Characterization of Edible Seaweed Harvested on the Galician Coast (Northwestern Spain) Using Pattern Recognition Techniques and Major and Trace Element Data

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Major and trace elements in North Atlantic seaweed originating from Galicia (northwestern Spain) were determined by using inductively coupled plasma−optical emission spectrometry (ICP-OES) (Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn), inductively coupled plasma−mass spectrometry (ICP-MS) (Br and I) and hydride generation−atomic fluorescence spectrometry (HG-AFS) (As). Pattern recognition techniques were then used to classify the edible seaweed according to their type (red, brown, and green seaweed) and also their variety (Wakame, Fucus, Sea Spaghetti, Kombu, Dulse, Nori, and Sea Lettuce). Principal component analysis (PCA) and cluster analysis (CA) were used as exploratory techniques, and linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA) were used as classification procedures. In total, t12 elements were determined in a range of 35 edible seaweed samples (20 brown seaweed, 10 red seaweed, 4 green seaweed, and 1 canned seaweed). Natural groupings of the samples (brown, red, and green types) were observed using PCA and CA (squared Euclidean distance between objects and Ward method as clustering procedure). The application of LDA gave correct assignation percentages of 100% for brown, red, and green types at a significance level of 5%. However, a satisfactory classification (recognition and prediction) using SIMCA was obtained only for red seaweed (100% of cases correctly classified), whereas percentages of 89 and 80% were obtained for brown seaweed for recognition (training set) and prediction (testing set), respectively.

KEYWORDS: Seaweed; trace elements; atomic spectrometry; principal component analysis; cluster analysis; linear discriminant analysis; soft independent modeling of class analogy

INTRODUCTION

Due to the current interest in health food in Western countries, the consumption of edible seaweed and the development of seaweed-based industries, especially in Europe, have increased in recent decades. According to the FAO (*1*, *2*), seaweed production in Europe represents approximately 10% of world seaweed production, and this percentage is expected to increase in the coming years. Seaweed has been well recognized as a food rich in essential minerals and fiber, as well as in other important bioactive compounds such as proteins, vitamins, carotenoids such as fucoxanthin, and polyunsaturated fatty acids (*3*, *4*). In addition, the continuous discovery of natural products with biological and pharmacological activity from seaweed has attracted the attention of pharmaceutical companies for use in drug development (*5*).

The high content of minerals is attributed to an efficient adsorption of metals and organometallic species from seawater.

Polysaccharides and proteins present in the cell wall of seaweed are mainly responsible for this excellent retention of minerals (*6*). In addition to adsorption mechanisms, elements can be bioaccumulated by biochemical (assimilation) process (*7*), and the elemental concentrations in the surrounding waters also play an important role in the whole bioaccumulation process (*8*). Although most minerals are essential elements for humans, especially iodine with a well-recognized bioactivity on the synthesis of thyroid hormones (*9*), some of them are potentially toxic elements, especially heavy metals. Therefore, the current interest in the concentrations of both essential and toxic elements in seaweed before and after commercialization of the products is continuously required. The establishment of reference values for minerals in seaweed is difficult because, as expected, major and trace element contents in these macroalgae can vary enormously among the different families, genera, and species of seaweed, even under similar environmental conditions, geographical origin, and harvesting time (*10*, *11*). Several works have been published dealing with total major and trace element levels in different seaweeds, mainly freshwater samples for environmental biomonitoring (*12−17*) and

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J. Agric. Food Chem., Vol. 58, No. 3, 2010   **1987**

also in edible seaweed (*9, 18−24*). However, few works have been published showing how the overall mineral data can help distinguish different seaweed types. For these latter studies, the correlation between the magnitudes of a certain variable (a major or trace element concentration in different seaweed types) cannot be carried out with conventional univariate techniques because to classify a certain seaweed type, we must take into account many variables at once. Therefore, the use of multivariate statistical methods applied to the values adopted by different parameters (variables), for example, major and trace element concentrations, are necessary to find the differences or similarities among several samples. On this basis, Besada et al. (*24*) have recently applied unsupervised (principal component analysis, PCA, and cluster analysis, CA) and supervised (linear discriminant analysis, LDA) pattern recognition techniques for distinguishing edible seaweed harvested in two different areas (Atlantic and Pacific Oceans).

One of the objectives of the current work has been the establishment of levels of major and trace elements in edible seaweed from Galicia (northwestern Spain). A second aim is the use of the levels of those elements in edible seaweed to observe differences among seaweed families harvested in the same area, under similar environmental conditions, and for the same harvesting time. The characterization has been performed by applying unsupervised pattern recognition techniques (PCA and CA) and supervised pattern recognition methods (LDA and soft independent modeling of class analogy, SIMCA) as multivariate approaches. Variables such as As, Ba, Br, Ca, Cu, I, K, Mg, Mn, Na, Sr, and Zn have been used as discriminating factors. Because of the small number of samples under study, the results obtained must be considered as a preliminary attempt to classify seaweed.

