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Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain

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Abstract

Total arsenic, inorganic arsenic, lead and cadmium contents were determined in 112 samples of seaweed preparations sold in Spain (seaweed packed in plastic or cardboard box, seaweed in the form of tablets and concentrates, foods containing seaweed, and canned seaweed). The concentration ranges found, expressed in mg/kg, dry weight, were: total As (0.031–149), inorganic As (<0.014–117), Pb (<0.050–12.1) and Cd (<0.003–3.55). For all the contaminants there were failures to comply with legislated values. In particular, all the samples of *Hizikia fusiforme* exceeded the inorganic As limit established in some countries, and a considerable number of species exceeded the Cd limit set by international regulations. With respect to food safety, consumption of 3 g/day of the samples analysed could represent up to 15% of the respective Tolerable Daily Intakes (TDI) established by the WHO. The situation is especially alarming for intake of inorganic As from *H. fusiforme*, which can be three times the TDI established.

Keywords: Lead; Cadmium; Arsenic; Inorganic arsenic; Edible seaweed

1. Introduction

In Western countries, the use of seaweed has traditionally concentrated on the extraction of compounds used by the pharmaceutical, cosmetics and food industries (production of agar, alginates, carrageenan, etc.) (Mabeau and Fleurence, 1993; Caliceti et al., 2002). In recent decades there has been an increase in direct consumption of seaweed as food, partly because of the nutritional (Mabeau and Fleurence, 1993; Darcy-Vrillon, 1993) and therapeutic (van Netten et al., 2000) benefits that these products provide. The mean intake of seaweed in Western countries is far from equalling that of Eastern countries, estimated for the Japanese population as 3.3 g dry seaweed per day (Darcy-Vrillon, 1993), but we must not forget the existence in all countries of extreme consumers, such as those who follow a macrobiotic diet.

From a nutritional point of view, seaweeds are interesting because of their high content of dietary fibre (33–50%), rich in soluble fractions with hypocholesterolemic and hypoglycemic effects (Mabeau and Fleurence, 1993; Jiménez-Escrig and Sánchez-Muniz, 2000). They are a source of proteins, with an amino acid composition of nutritional interest (Fleurence, 1999; Wong and Cheung, 2000). Minerals also attain considerable levels (8–40%), so that seaweed could be used as a food supplement in order to reach the recommended daily intakes of some macrominerals and trace elements (Rúperez, 2002). Finally, because of their low lipid content, 1–2%, they constitute a negligible energy source (Darcy-Vrillon, 1993). On the other hand, seaweed has a high metal pollution accumulation capacity. This reason it has been used as a bio-indicator for marine environment contamination (Riget et al., 1997), and there are studies on heavy metal contamination in different species of environmental and commercial importance (Hou

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and Yan, 1998; Sánchez-Rodríguez et al., 2001; Caliceti et al., 2002). Metal contamination is an aspect that can condition the safety of edible seaweeds as a food, but there are very few studies on inorganic contaminants in commercially available seaweed preparations (Norman et al., 1987; Ortega-Calvo et al., 1993; Munilla et al., 1995; van Netten et al., 2000; Hsu et al., 2001; Almela et al., 2002).

France, the USA, Australia and New Zealand have established specific regulations for toxic elements in edible seaweed (Mabeau and Fleurence, 1993; ANZFA, 1997). Other countries, such as Spain, do not legislate specifically for seaweed. At present, the European Community has not fixed maximum contents of contaminants in seaweed (Official Journal of the European Communities, Regulation (EC) No 466/2001).

The edible seaweeds sold in Spain come from regions in the north of the country or are imported from Japan, China, Korea and Chile. Existing data on contents of heavy metals and arsenic in edible seaweed sold in Spain (Ortega-Calvo et al., 1993; Almela et al., 2002) show that several samples do not comply with existing legislation. The cited studies provided a first approach to evaluating the food safety of seaweeds, but they are not sufficiently extensive in terms of number of samples and periodicity of sampling to permit characterization of the products to which the consumer has access. The aim of the present study is to evaluate the food safety, in terms of total arsenic, inorganic arsenic, Pb and Cd contents, of an extensive, representative range of edible seaweeds sold in Spain in various forms: dried seaweed, seaweed tablets and concentrates, seaweed incorporated in other foods (noodles, hamburgers, pizzas, soups, biscuits, etc.) and canned seaweed. We also wish to provide a database for future legislative enforcements.

