Trace Metal Determination of Edible Seaweeds by Pressurized Hot Water Extraction and Determination by Inductively Coupled Plasma Mass Spectrometry

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INTRODUCTION

Seaweeds (and also shellfish and fish) were used to study the quality of the marine environment (1). The use of seaweeds is of particular advantage since they are suitable bio-monitors due to their high pollutant accumulation capacities compared with other species (2). Moreover, seaweed has been recognized as an important source of nutrients in the human diet since ancient times. Sea vegetables are an important source of essential minerals, vitamins, proteins, carbohydrates, and polyunsaturated fatty acids (3). These properties make seaweeds and seaweed by-products suitable for a large number of applications in the food and pharmaceutical industries. Due to the introduction of seaweed in our daily diet, an elemental composition study of these foods becomes necessary.

The determination of metals in organic matrices requires analytical techniques with low detection limits. Inductively coupled plasma mass spectrometry (ICP-MS) stands out as a very powerful and attractive technique for carrying out the determination of metals because of its multi-element capacity and high sensitivity. However, the determination of metals in solid biological samples by spectroscopy methods commonly requires the total dissolution of the sample. Metal extraction has generally been performed

ABSTRACT

A fast, simple, automated, and “green” method, using pressurized hot water extraction, has been developed for the determination of total As, Ba, Cd, Cr, Mn, Ni, Pb, and Zn in edible seaweeds (Dulse, Nori, Kombu, Wakame, Sea Spaghetti, Sea Lettuce, and Furbelows). Pressurized hot water modified with acetic acid (150 mM) was tested as a solvent to extract these metals from several seaweed samples. Two-level factorial designs were used to optimize the pressurized hot water extraction (PHWE) procedure. The effects of several parameters were evaluated using a Plackett-Burman $2^9 \times 3/32$ experimental design, type III resolution, and four degrees of freedom. The significant parameters (extraction temperature and extraction pressure) obtained were optimized by using $2^2 +$ star central composite design.

The accuracy of the methods was verified by analyzing BCR-249 Sea Lettuce. Detection and quantification limits in the range of 0.02–2.2 $\mu\text{g g}^{-1}$ and 0.05–7.3 $\mu\text{g g}^{-1}$ were achieved, respectively. The developed method was applied to several Atlantic seaweeds harvested in the Galician coast (Northwestern Spain).

by acid digestion assisted by ultrasonic probes or baths or by microwave energy (4–6). Recent trends in sample treatment for solid-liquid extraction of metals and metalloids include pressurized hot water (PHWE)/liquid extraction

(PLE) (7) and matrix solid phase extraction (MSPD) (8).

Water can be used effectively as solvent in pressurized hot water extraction (PHWE) because the physio-chemical properties of water are readily altered through changes in temperature and pressure. The addition of several organic (ethanol, methanol, acetone, acetonitrile, sodium dodecyl sulphate, hydrocarbons or carbon dioxide) and inorganic (nitric acid at 1.0–4.0% v/v) modifiers to pressurized hot water alters both the properties of water and the critical temperature and pressure, which enhance the solubility of analytes in water or increase interactions of water with the analytes (9).

PHWE pre-treatments reduce analysis time, sample manipulation (filtration is avoided), solvent consumption, and the amount of sample required for analysis, which increases sample throughput and shortens turn-around time. In addition, the use of water or water modified with dilute acids could be linked to the principles of “Green Chemistry”.

The aim of the current work is the optimization of a PHWE method to extract metals (As, Ba, Cd, Cr, Mn, Ni, Pb, and Zn) from edible seaweeds. The fast, simple, and safe element extraction combined with simultaneous ICP-MS detection can offer a convenient method for assessing the presence of several metals in edible seaweed samples in a relatively short analysis time.

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EXPERIMENTAL

Instrumentation

Pressurized hot water extraction was conducted using a Dionex ASE®-200 system (Sunnyvale, CA, USA), equipped with 11-mL stainless steel extraction cells (Dionex) and cellulose filters at the bottom and top of the extraction cells (D28, 1.983 cm diameter, Dionex). A Thermo Finnigan X Series ICP-MS (Thermo Instruments, Austin, TX, USA) was used for analysis. An Anton Paar® Multiwave® (Graz, Austria) with closed Teflon® vessels was used for assisting the acid digestion. The chemometrics package used for data analysis was a Statgraphics Plus 5.1 (Manugistics Inc., Rockville, MD, USA).