MATERIALS AND METHODS

Instrumentation. An ICP-OES instrument (Optima 3300 DV, Perkin-Elmer, Norwalk, CT) equipped with an autosampler AS 91 (Perkin-Elmer) and a GemCone nebulizer-cyclonic spray chamber (Perkin-Elmer) was used for Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn measurements. An ICP-MS instrument (820-MS, Varian, Mulgrave, Australia), equipped with an SPS3 autosampler (Varian), a MicroMist nebulizer type (Varian), and nickel skimmer and sample cones, was used for Br and I determinations. Quantification of As was performed by using an atomic fluorescence spectrometer coupled with a hydride generation system, PSA 10.055 Millennium Excalibur (Kent, U.K.) and an arsenic high-intensity hollow cathode lamp (HI-HCL) (PSA). Other equipment used included an Ethos Plus microwave lab-station (Milestone, Sorisole, Italy), delivering a maximum power and temperature of 1000 W and 300 °C, respectively, and with internal temperature control, which was used to assist the acid digestion process; a Centromix centrifuge (Selecta, Barcelona, Spain); a Retsch agata automatic mortar (model RM100, Haan, Germany); and a LYPH-LOCK 6 L freeze-dry system model 77530 from Labconco Corp. (Kansas City, MO). The chemometrics packages used for data analysis were Statgraphics Plus 5.0 (Manugistics Inc., Rockville MD), Statistica 6.0 (StatSoft Inc., Hamburg, Germany), and Unscrambler 7.01 (CAMO ASA, Trondheim, Norway).

Reagents. All solutions were prepared by using ultrapure water of resistance 18 MΩ cm^{-1} obtained from a Milli-Q purification system (Millipore Corp., Bedford, MA). Stock standard solutions of the metallic elements (1000 mg L^{-1}) were of ultrapure grade: $Cu(NO_3)_2$ and $NaNO_3$ were purchased from Scharlau (Barcelona, Spain); $Ba(NO_3)_2$, $Ca(NO_3)_2$, KNO_3, $Mg(NO_3)_2$, $Mn(NO_3)_2$, $Sr(NO_3)_2$, and $Zn(NO_3)_2$ were from Merck (Poole, U.K.); and As_2O_3 was from Sigma-Aldrich-Fluka (Steinheim, Germany). Bromide and iodide stock standard solutions (1000 mg L^{-1}) were prepared from 99.0% potassium bromide (Probus, Barcelona, Spain) and 99.5% potassium iodide (Merck). Tellurium chloride and yttrium nitrate standard solutions (1000 mg L^{-1}), used as

Table 1. Analysis of NIST-09 (Sargasso) Certified Reference Material

	certified value (μg g^{-1})	found value (μg g^{-1})	% recovery
As	115 ± 9	103 ± 5	90 ± 8.3
Ba	—[a]	16.0 ± 0.8	—
Br	270[b]	274 ± 17	102
Ca	13400 ± 536	13216 ± 52	101 ± 4.06
Cu	4.9 ± 0.2	5.1 ± 0.1	104 ± 4.70
K	61000 ± 1830	59431 ± 206	97 ± 2.9
I	520[b]	525 ± 59	101
Mg	6500 ± 325	5978 ± 258	92 ± 6.1
Mn	21.2 ± 1.1	20.2 ± 0.1	95 ± 4.9
Na	17000 ± 850	16399 ± 374	97 ± 5.3
Sr	1000 ± 30	975 ± 4	98 ± 3.0
Zn	15.6 ± 1.2	16.1 ± 0.2	103 ± 8.03

[a] Not given. [b] Indicative value.

Table 3. Mean Concentrations and Standard Deviations (in Parentheses), Expressed as Micrograms per Gram (As, Ba, Br, Cu, I, Mn, Sr, and Zn) and Percent (m/m) (Ca, K, Mg, and Na), for Different Edible Seaweeds

	brown seaweed (*N* = 20)	red seaweed (*N* = 11)	green seaweed (*N* = 4)
As	112 (124)	11.8 (6.26)	3.97 (1.47)
Ba	8.07 (8.21)	3.44 (2.26)	14.6 (11.6)
Br	681.4 (415.3)	179.9 (150.3)	504.6 (28.56)
Ca	1.1 (0.22)	0.38 (0.13)	0.72 (0.35)
Cu	3.13 (1.32)	6.79 (3.36)	14.4 (2.97)
I	2048 (2459)	77.7 (36.2)	100.1 (29.79)
K	6.9 (3.0)	4.8 (3.0)	1.7 (0.3)
Mg	0.83 (0.31)	0.24 (0.079)	2.9 (0.44)
Mn	21.7 (26.3)	45.0 (45.9)	77.8 (33.8)
Na	5.0 (3.0)	0.88 (0.53)	2.9 (2.0)
Sr	54.5 (37.0)	4.6 (1.1)	0.634 (0.136)
Zn	47.4 (44.8)	66.8 (24.3)	40.5 (15.0)

