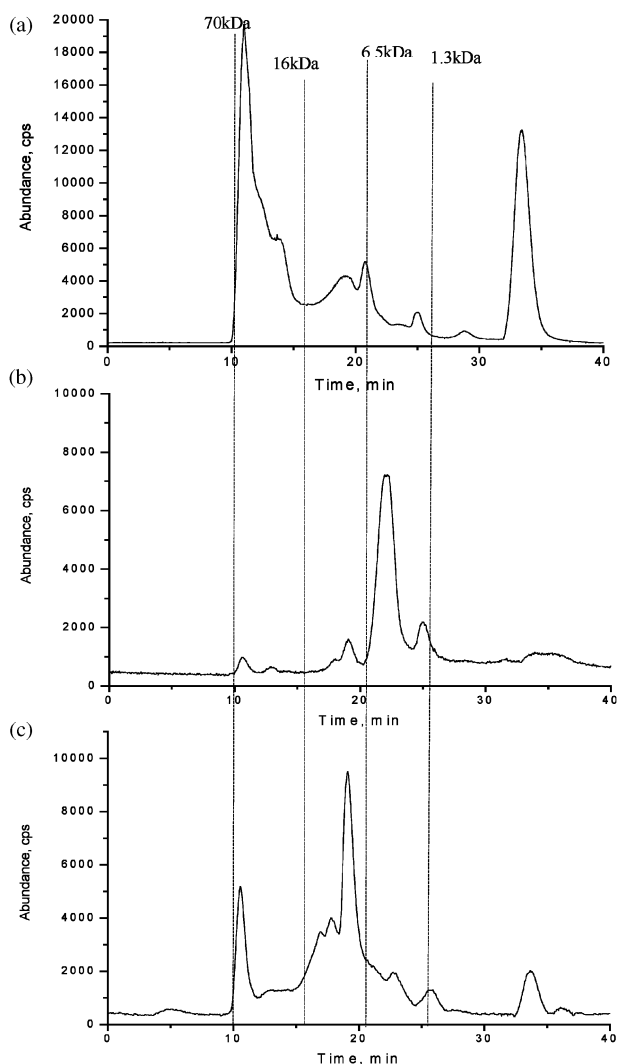


Fig. 1 SEC-ICP-MS chromatograms of Wakame: (a) NaOH extract; (b) HCl extract; (c) enzymatic extract.

It is clear from Fig. 1(a) that in Wakame iodine is associated with both low and high molecular weight fractions. The fractionation profile shows that three peaks of significant intensities are found in the size exclusion chromatogram. The first peak eluting at the dead volume of the column (≥ 70 kDa) represents the association of iodine with the high molecular weight compounds, presumably proteins and/or carbohydrates, as these species are soluble in alkaline media. Another peak at 20.8 min indicates association of iodine with a medium molecular weight fraction of about 6.5 kDa. This peak may be due to the presence of peptides containing iodoamino acids and iodinated polyphenolic species. The last peak eluting after the elution volume of the size exclusion column is identified as the inorganic iodine species, which represents a significant portion of iodine in the Wakame algae. Delayed elution of iodide is attributed to its non-specific interaction between polysaccharide based stationary phases of the size-exclusion column. Since the size exclusion column does not have the capability to separate inorganic forms of iodine, further speciation of the inorganic iodine fraction was performed by anion exchange chromatography, which is discussed later in the text.

Extraction of iodine species in 0.1 mol l^{-1} HCl and in Tris-HCl solution was also evaluated. In an acidic medium, fractions containing only low molecular weight iodinated species are extracted, which is depicted in a corresponding size-exclusion chromatogram Fig. 1(b). Only one significant peak is depicted at 21.7 min at a molecular weight of about 3.1 kDa. This peak might be due to iodine containing peptides. Since the

oxidation of iodide to elemental iodine is enhanced in acidic solutions, a peak corresponding to the inorganic form of iodide could be diminished. It can also be noted from the chromatogram that high molecular weight species eluting at the dead volume of the column are not extracted in acidic media. This is probably due to low solubility of biological macromolecules, such as proteins, at acidic pH.

A comparison of the chromatograms shown in Figs. 1(a) and (b) indicates an association of iodine to high molecular weight fractions, which can be presumed to be proteins based on their different solubility in acidic or basic media. An aqueous buffer Tris-HCl (pH 8.0) was investigated for extracting proteins from algae as it is known to solubilize water soluble proteins.²⁸ It can be noted from the size-exclusion chromatogram Fig. 1(c) that a high molecular weight fraction is extracted in this medium, eluting at the dead volume of the column. This suggests that in marine algae Wakame iodine is incorporated into water soluble high molecular weight proteins.