Reagents and Standards Solutions

Arsenic, barium, cadmium, chromium, manganese, nickel, lead, and zinc stock standard solutions of 1000 mg L⁻¹ each (Panreac, Barcelona, Spain) were used. Glacial acetic acid 99.99% from Aldrich Chemical Co. (Milwaukee, WI, USA) was used as modifier or additive. Nitric acid 69–70% (Baker, Phillipsburg, PA, USA) and hydrogen peroxide 33% (m/v) (from Panreac) were used for acid extraction. Diatomaceous earth (DE), 95% SiO₂ (Aldrich) was used as a dispersing agent. BCR 279 (Sea lettuce, *Ulva lactuca*) from Community Bureau of Reference – Commission of the European Communities (Brussels, Belgium) was used for method validation. Nitrogen Alphagaz™ (Air Liquide, Madrid, Spain) was used as a purge gas. For all solutions, ultra pure water with a resistance 18 MΩ cm⁻¹, obtained from a Milli-Q™ purification device (Millipore Co., Bedford, MA, USA), was used.

Edible Seaweed Samples

Seven different types of edible seaweed, harvested on the Galician coast (Northwestern Spain), were

obtained from a local manufacturer. One sample is commercialized as cooked and canned in brine (~ 25 g) and consists of a mixture of two brown seaweeds: Sea Spaghetti (*Himanthalia elongata*) and Furberlows (*Saccorbiza polyschides*). This sample was previously freeze-dried. The remaining samples are commercialized as dehydrated products (approximately 100 g): Dulse, *Palmaria palmata*; and Nori, *Porphyra umbilicalis* (red seaweeds); Kombu, *Laminaria ochroleuca*; Wakame, *Undaria pinnatifida*; and Sea Spaghetti, *H. elongata* (brown seaweed); and Sea lettuce, *Ulva rigida* (green seaweed). Dehydrated samples were kept in an oven at 40 °C for 2 hours to eliminate water traces before grinding and were preserved in pre-cleaned polyethylene bottles.

Microwave-Assisted Acid Digestion Procedure

Powdered seaweed samples were subjected to an acid digestion assisted by microwave energy to discover the total metal content and for comparative purposes. A 0.25 g sample was weighed and placed into PTFE closed bombs. Then 2.0 mL of ultra-pure water, 2.0 mL of concentrated H₂O₂, and 4.0 mL of concentrated HNO₃ were added, and the mixture subjected to microwave energy. The microwave energy program involved three steps: first, from 0 to 800 W (15 min), 15.0 min at 800 W, and

finally from 800 to 0 W (5.0 min) (maximum temperature of 200 °C), and a venting stage of 40 min to cool the reactors. After microwave digestion, the acid liquid phase was filtered (Whatman n° 40 paper filter). The solutions were diluted to 25 mL with ultra-pure water and kept in polyethylene bottles at 4 °C before measurement.

PHWE Procedure

Stainless-steel cells with cellulose filters on both the bottom and top (11 mL) were filled with approximately 0.50 g seaweed thoroughly mixed with 2.5 g inert diatomaceous earth (DE) (proportion 1:5). DE was used to prevent aggregation of the sample particles and blockage of the outlet of the extraction cell. After closure, the cells were situated in the carousel of the ASE 200 system, and the samples were extracted under the PHWE conditions shown in Table I. The cells were purged for 60 seconds with N₂ gas after the extraction, and the displaced solvent was collected in pre-cleaned glass vials. Finally, the extracts were transferred to 25-mL volumetric flasks, diluted to volume with Milli-Q™ water, and then placed in polyethylene bottles at 4 °C. At least two different blanks were performed for each set of PHWE conditions.