Table 2. Mean Concentrations and Standard Deviations (in Parentheses), Expressed as Micrograms per Gram (As, Ba, Br, Cu, I, Mn, Sr, and Zn) and Percent (m/m) (Ca, K, Mg, and Na), for Different Edible Seaweeds

	brown seaweed					red seaweed		green seaweed
	canned seaweed	Fucus	Wakame (*N* = 5)	Kombu (*N* = 8)	Sea Spaghetti (*N* = 5)	Dulse (*N* = 4)	Nori (*N* = 7)	Sea Lettuce (*N* = 4)
As	15.6 (0.565)	37.9 (1.22)	31.4 (8.32)	245 (87.6)	13.7 (2.22)	5.77 (1.93)	15.3 (4.97)	3.97 (1.47)
Ba	20.8 (0.0512)	13.0 (0.324)	13.8 (13.4)	5.03 (2.20)	3.93 (1.47)	4.07 (3.33)	2.96 (1.78)	14.6 (11.6)
Br	111.6 (8.253)	438.2 (19.60)	403.7 (215.6)	1137 (183.3)	393.1 (47.25)	358.8 (78.43)	77.36 (29.02)	504.6 (28.56)
Ca	1.3 (0.037)	1.7 (0.088)	1.1 (0.21)	0.96 (0.087)	1.0 (0.10)	0.38 (0.12)	0.37 (0.079)	0.72 (0.35)
Cu	3.27 (0.305)	4.86 (0.245)	4.30 (0.698)	1.81 (0.565)	3.77 (0.772)	3.63 (0.655)	8.61 (2.88)	14.4 (2.97)
I	36.07 (1.193)	548.6 (19.21)	139.1 (95.66)	4870 (1102)	143.3 (78.60)	110.4 (24.04)	58.73 (29.39)	100.1 (29.79)
K	2.0 (0.084)	3.1 (0.051)	6.4 (4.8)	7.8 (2.1)	4.0 (0.2)	8.1 (2.5)	2.9 (0.8)	1.7 (0.3)
Mg	0.48 (0.012)	0.80 (0.021)	1.2 (0.4)	0.63 (0.1)	0.86 (0.047)	0.16 (0.067)	0.29 (0.026)	2.9 (0.44)
Mn	29.0 (0.395)	81.7 (1.81)	5.68 (0.804)	3.70 (0.96)	52.7 (14.3)	67.7 (76.2)	32.0 (9.40)	77.8 (33.8)
Na	8.3 (0.088)	3.6 (0.072)	9.0 (1.0)	3.0 (0.066)	4.0 (0.22)	1.0 (1.0)	0.82 (0.14)	2.9 (2.0)
Sr	18.0 (0.688)	8.68 (0.182)	8.68 (2.12)	77.6 (25.3)	79.5 (8.90)	4.86 (0.585)	4.36 (1.36)	0.634 (0.136)
Zn	49.0 (2.78)	44.5 (3.16)	70.6 (87.8)	30.1 (8.46)	52.3 (16.8)	64.0 (42.3)	68.4 (9.36)	40.5 (15.0)

Table 4. Results from Multiple-Range Tests by Using the Student−Newman−Keuls Method (95.0% Confidence Level) for Ca, As, Br, and Zn Concentrations in Brown, Red, and Green Seaweed Types

	Ca		As		Br		Zn	
	mean (% m/m)	homogeneous groups	mean (μg g^{-1})	homogeneous groups	mean (μg g^{-1})	homogeneous groups	mean (μg g^{-1})	homogeneous groups
red	0.38	X	11.8	X	180	X	66.8	X
green	0.72	Y	4.0	X	505	XY	40.5	X
brown	1.1	Z	112	Y	681	Y	47.4	X
contrast	difference[a]		difference		difference		difference	
brown-red	0.72*		100.2*		501*		−19.4	
brown-green	0.38*		108.0*		176		6.9	
red-green	−0.34*		7.8		−325		26.3	

[a] * denotes a statistically significant difference between the pair.

internal standard for ICP-MS measurements, were from SCP Science (Montreal, Canada). Nitric acid (69%), hydrogen peroxide (33% w/v), and hydrochloric acid (37%) were from Panreac (Barcelona, Spain). Tetramethylammonium hydroxide (TMAH, 25% w/v) was supplied by Merck. Sodium borohydride solutions were prepared from sodium borohydride purchased from Alfa Aesar (Karlsruhe, Germany). Fresh sodium borohydride solutions were prepared in sodium hydroxide (Panreac) solution. The certified reference material was NIES-09 (sargasso, *Sargassum fulvellum*) from the National Institute for Environmental Studies (Ibaraki, Japan).

Seaweed Samples. Seaweed samples were supplied by a local manufacturer. These products were harvested along the Galician coast (northwestern Spain) and include 6 main Atlantic seaweeds: 20 brown seaweed (5 *Undaria pinnatifida* or commercially named Wakame, 1 *Fucus vesiculosus* or Fucus, 6 *Himanthalia elongata* or Sea Spaghetti, and 8 *Laminaria ochroleuca* or Kombu), 11 red seaweed (4 *Palmaria palmata* or Dulse and 7 *Phorphyra umbilicalis* or Nori), 4 green seaweed (4 *Ulva rigida* or commercially named Sea Lettuce), and 1 canned seaweed sample (brown seaweed, Sea Spaghetti and Furbelows (*Saccorhiza polyschides*) mixture).

Dried seaweed samples, approximately 100 g, were oven-dried overnight at 40 °C to remove water traces. Leaves were cut by hand into small pieces inside a Class-100 clean fume hood (Telstar S.A., Terrassa, Spain) and then pulverized with an automatic mortar to obtain particle sizes of < 300 μm. The particle size was established after sieving (250 μm). The canned seaweed in brine, approximately 25 g wet weight, was lyophilized at −40 °C for 1 week and was then pulverized. Dried powdered samples were finally stored in polyethylene bottles before analysis.