2. Materials and methods

2.1. Instruments

Pb and Cd were determined by graphite furnace atomic absorption spectroscopy (GFAAS) with longitudinal AC Zeeman (AAnalyst 600, Perkin–Elmer, Madrid, Spain), equipped with a transversely heated graphite atomizer and a built-in, fully computer-controlled AS-800 autosampler (Perkin–Elmer). Pyrolytic graphite coated tubes with an inserted L'vov platform were used. Total and inorganic As were determined with an AAS model 3300 (Perkin–Elmer) equipped with an autosampler (AS-90, Perkin–Elmer) and a flow injection hydride generation system (FIAS-400, Perkin–Elmer).

Other equipment used included a domestic microwave oven (Optiquick DUO, Moulinex, Spain) with a maximum power of 900 W, a sand bath (PL 5125, Raypa, Scharlau, S.L., Spain), a muffle furnace equipped with a Eurotherm Controls 902 control program (K 1253, Heraeus, Spain), a mechanical shaker (KS 125 Basic, IKA Labortechnik, Merck Farma y Química, Spain) and an Eppendorf 5810 centrifuge (Merck).

2.2. Reagents

Commercial standard solutions (1000 mg L^{-1}) of As(V), Pb and Cd were used (Merck). Deionized water (18.2 MΩW cm) was used for the preparation of reagents and standards. All chemicals were of at least *pro analysis* quality or better. All glassware was treated with 10% v/v HNO_3 for 24 h, and then rinsed three times with deionized water before use.

2.3. Samples

A total of 112 samples were analysed: 52 samples were seaweed packed in plastic or cardboard box, 11 seaweed tablets and concentrates, 28 foods containing seaweed and 21 canned seaweed.

The products, which came from Spain, Chile, China, Korea and Japan, were purchased in shops in the city of Valencia (Spain) during the year 2002. The products with a high humidity content, e.g. canned seaweed, were freeze-dried and then crushed to a fine powder in a mill. The samples sold in dried form were only crushed in a mill. All samples were stored in previously decontaminated twist-off flasks and kept at 4 °C until analysis.

2.4. Total arsenic determination

Dry ashing mineralization and quantification by flow injection-hydride generation atomic-absorption spectrometry (FI-HG-AAS) were employed (Almela et al., 2002). Samples (0.25 g) were treated by 2.5 mL of ashing aid suspension (20% w/v $MgNO_3 + 2\%$ w/v MgO) and 5 mL of nitric acid (50% v/v). The mixture was evaporated to dryness and mineralized at 450 °C with a gradual increase in temperature. The white ash was dissolved in 5 mL 6 M HCl, reduced with 5 mL of reducing solution (5% w/v KI and 5% w/v ascorbic acid) and made up to 25 mL with 6 M HCl. The analytical conditions used for arsenic determination by FI-HG-AAS were the following: loop sample 0.5 mL; reducing agent 0.2% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH, 5 mL min^{-1} flow rate; HCl solution 10% (v/v), 10 mL min^{-1} flow rate; carrier gas argon, 100 mL min^{-1} flow rate; wavelength 193.7 nm; spectral band-pass 0.7 nm; electrodeless discharge lamp system 2; lamp current setting 400 mA; cell temperature 900 °C.

Calibration standard solutions of As(III) were prepared from a reduced standard solution of As(V), using a mixture containing 5% (w/v) KI and 5% (w/v) ascorbic acid as reducing solution. Triplicate analyses were performed for each sample. The limit of detection for total As was 0.025 mg/kg dry weight (dw).

2.5. Inorganic arsenic determination

Analysis was performed by acid digestion, solvent extraction FI-HG-AAS (Almela et al., 2002). Deionized water (4.1 mL) and concentrated HCl (18.4 mL) were added to the samples (0.5 g) and the mixture was left overnight. After reduction by HBr (2 mL) and hydrazine sulphate (1.5% w/v, 1 mL), the inorganic arsenic (i-As) was extracted into chloroform (3×10 mL) and back-extracted into 1 mol L^{-1} HCl (2×10 mL). For the determination of i-As in the back-extraction phase 2.5 mL of ashing aid suspension (20% w/v $MgNO_3 + 2\%$ w/v MgO) and 10 mL of concentrated HNO_3 were added. The mixture was evaporated to dryness and then treated in the same way as for total As (dry ashing FI-HG-AAS). Calibration standard solutions of As(III) were used. Triplicate analyses were performed for each sample. The limit of detection for inorganic As was 0.014 mg/kg dw.