Study of the association of iodine to biological molecules

In order to confirm that an iodine containing peak corresponding to the high molecular weight fraction in a size-exclusion chromatogram, Fig. 1(c) is only due to the presence of iodine containing proteins, a systematic approach leading to specific precipitation of proteins was followed as described above. Interferences from other high molecular weight molecules, such as pigments, carbohydrates and polyphenols, were avoided by removing them from the plant matrix using acetone, calcium chloride and PVPP, respectively, according to Hou *et al.*¹⁸ SDS, an anion surfactant, was added in the Tris buffer to extract the high molecular weight proteins. Precipitation of proteins extracted in the above solution was achieved by addition of 80% acetone. High organic solvent concentrations have been used in the past for the precipitation of both high as well as low molecular weight proteins.^{28,29}

The proteins thus extracted were injected into the SEC-UV-ICP-MS system and, as shown in Fig. 2(a), only one peak containing high molecular weight iodinated species is eluted at the dead volume of the column. The elution profile of this peak is similar to the elution profile of the high molecular weight peak eluting at the column dead volume in the size-exclusion chromatogram Fig. 1(a). Therefore, it can be concluded that the high molecular weight species extracted in the alkaline solution is due to iodine-containing proteins. The presence of a peak at the column dead volume in the UV elution profile (295 nm), shown in the same chromatogram, also leads to the presence of proteins in the extract.

A further experiment was performed to understand the nature of iodine bonding to proteins in Wakame. Proteinase K was employed for the hydrolysis of proteins. The extract was analyzed by SEC-ICP-MS. The chromatographic fractionation profile revealed the conversion of high molecular weight proteins in Fig. 2(a) into a low molecular weight fraction eluting at about 26.7 min. These species having molecular weight below 1.3 kDa may correspond to the iodinated peptides and iodinated amino acids released after protein hydrolysis. This also suggests that iodine is associated with proteins through covalent bonding.

The presence of iodinated polyphenolic species was also investigated in Wakame seaweed. Extraction of polyphenolic species was performed with 75% (v/v) ethanol according to Hou *et al.*¹⁸ A volume of 100 μl of lyophilized solution was injected into the SEC-ICP-MS system. It is suggested from Fig. 2(c) that in Wakame seaweed iodine is also associated to polyphenolic species. The intensity of the peak eluting at the column dead volume is very low, which can be explained by the low solubility of proteins in organic solvent: major polyphenolic species associated with iodine elute at 18.7 min, which corresponds to a molecular weight of approximately 7.9 kDa.