Microwave-Assisted Acid Digestion Procedure for As, Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn Determination. The microwave-assisted acid digestion proposed by Ruiz-Chancho et al. (*25*) with some modifications was used. The procedure consisted of adding 8 mL of concentrated nitric acid and 2 mL of concentrated hydrogen peroxide to 0.2 g of powdered seaweed sample. Capped vessels were subjected to microwave irradiation according to the following program: room temperature was raised by 14 °C/min to reach 90 °C and remained at 90 °C for 5 min. Temperature was also increased to 120 at 3 °C/min and then to 190 at 7 °C/min, remaining at 190 °C for 5 min. After cooling, acid digests were made up to 25 mL with Milli-Q water. Each sample was treated three times, and two reagent blanks were prepared for each set of samples.

Microwave-Assisted TMAH Extraction for Br and I Determination. Due to the generation and loss of volatile bromine and iodine species when using acid-based sample pretreatments, which commonly lead to lack of accuracy for both iodine and bromine determinations (*26, 27*), a microwave-assisted alkaline digestion method was used to assess these elements in seaweed (*9*). Approximately 0.1 g of powdered seaweed sample was directly weighed into high-pressure Teflon bombs, and 5 mL of Milli-Q water and 5 mL of TMAH were added. Vessels were then capped and subjected to microwave irradiation according to the following program: room temperature was raised at 18 °C/min to reach 200 °C,

Table 5. Factor Loadings after a Normalized Varimax Rotation for the Three First Principal Components

	loadings		
	PC1	PC2	PC3
As	**0.9149**	−0.0077	0.1236
Ba	−0.0295	0.6391	−0.5977
Br	**0.8946**	0.1092	0.1789
Ca	0.2794	**0.8523**	0.1479
Cu	−0.3900	−0.2293	**-0.7486**
I	**0.9323**	−0.0364	0.2019
K	0.2444	0.1008	**0.8518**
Mg	0.0009	0.3847	−0.6166
Mn	−0.5136	−0.2094	−0.0943
Na	−0.1005	**0.8511**	0.0753
Sr	0.5985	0.1695	0.5265
Zn	−0.3783	−0.0104	−0.1062

remaining at 200 °C for 5 min. After cooling, sample extracts were centrifuged at 3000 rpm for 10 min, and the supernatant was transferred into 50 mL flasks. The solid residue was rinsed with a small volume of Milli-Q water, and after centrifugation the water rinses were combined with the above-mentioned supernatant. Finally, extracts were filtered through 0.45 μm filters (Millipore) before ICP-MS measurements. Each sample was treated three times, and two reagent blanks were prepared for each set of samples.