TABLE I
Pressurized Liquid Extraction Conditions

Modifier concentration	150 mM
Extraction temperature	130 °C
Static time	5 min
Extraction step	3
Pressure	3000 psi
Flush volume solvent	60%
DE mass/sample mass ratio	5
N ₂ purge time	60 s
Cell size	11 mL

ICP-MS Measurements

Trace metals in aqueous extracts were measured by ICP-MS under the standard operating conditions given in Table II. Calibrations were based on 150 mM acetic acid aqueous standard solutions covering metal concentrations from 0 to 1000 $\mu\text{g L}^{-1}$. Yttrium and indium ($5.0 \mu\text{g L}^{-1}$), germanium ($10.0 \mu\text{g L}^{-1}$), and scandium ($50.0 \mu\text{g L}^{-1}$) were selected as internal standards.

Calibration and Sensitivity

After performing different calibrations over six different days, good repeatability of the calibration's slopes was obtained. The limit of detection LOD (3 SD criterion, SD standard deviation of 11 measurements of a reagent blank), expressed as $\mu\text{g g}^{-1}$ and the limit of quantification LOQ (10 SD criterion) were calculated. Low LOD and LOQ (see Table III) values were obtained for all elements so that metal determination in edible seaweed samples can be performed.

TABLE III
Mean Slopes for Calibration
Graphs and
Detection and Quantification
Limits

	Mean Slope $\pm$ SD ^a	LOD ^b	LOQ ^c
		$(\mu\text{g g}^{-1})$	
As	0.460 ± 1.5	0.48	1.68
Ba	0.120 ± 3.1	0.12	0.39
Cd	0.042 ± 3.2	0.03	0.09
Cr	0.712 ± 5.6	0.08	0.27
Mn	0.147 ± 3.6	2.14	7.30
Ni	0.202 ± 4.3	0.16	0.54
Pb	0.552 ± 10.8	0.12	0.05
Zn	0.062 ± 21.6	0.24	0.79

^a n = 6

^b LOD = 3 SD/m

^c LOQ = 10 SD/m

where SD is the standard deviation of 11 measurements of a blank and m is the slope of standard addition graph.

TABLE II
ICP-MS Operating Conditions

General		
	Radiofrequency power	1380 W
	Peristaltic pump speed	0.45 rpm
	Stabilization delay	35 s
	Number of replicates	3
	Nebulizer type	MicroMist
Gas flow rates		
	Plasma	17.0 L min ⁻¹
	Auxiliary	1.65 L min ⁻¹
	Sheath	0.27 L min ⁻¹
	Nebulizer	0.98 L min ⁻¹
Torch alignment		
	Sampling depth	7.0 mm
Elements		
	Mass	
	As	75
	Ba	138
	Cd	111
	Cr	52
	Mn	55
	Ni	60
	Pb	208
	Zn	66
Internal standards		⁴⁵ Sc, ⁷² Ge, ⁸⁹ Y, ¹¹⁵ In

RESULTS AND DISCUSSION

Optimization of Pressurized Hot Water Extraction Procedure

Several variables such as modifier (acetic acid) concentration (M), extraction temperature (T), static time (t), extraction steps (S), pressure (P), mean particle size (s), and diatomaceous earth mass/sample mass ratio (R) were studied by using factorial designs. A "dummy" variable (D), which has no influence on the experiment, is employed to evaluate the standard errors of the experiments. Flush solvent volume and N₂ purge time were fixed at 60% and 60 s, respectively. Low and high values were selected for each factor according

to previous studies and taking into account instrumental limitations (Table IV). The experimental design matrix corresponding to the selected factors is shown in Table V. A homogenized pooled seaweed sample was used for optimization, and several aliquots of this pooled sample were weighed and extracted to obtain the optimum PHWE extraction conditions. The target elements were As, Ba, Cd, Cr, Mn, Ni, Pb and Zn, since their concentrations are higher than the LOQs. In order to establish the reference metals concentrations, the pool was acid-digested four times and the metal concentrations measured twice by ICP-MS (Table VI). Therefore, the response variable was the

TABLE IV
Experimental Field Definition for the Plackett–Burman (PBD) and Central Composite (CCD) Designs^a

Variable	Symbol	Low Level (–)	High Level (+)	Unit
Modifier concentration	M	0.15	0.45	mM
Extraction temperature	T	50	100	°C
Static time	t	5	10	min
Extraction step	S	1	3	
Pressure	P	500 ^b	1500 ^c	psi
Particle size	s	600	900	µm
DE mass/sample mass ratio	R	2	5	
Dummy factor	D	– 1	+ 1	

^a The seaweed mass was 0.50 g for all experiments.