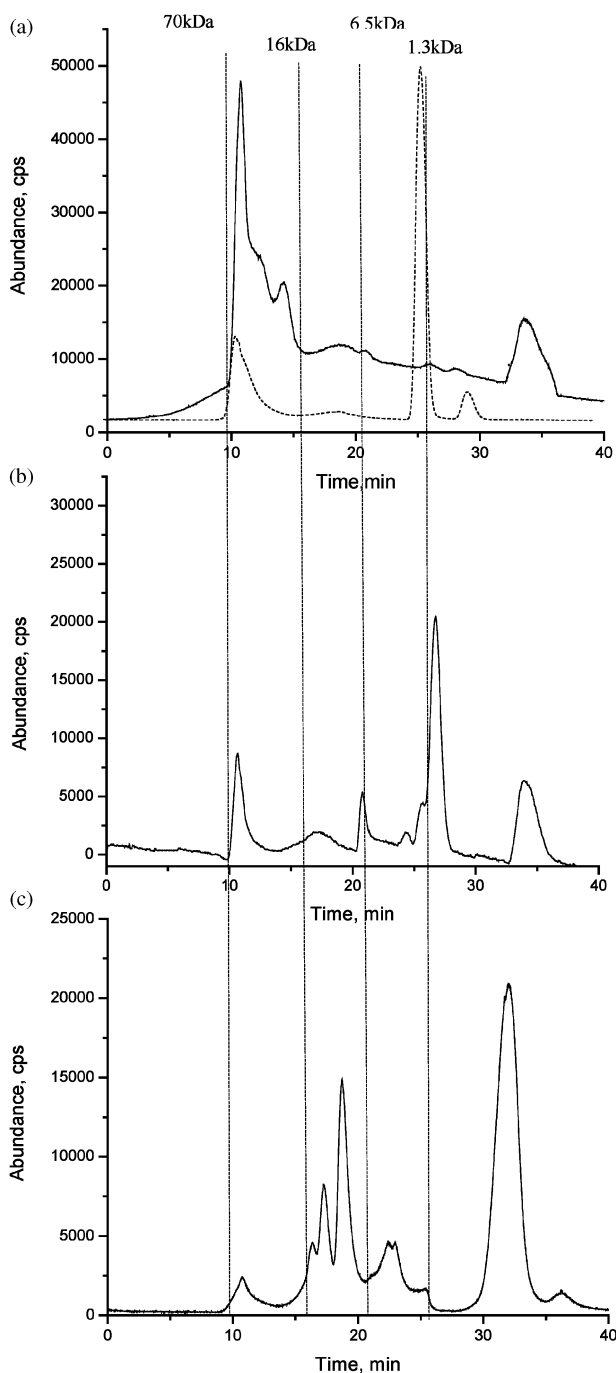


Fig. 2 SEC-ICP-MS chromatograms of Wakame: (a) protein extract (—) spectrophotometric detection (295 nm); (b) enzymatic extract; (c) polyphenolic extract.

Iodine bound polyphenolic species have also been reported by Hou *et al.* in *Sargassum kjellmanianum*.¹⁸ These polyphenolic species may correspond to the polymer of iodine substituted *p*-chloroglucinol, since they are commonly found in brown seaweed.¹⁸

SEC-ICP-MS of Kombu extracts. A similar approach to that developed for Wakame seaweed was also followed for studying the distribution of iodine in Kombu seaweed. Three different extractions: alkaline, acidic and Tris-HCl, as in case of Wakame, were performed. 100 μ l of extracted solution was injected into the SEC-ICP-MS system for speciation of iodine. The fractionation profile of iodine species extracted in alkaline medium shows only one, predominant, low molecular weight species corresponding to the retention time of inorganic iodine

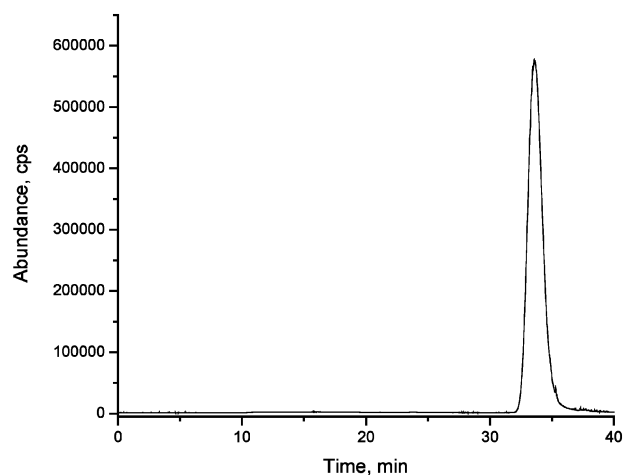


Fig. 3 SEC-ICP-MS chromatogram of Kombu seaweed NaOH extract.

species (Fig. 3). Evaluation of iodine species extraction in acidic and Tris-HCl media reveals the same results. This leads to the conclusion that metabolism of iodine in Kombu does not lead to formation of any significant organoiodine species and it is significantly different from the results obtained for Wakame. The major iodine species found in Kombu corresponds to inorganic iodide species and, as indicated below, has been further identified by IC-ICP-MS.

IC-ICP-MS for speciation of inorganic iodine

Ion chromatography has previously been applied for the separation of anionic iodine species.^{21,22} In the present study, separation between iodide and iodate was performed with an anion-exchange column. The composition of the mobile phase and the sample solution in which standards were prepared was optimized. Complete separation of iodide ($1 - t_{\text{ret}} = 1.9$ min) and iodate ($2 - t_{\text{ret}} = 6.7$ min) was performed using 5 mM sodium hydroxide as the mobile phase (Fig. 4(a)). Since the stability of the iodine species is affected by the pH of the solution, the stability of iodine and iodate in alkaline medium (0.1 mol l^{-1} NaOH) was performed. This study was necessary in alkaline medium because the maximum extraction efficiency of iodine from the seaweed samples was obtained in this medium (Table 2). The composition of the mixture of the two species remains stable in this medium. The peak area precision (% RSD) of iodide and iodate standards injected at 10 ng ml^{-1} , based on three replicate measurements, were well below 1.0% and 1.2%, respectively. Absolute limits of detection for both the species, calculated based on 3σ of the blank signal were found to be $0.12 \mu\text{g l}^{-1}$ and $0.2 \mu\text{g l}^{-1}$.

Peak areas of a particular concentration of iodide and iodate in alkaline medium were found to be similar to the peak areas of corresponding species when injected individually at the same concentration. This suggests that in 0.1 mol l^{-1} NaOH solution, no significant loss or interconversion of one form of iodine to another takes place, which makes it a suitable medium for speciation of inorganic iodine species.