Determination of Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn by ICP-OES. Ca, K, Mg, and Na were measured by ICP-OES (radial configuration), whereas Al, Ba, Cu, Fe, Mn, Sr, and Zn were measured with axial plasma configuration. Standard plasma conditions (1300 W for radio-frequency power, 1.5 mL min^{-1} for sample introduction, and 15.0, 0.5, and 0.8 L min^{-1} for plasma, auxiliary, and nebulizer gas flows, respectively) were used. Detection wavelengths were 455.403, 393.366, 327.393, 766.490, 279.553, 257.610, 588.995, 407.771, and 206.200 nm for Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn, respectively. The standard addition technique as a calibration method gave accurate results for all elements. Standard addition graphs covered analyte concentration ranges between 0 and 50 mg L^{-1} (Ca, K, Mg, and Na), between 0 and 1.5 mg L^{-1} (Al, Fe, and Sr), and between 0 and 0.5 mg L^{-1} (Ba, Cu, Mn, and Zn). Acid digests were diluted 10 times to assess Ca, K, Mg, Na, Al, Fe, and Sr, whereas Ba, Cu, Mn, and Zn were measured in acid digests diluted twice. Reagent blanks were low for all cases, and the limits of detection (LODs) based on the 3SD/m criteria (SD is the standard deviation of 11 measurements of a blank, and m is the slope of the standard addition graph) were found to be 0.015, 0.92, 0.30, 17, 0.13, 0.012, 0.36, 0.011, and 0.018 μg g^{-1} for Ba, Ca, Cu, K, Mg, Mn, Na, Sr, and Zn, respectively. The accuracy of the method (microwave-assisted acid digestion and ICP-OES determination) was assessed by analyzing NIES-09 CRM (Sargasso). The material was subjected to the microwave-assisted acid digestion procedure three times (first set of samples and last set of samples), and each acid digest was
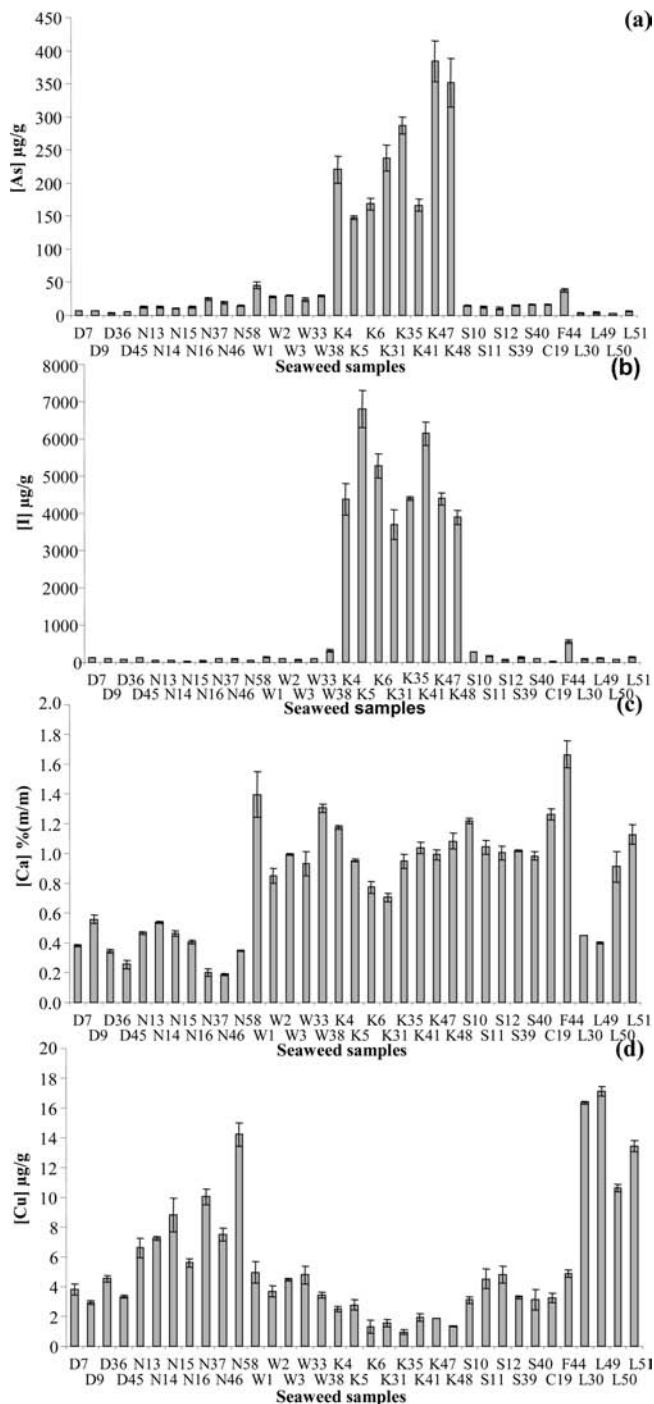
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Figure 1. As (**a**), I (**b**), Ca (**c**), and Cu (**d**) concentrations in edible seaweed samples ($N = 6$): canned seaweed (C), Dulse (D), Fucus (F), Kombu (K), Nori (N), Sea Lettuce (L), Sea Spaghetti (S), and Wakame (W).

Figure 2. Three-dimensional scores plot according to element contents data: canned seaweed (C), Dulse (D), Fucus (F), Kombu (K), Nori (N), Sea Lettuce (L), Sea Spaghetti (S), and Wakame (W).

analyzed twice by ICP-OES in each set of measurements. Results (**Table 1**) show good agreement between metal concentrations found and certified concentration values.

Determination of Br and I by ICP-MS. Iodine and bromine determinations were performed by ICP-MS using a radiofrequency power of 1380 W and sample introduction rate of 0.45 mL min^{-1}. Gas flows were fixed at 0.98, 17.0, 1.65, and 0.27 L min^{-1} for nebulizer, plasma, auxiliary, and sheath gases, respectively. H$_2$ was used in the collision-reaction cell at a flow rate of 80 mL min^{-1}. Measurements were performed by monitoring the mass-to-charge-ratios of 79 and 127 for Br and I, respectively. TMAH extracts were conveniently diluted before ICP-MS measurement, and the dilution was varied from 1:1 (Kombu, Sea Spaghetti, Wakame, and Nori samples) to 1:5 (Sea Lettuce and Dulse samples). Tellurium at a
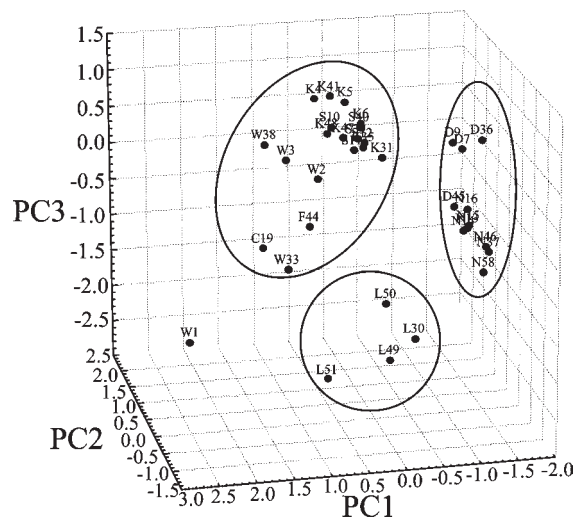
concentration of 2 mg L^{-1} was used as an internal standard for iodine determinations, whereas yttrium at 2 μg L^{-1} was chosen as an internal standard for bromine determinations. Similarly, the use of H$_2$ in the collision cell at a flow rate of 80 mL min^{-1} gave the best sensitivity and linear ranges for iodine and bromine determinations (*9*). Calibrations covered iodide and bromide concentrations between 0 and 400 μg L^{-1} for Dulse, Sea Spaghetti, Sea Lettuce, Wakame, and Nori samples, and between 0 and 2000 μg L^{-1} for Kombu samples. Low reagent blanks were obtained for Br and I, and the LODs of the method were 0.015 and 0.025 μg g^{-1} for Br and I, respectively. The accuracy of the method (microwave alkaline digestion and ICP-MS determination) was assessed by analyzing NIES-09 CRM. Results for three independent TMAH extracts (**Table 1**) show good agreement with indicative Br and I concentration values.