^b 1000 psi for CCD

^c 2500 psi for CCD

TABLE V
Plackett-Burman Design for the Determination of Significant Parameters Involved on PHWE

Run	M	T	t	S	P	s	R	D	Mean ^a AR(%)
1	+	–	+	–	–	–	+	+	58
2	+	+	–	+	–	–	–	+	94
3	–	+	+	–	+	–	–	–	60
4	+	–	+	+	–	+	–	–	56
5	+	+	–	+	+	–	+	–	44
6	+	+	+	–	+	+	–	+	48
7	–	+	+	+	–	+	+	–	78
8	–	–	+	+	+	–	+	+	47
9	–	–	–	+	+	+	–	+	37
10	+	–	–	–	+	+	+	–	29
11	–	+	–	–	–	+	+	+	71
12	–	–	–	–	–	–	–	–	43

^a Mean Analytical Recovery(%).

mean percentage of released elements in accordance with the equation:

$$\text{Mean Recovery(\%)} = \sum \frac{(C_{\text{PHWE}}/C_{\text{MAE}})}{N} \times 100$$

where C_{PHWE} is the element concentration obtained after the PHWE procedure, C_{MAE} is the element concentration after the microwave acid digestion procedure, and N is the number of elements studied (N=8).

Analysis of the results given in Table V produces the Pareto chart (P=95.0%) shown in Figure 1. In these charts, the length of each bar is proportional to the standardized effect. The vertical line on the plot judges the effects that are statistically significant. Bars that extend beyond the line correspond to effects that are statistically significant at the 95% confidence level. The results showed statistical significance for some factors, such as extraction temperature (T) and

TABLE VI
Mean Metal Concentrations and SD (N=5) Found in Edible Seaweed Pool Sample After Microwave-assisted Acid Digestion

	Conc. (µg g ⁻¹)	SD (µg g ⁻¹)
As	19.7	0.7
Ba	8.31	0.6
Cd	0.86	0.03
Cr	0.56	0.03
Mn	19.3	0.1
Ni	2.42	0.2
Pb	0.02	0.001
Zn	70.6	1.9

extraction pressure (P). The variable static time (t) was not found to be statistically significant. This fact is important because short static times (5 min) can be chosen to perform the extractions. Extraction steps (S), particle size (s), DE mass/sample mass ratio (R), and modifier concentration were also statistically non-significant within the studied ranges. Finally, the dummy factor (D) was not significant for any case, meaning that there is neither systematic error nor unknown variables affecting the system.

The optimization of T and P factors was accomplished by an orthogonal central composite design (2² + star) which studied the effects of these factors in 10 randomized runs with 5 degrees of freedom. The remaining variables, modifier concentration, extraction time, number of extraction steps, DE mass/sample mass ratio and particle size, were fixed at convenient values in accordance with operating aspects and pre-treatment time. Therefore, acetic acid concentration and extraction time were fixed at the low values, 150 mM and 5.0 min, respectively. The variable number of extraction steps was fixed at the high values (3 extraction steps) because the effect of

TABLE VII
Central 2² + Star Orthogonal
Composite Design
for Extraction Temperature
Extraction Pressure Variables

Run No.	T	P	Mean ^a AR(%)
1	K ₀ ^b	K ₀ ^c	70
2	-	-	46
3	+	-	84
4	-	+	56
5	+	+	100
6	- α ^c	K ₀ ^c	41
7	+ α ^d	K ₀ ^c	81
8	K ₀ ^b	- α ^f	89
9	K ₀ ^b	+ α ^g	99
10	K ₀ ^b	K ₀ ^c	83

^a Mean Analytical Recovery(%).

^b K₀ = 100 °C

^c - α = 46 °C

^d + α = 154 °C

^e K₀ = 1750 psi

^f - α = 940 psi

^g + α = 2600 psi

this variable is positive. Finally, the DE mass/sample mass ratio was fixed around 5, which implies a weight around 2.5 g of DE for a fixed sample mass of 0.50 g.