Alkaline extracts of both Wakame and Kombu seaweed were analyzed for the possible presence of iodide and iodate. A volume of 20 μ l of diluted solution was injected into the IC-ICP-MS system. The chromatographic profile shown in Fig. 4(b) reveals that the predominant inorganic iodine species present in Wakame in alkaline extraction medium is iodide, with a low amount of iodate also present. While in the case of Kombu, the only iodine species present is iodide, which is depicted in the IC-ICP-MS chromatogram (Fig. 4(c)).

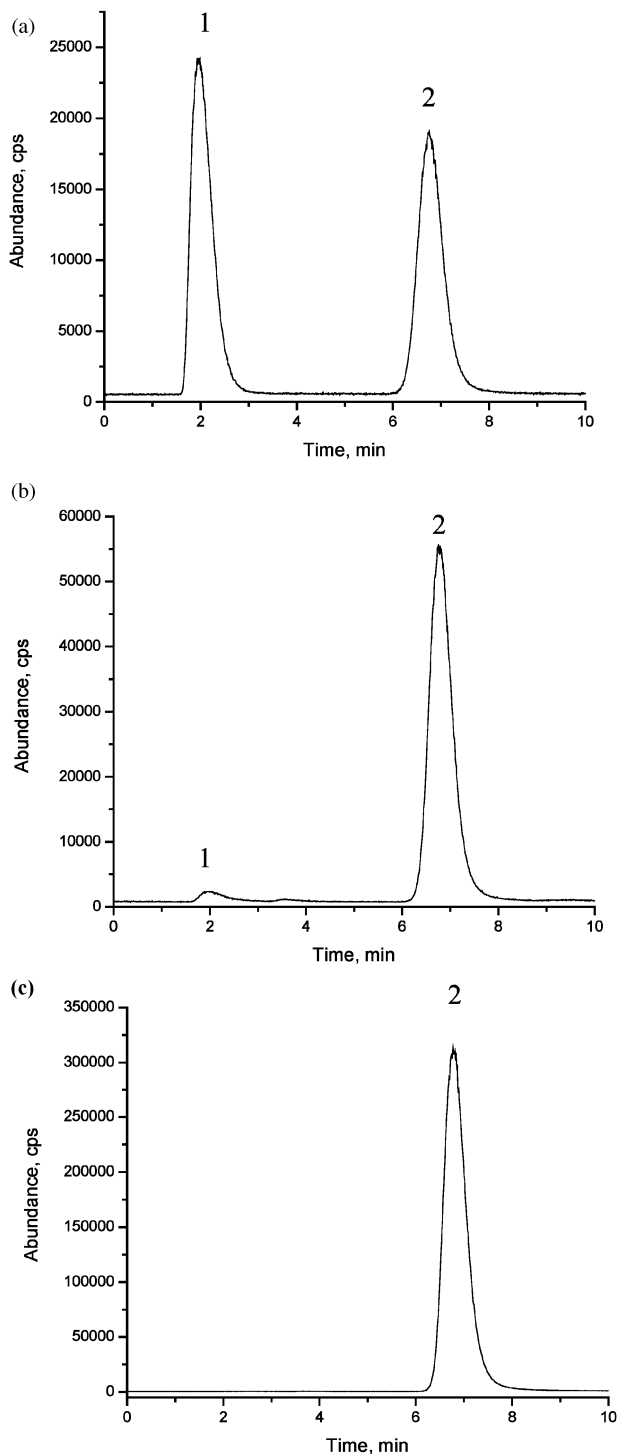


Fig. 4 IC-ICP-MS chromatogram of (a) mixed iodine standards 10 ng ml^{-1} , each prepared in 0.1 mol l^{-1} sodium hydroxide; (1) iodate; (2) iodide. (b) NaOH extract of Wakame. (c) NaOH extract of Kombu.