Determination of As by HG-AFS. Arsenic determination was performed by continuous flow (CF) HG-AFS by monitoring the wavelength 193.7 nm. The HI-HCL was operated at 27.5 mA (primary current) and 35 mA (boost current), whereas Ar flow rate was 0.3 L min^{-1} and N$_2$ flow rate (Permapure membrane) was fixed at 2.5 L min^{-1}. Diffusion flame was maintained with an additional H$_2$ flow rate of 25 mL min^{-1}. Acquisition was performed by fixing delay, analysis, and memory times at 15, 30, and 30 s, respectively. Acid digests were conveniently diluted with a 0.1 M hydrochloric acid solution, and 1% (m/v) sodium tetrahydroborate, prepared in a 0.05% (m/v) sodium hydroxide solution (2 mL min^{-1}), and 0.5 M hydrochloric acid (4 mL min^{-1}) were used as reducing and carrier solutions, respectively. The standard addition technique, covering As concentrations within the $0-20$ μg L^{-1} range, was used for measurements. Low reagent blanks were obtained for all cases. The LOD of the method was 2.3 μg g^{-1}. The accuracy of the method (microwave-assisted acid digestion and HG-AFS determination) was assessed by analyzing NIES-09 CRM. After the microwave-assisted acid digestion procedure (by triplicate), As concentrations found (**Table 1**) were in good agreement with the As certified value.

Data Analysis. Data matrices, where rows are the objects (edible seaweed samples) and columns are the variables (As, Ba, Br, Ca, Cu, K, I, Mg, Mn, Na, Sr, and Zn concentrations) were built. The statistical analysis has been performed by means of Statgraphics 5.0 (Bartlett's and Cochran's C tests for variance comparison and multiple-range test for means comparisons), Statistica 6.0 (PCA, CA, and LDA), and Unscrambler (SIMCA). Before pattern recognition techniques were applied, the half-range central value transformation (*28*) was used for data pretreatment.

RESULTS AND DISCUSSION

Univariate Approaches. Tables **2** and **3** list the mean element concentrations and standard deviations for each group of samples (brown, red, and green seaweed in **Table 2**) and all seaweed types
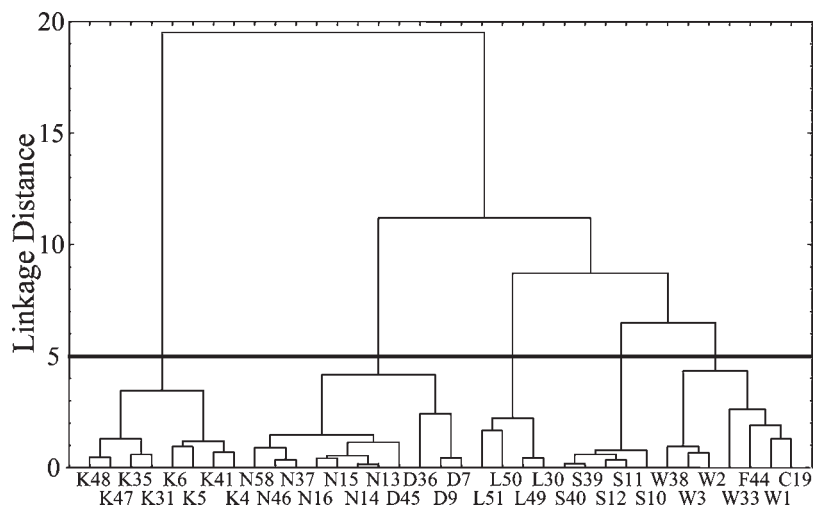
Figure 3. Dendrographic classification of edible seaweed after cluster analysis using elements concentrations: canned seaweed (C), Dulse (D), Fucus (F), Kombu (K), Nori (N), Sea Lettuce (L), Sea Spaghetti (S), and Wakame (W).

(canned seaweed, Fucus, Wakame, Kombu, Sea Spaghetti, Dulse, Nori, and Sea Lettuce in **Table 3**). High differences can be observed in the elemental composition among the samples, especially for some elements such as As and I, which offer high concentrations in brown seaweed, mainly in Kombu types. As an example, the arsenic concentration varies from a mean concentration of 5.8 μg g^{-1} in red seaweed Dulse to 245 μg g^{-1} in brown seaweed Kombu, whereas iodine concentration is from 59 μg g^{-1} in red seaweed Nori to 4870 μg g^{-1} in brown seaweed Kombu (**Tables 2** and **3**).