Table VII gives the central composite design matrix. After performing the CCD experiments, the statistical evaluation of quadratic terms has proven significant for some cases, which implied response surfaces with curvature. The results of the experiments were analyzed by the response surface plot which is shown in Figure 2. It can be seen that 130 °C is the optimum value for extraction temperature factor. The maximum values for the factor extraction pressure has not been included in the experimental region (Figure 2). A new factorial design shifted in the direction of the maximum gradient would reach practical limitations. The highest pressure that could be used is 3000 psi. Thus, we selected this pressure as an ade-

TABLE VIII
Analysis of BCR-279 (Sea Lettuce) Certified Reference Materials
After PHWE Procedure

	Certified Value (μg g ⁻¹)	Found Value (μg g ⁻¹)
As	3.09 ± 0.21	2.92 ± 0.1
Ba	- ^a	12.9 ± 1.3
Cd	0.274 ± 0.022	0.259 ± 0.01
Cr	8.1-10.7 ^b	8.9 ± 0.4
Mn	2090-2150 ^c	2043 ± 60
Ni	12.8 ^d	14.9 ± 0.2
Pb	13.5 ± 0.4	13.9 ± 0.2
Zn	51.3 ± 1.2	50.8 ± 2.1

^a Not certified.

^b Not certified. Cr concentration range reported by several authors (10,11).

^c Not certified. Mn concentration range reported by several authors (12).

^d Not certified. Ni concentration range reported by several authors (11).

Fig. 1. Standardized (P=95%) main effects Pareto Charts for the Plackett-Burman Design using mean metal extraction percentages.

quate value. The fixed values for the insignificant factors (results from Plackett-Burman design) are also given in Table I.

Validation

Accuracy of the proposed method was assessed by analyzing BCR-279, which had certified concentrations for some metals (As, Cd, Pb, and Zn). The CRM was prepared seven times following the optimized PHWE procedure, and each extract was analyzed twice by ICP-MS. After subtracting reagent blanks, the concentrations found are listed in Table VIII. Good agree-

ment can be seen between the found As, Pb, and Zn concentrations and the certified As, Pb, and Zn contents. This fact has been confirmed after applying a *t*-test (95% confidence interval). Ba, Cr, Mn, and Ni contents are not certified in BCR-279, but some reported concentrations can be found in the literature. As listed in Table VIII, the Cr, Mn, and Ni concentrations found in BCR-279 are quite similar to the reported values (10-12).

Fig. 2. Estimated Response Surface from the Central Composite Design using mean metal extraction percentages.

Application to Edible Seaweeds

The optimized procedure was applied to several edible seaweed samples. The solid samples were subjected twice to PHWE. The results are shown in Figure 3. Metal contents (dry weight) were within the 4.9–42.6, <0.39–17.7, <0.09–3.56, <0.27–0.60, <7.30–53.7, <0.54–13.9, <0.05–1.50, and 1.5–48.3 $\mu\text{g g}^{-1}$ ranges for As, Ba, Cd, Cr, Mn, Ni, Pb, and Zn, respectively. Low concentrations were observed for Cd, Cr and Pb (<0.09–3.56, <0.27–0.60, and <0.05–1.50 $\mu\text{g g}^{-1}$, respectively).

Fig. 3. Analysis of several Atlantic seaweeds from the Galician Coast.

CONCLUSION

The use of pressurized hot water extraction (PHWE) is a simple and fast (~ 17 minutes) extraction procedure for metal extraction from edible seaweeds. The most important variables affecting the extraction method were the extraction temperature and pressure. Quantitative recoveries were reached for all metals studied (As, Ba, Cd, Cr, Mn, Ni, Pb, and Zn) by using pressurized hot water modified with acetic acid (150 mM) at an extraction temperature of 130 °C and at a pressure of 3000 psi, for a static time of 5 minutes and three cycles. Filtration or centrifugation stages to separate the extract and the solid residues are avoided, thus minimizing leachate handling. Apart from the efficiency of the proposed analytical alternative, the use of this method not only reduces the cost of analysis by saving time and acids, but also reduces the environmental risk by minimizing acid wastes in the laboratory.

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