RP-HPLC-ICP-MS for studying iodine species

Reversed-phase high-performance liquid chromatography was used for the speciation of low molecular weight iodine species. Since association of iodine with proteins has been proved in Wakame seaweed, the extract obtained after enzymatic hydrolysis of the protein fraction was analyzed for the presence of iodoamino acids. Separation of iodoamino acids has been previously achieved by RP-HPLC.^{30,31} For the present study, a similar separation procedure to that published by Michalke²⁰ was followed with some variation in the flow rate of mobile phase and the gradient program. In Fig. 5(a) chromatograms of three laboratory-available iodine standards are presented. It

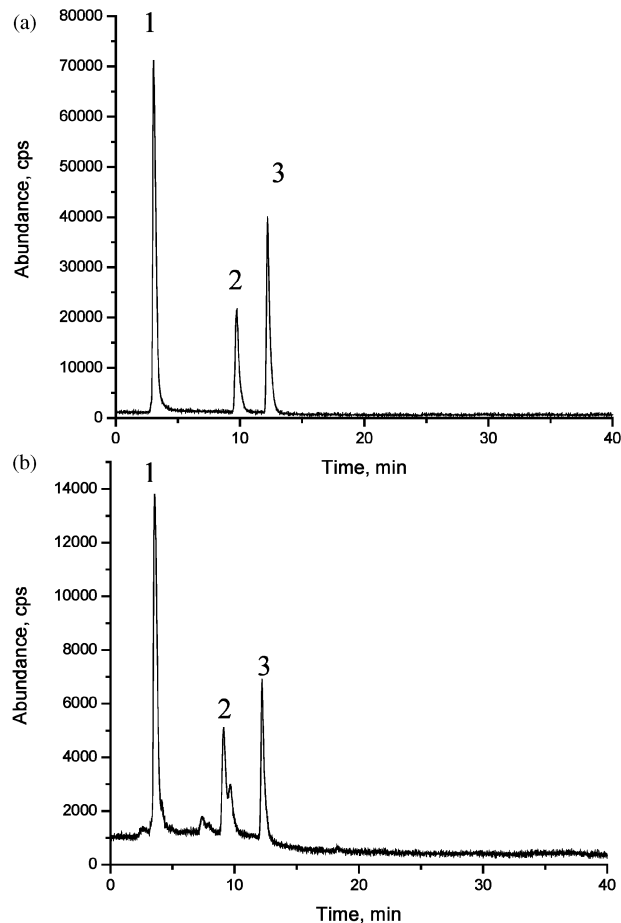


Fig. 5 RP-HPLC-ICP-MS chromatogram of (a) mixed iodine standards 100 ng ml^{-1} ; (1) iodide; (2) 3-iodotyrosine; (3) 3,5-diiodotyrosine. (b) Enzymatic extract of Wakame.

is shown that baseline resolution was obtained between inorganic iodine ($1 - t_{\text{ret}} = 2.9 \text{ min}$), monoiodotyrosine ($2 - t_{\text{ret}} = 9.6 \text{ min}$) and diiodotyrosine ($3 - t_{\text{ret}} = 12.1 \text{ min}$). Also, it can be noted from Table 1 that about 50% methanol was required for elution of iodoamino acids from the column. In order to reduce the organic load introduced into the plasma, online dilution of the chromatographic eluent, using 2% HNO_3 , was performed with a dilution factor of 1 : 2. Dilution using HNO_3 not only increases the MeOH tolerance of the plasma, since it partially oxidizes the methanol,²⁰ but also reduces the deposition of carbon residue on the sampling and skimmer cones surfaces. Rf plasma power was also increased to 1500 W to circumvent the cooling effect due to the introduction of organic solvent.

Before injection, the hydrolysate was filtered through Centricon-10 (molecular weight cut-off filter) to remove unhydrolysed higher MW material and Proteinase K to avoid column loading. A volume of $50 \mu\text{l}$ -filtered solution was introduced into the RP-HPLC-ICP-MS system. From the chromatographic information shown in Fig. 5(b), it is depicted that iodide, monoiodotyrosine and di-iodotyrosine are the primary species present in the enzymatic extract. Standard addition also gave enough evidence for the presence of these species. It can be noted that no other iodoamino acids are eluted from the column, which suggests that iodine metabolism in Wakame leads to the formation of only mono- and di-iodinated tyrosines, unlike human metabolism where iodination can lead to formation of tetraiodinated species.

Conclusions

In this study, the applicability of several chromatographic techniques, including SEC, IC-HPLC and RP-HPLC, coupled

to ICP-MS to iodine speciation in seaweed has been demonstrated. Moreover, the use of hyphenated techniques for iodine speciation in seaweed extracts allowed us to obtain important information on the association of iodine to the various matrix components of seaweed. Whereas iodide is about the most predominant species present in Kombu, a more complicated distribution of iodine is present in Wakame seaweed. This study shows that incorporation of iodine in different seaweeds follows different metabolic pathways, notwithstanding that both of them belong to same class, Phaeophyceae. The presence of iodide was proved in Kombu, while in the case of Wakame, monoiodotyrosine and diiodotyrosine are also present and probably bound to the proteins. Since the bioavailability of iodide is better than any other form of iodine, Kombu seaweed would be preferred as a natural dietary supplement.

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