A preliminary univariate approach based on the statistical comparison of the standard deviations and means of the different element concentrations was performed before multivariate analysis. Bartlett's and Cochran's C tests were used first to prove homogeneity of variance. They test the null hypothesis, the hypothesis being that variances across different groups are equal. After p value calculation by each test at the 95.0% confidence level, standard deviations for all element concentrations (brown, red, and green types), except for Ca and Cu, were statistically significant different (p values < 0.05). This violates one of the important assumptions underlying the analysis of variance, and ANOVA cannot be applied for statistical comparisons of means. In these cases the multiple-range test, commonly performed using the Student–Newman–Keuls (SNK) method, was used.

After the multiple-range test had been applied, four different results (shown in **Table 4**) were obtained. In the first case (details for Ca in **Table 4**) the three groups (brown, red, and green seaweed) offer means statistically significant different among them, and three independent homogeneous groups (left side of the table) are observed. This result was also obtained for Cu and Mg concentrations. The second case (data for As in **Table 4**) implies the statistically significant difference of one group (brown seaweed) with respect to the other two groups (red and green types), which offer statistically significant similar element concentrations. This result was also obtained for Sr concentration, and Sr levels in brown seaweed were statistically significant different from Sr concentration in red and green types. The third situation (data for Br in **Table 4**) shows two different homogeneous groups, the means of which are statistically significant different (brown and red types for Br), and the third group (green seaweed samples for Br in **Table 4**), which can form homogeneous groups with the other groups. This third group offers mean concentrations statistically significant similar to those mean concentrations found in the other two independent

groups. The same result (red and brown types as independent homogeneous groups and green seaweed as belonging to both independent groups) was also observed for I and Na. Similarly, K and Mn levels gave independent homogeneous groups for green and brown types and red seaweed as belonging to both independent groups, and Ba concentrations offered statistically significant differences between red and green type (independent homogeneous groups) and statistically significant similarities between brown and red types and between brown and green types. Finally, the fourth result (multiple-range test for Zn in **Table 4**) gave only one independent homogeneous group formed by brown, red, and green types. This means that Zn concentrations are statistically significant similar for all sample types.

In conclusion, it can be said that the Ca, Cu, and Mg concentrations are those variables that can offer the highest discrimination power for distinguishing among brown, red, and green seaweeds. In contrast, Zn concentration is expected to offer poor discrimination capabilities.

Multivariate Characterization by PCA and CA. A data set, where As, Ba, Br, Ca, Cu, I, K, Mg, Mn, Na, Sr, and Zn concentrations are the discriminating variables and 35 seaweed samples are the objects, was established. The data set was homogenized before unsupervised (PCA and CA) and supervised (LDA and SIMCA) pattern recognition techniques were applied (28).

PCA was performed using cross-validation as a validation method, and after a normalized Varimax rotation, 71% of the total variance was explained by three principal components (PCs), which show eigenvalues of > 1.0 (4.6, 2.3, and 1.6 for PC1, PC2, and PC3, respectively). From loadings of variables along the first three PCs listed in **Table 5**, it can be said that As concentration, Br concentration, and I concentration are the dominating features in PC1, and they explain 38% of total variance. As can be seen in **Figure 1a,b** for As and I, respectively, these three variables offer the highest values for brown seaweed, especially for Kombu samples. The second PC offers the highest weights for Na and Ca contents (**Table 5**) and explains 19% of the total variance of the data set. Concentration levels for Ca (**Figure 1c**) show higher values in brown seaweed, but green and red seaweed also offer high contents. A similar pattern has been obtained for Na (figure not given). Finally, the third PC is mainly formed by the variables K concentration and Cu concentration (explaining 14% of total variance). Cu levels plotted in **Figure 1d** indicate that Cu concentrations are higher in red and green seaweed than in brown seaweed.

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Examination of the three-dimensional plot of the samples in the space defined by the three first PCs (scores plot shown in **Figure 2**) reveals four main groups. A first group (upper right part of the plot) comprises red seaweed (Dulse samples D7, D9, D36 and D45; and Nori type N13, N14, N15, N16, N37, N46, and N58). A second spread group located at the upper-middle part of the plot is formed by brown seaweed. Kombu samples (K4, K5, K6, K31, K35, K41, K47, and K48) and Sea Spaghetti (S10, S11, S12, S39, and S40) appear to form a compact group, whereas Wakame (W2, W3, W33, and W38), one Fucus sample (F44), and one canned seaweed (C19) are spread. Wakame sample coded W1 appears to be independent. The third group located at the bottom-middle part of the scores plot is formed by green seaweed (Sea Lettuce L30, L49, L50, and L51).

Results from CA (Euclidean distance between objects as a measure of similarity and Ward's method as clustering method) are shown as a dendrogram plot in **Figure 3**. Five main clusters can be established at a linkage distance of five. The first one

Table 6. Classification of Brown, Red, and Green Seaweed for Correct Recognition and Prediction with LDA and SIMCA (around 25% Samples in Prediction Set)

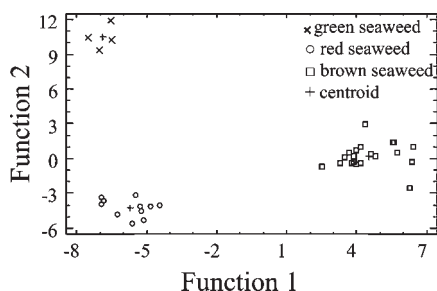|		recognition ability (%)	prediction ability (%)
LDA	brown seaweed	100	
	red seaweed	100	
	green seaweed	100	
SIMCA	brown seaweed	93	80
	red seaweed	89	100

Figure 4. Plot of discriminant functions after LDA.

(beginning from the right) is composed of seven brown seaweeds: five Wakame samples (W1, W2, W3, W33, and W38), one Fucus sample (F44), and one canned seaweed (C19). The second cluster in **Figure 3** (from the right) is formed by five Sea Spaghetti samples (brown type), coded S10, S11, S12, S39, and S40, whereas the third cluster is formed by four green seaweeds (Sea Lettuce L30, L49, L50, and L51). The fourth cluster comprises all red seaweed types (Dulse and Nori samples D7, D9, D36, D45, N13, N14, N15, N16, N37, N46, and N58). Finally, the fifth cluster is formed by Kombu samples (brown seaweed) coded K4, K5, K6, K31, K35, K41, K47, and K48.

Multivariate Characterization by LDA and SIMCA. The exploratory techniques applied above show that a certain difference can be observed between brown, red, and green seaweed. To obtain a suitable classification, LDA (with a significance level of 5%) was applied to the data set (35 samples), and three categories (red, brown, and green seaweed) were established. As can be seen in **Table 6** and **Figure 4**, the recognition ability to distinguish among brown, red, and green seaweed was highly satisfactory, and 100% of correctly classified cases was obtained for the three categories.

SIMCA was applied to the data set (35 samples), which was randomly divided into a training set and a testing or prediction set. The testing set, usually formed by approximately 25% of the total samples, was formed by 11 seaweed samples: 4 green seaweeds (Sea Lettuce), 5 brown seaweeds (1 Kombu, 1 Wakame, 1 Sea Spaghetti, and also the canned seaweed and Fucus samples), and 2 red seaweeds (1 Dulse and 1 Nori sample). The training set was formed by 24 seaweed samples: 15 brown seaweeds (7 Kombu and 4 each Wakame and Sea Spaghetti samples), and 9 red seaweeds (3 Dulse and 6 Nori samples). The establishment of both training and prediction sets from green seaweed (only four samples) was not considered because of the small number of samples.

Results from SIMCA (with a significance level of 5%) are also shown in **Table 6** and **Figure 5** (Cooman's plot). Recognition percentages of samples correctly classified of 89 and 93% were obtained for red and brown seaweed, respectively, being that one red seaweed (Dulse) and one brown seaweed (Wakame) were misclassified. The prediction ability was good, and 100% of samples correctly classified was obtained for red seaweed (two samples), whereas 80% of prediction ability was obtained for brown seaweed (five samples). Brown seaweed coded C19
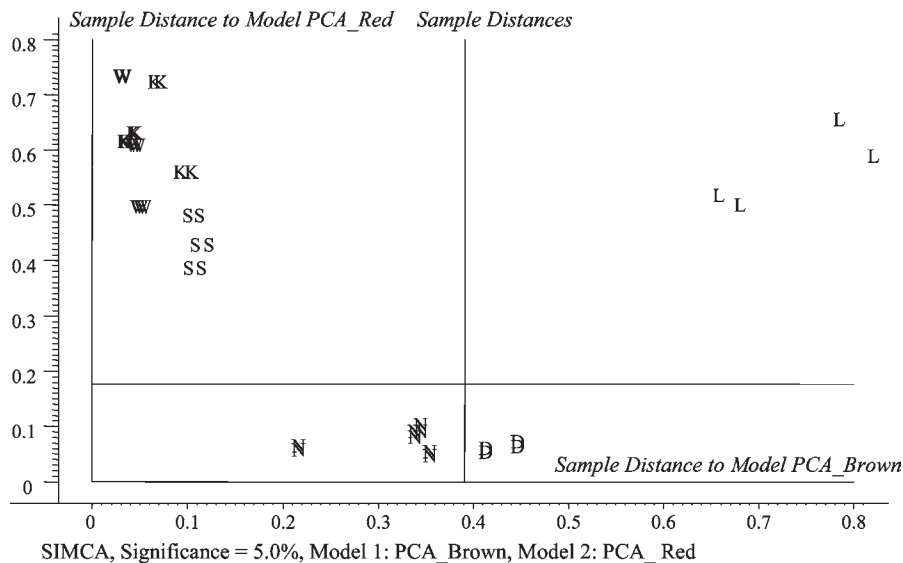

Figure 5. Cooman's plot after SIMCA: Kombu (K), Wakame (W), Sea Spaghetti (S), Nori (N), Dulse (D), and Sea Lettuce (L).

(canned seaweed) and all green seaweed samples were not found to belong to either red or brown seaweed groups or classes, and they were recognized as independent samples (new groups).

ABBREVIATIONS USED

ANOVA, analysis of variance; CA, cluster analysis; CF, continuous flow; CRM, certified reference material; HG-AFS, hydride generation−atomic fluorescence spectrometry; ICP-MS, inductively coupled plasma−mass spectrometry; ICP-OES, inductively coupled plasma−optical emission spectrometry; LDA, linear discriminant analysis; PCA, principal component analysis; SIMCA, soft independent modeling of class analogy; TMAH, tetramethylammonium hydroxide.

ACKNOWLEDGMENT

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