2.6. Cadmium and lead determination

Analysis was performed by wet digestion GFAAS (Almela et al., 2002). The sample (0.20 g) was placed in a high pressure poly(tetrafluoroethylene) (PTFE) vessel, and 2 mL of 65% HNO_3 and 1 mL of 35% H_2O_2 were added. The vessel was sealed with the screw cap and placed inside the microwave oven. Samples were irradiated at a 700 W power setting for 3 cycles of 1 minute. After digestion, the vessel was cooled, filtered and diluted with water to a final volume of 25 mL. The furnace programme [temperature (°C)/ramp time (s)/hold time (s)] employed for Cd determination was: drying (90 °C/10 s/20 s; 120 °C/10 s/20 s; 130 °C/5 s/40 s; 300 °C/5 s/5 s); pyrolysis (500 °C/10 s/20 s); cooling (20 °C/10 s/20 s); atomization (1400 °C/0 s/5 s); cleaning (2450 °C/1 s/5 s). For the determination of Pb the same furnace programme was used, with the exception of the temperatures for pyrolysis (850 °C) and atomization (1600 °C).

The matrix modifier used for determining the two metals was a mixture of 0.067 mg $H_2PO_4NH_4$ and 0.003 mg $Mg(NO_3)_2$ in 10 μL 1% HNO_3 (v/v). The quantification of Pb and Cd was performed using a calibration curve of the corresponding standards. Triplicate analyses were performed for each sample. The limit of detection was 0.05 mg/kg dw for Pb and 0.003 mg/kg dw for Cd.

2.7. Quality assurance–quality control

The suitability of the analytical methods employed for total As, inorganic As, Pb and Cd determination has been checked previously by evaluating their analytical characteristics (limit of detection, precision and accuracy) (Almela et al., 2002). The limit of detection for each element was calculated by dividing three times the standard deviation of the absorbance of nine reagent blanks by the slope of the standard calibration curve. In the present work, three certified reference materials (CRMs) were employed to evaluate accuracy: *Fucus sp.* certified for As, Pb and Cd (International Atomic Energy Agency, Vienna, Austria); *Lagarosiphon major*, certified for Pb and Cd (BCR-060, Institute for Reference Materials and Measurements, IRMM, Brussels, Belgium); *Ulva lactuca*, certified for As, Pb and Cd (BCR-279, IRMM). These CRMs cover a wide range of concentrations of the contaminants of interest. There are no certified reference materials available for inorganic As content. The quality criterion adopted, therefore, was the overlapping of the ranges of inorganic As found in the CRMs just mentioned and the values reported in these samples in a previous work carried out in our laboratory (Almela et al., 2002).

In each analytical session, contents were determined in two blanks, a CRM and several samples of edible seaweed. If the metal contents obtained for the CRM samples were within the ranges certified, the measuring process was considered as being under control and we went on to quantify the samples. The precision, expressed as the relative standard deviation for three independent analyses, was less than 10% for the determination of total arsenic, inorganic arsenic and cadmium, and less than 20% for the determination of lead.

3. Results

3.1. Edible seaweed

Thirteen different genera of edible seaweed were analysed (Table 1). An effort was made to acquire a wide variety of different species. Very few genera of green seaweed are sold as dried seaweed packed in plastic or cardboard box, and therefore most of the samples analysed were red ($n = 16$) or brown seaweed ($n = 31$). The concentrations of total As varied over a wide range (2.2–149 mg/kg dw). Green seaweed had the lowest concentrations (mean ± SD = 2.70 ± 0.80 mg/kg dw), followed by red (23.3 ± 13.3 mg/kg dw) and brown seaweed (60.6 ± 40.7 mg/kg dw). This gradation of total As contents in relation to the type of seaweed (brown > red > green) has been shown previously by our work group (Almela et al., 2002). The contents in *Hizikia fusiforme* were particularly high (68.3–149 mg/kg dw), results that cannot be considered anomalous, being customary in this type of seaweed, as reflected by the high values reported in the literature, which can attain 179 mg/kg dw (Yasui et al., 1983; Watanabe et al., 1979; Munilla et al., 1995; van Netten et al., 2000; Hanaoka et al., 2001; Almela et al., 2002; Laparra et al., 2003).

For inorganic As contents the concentrations varied between 0.014 mg/kg dw, the limit of detection of the methodology, and 117 mg/kg dw. The range was as wide as that obtained for total As, although the gradation between green, red and brown seaweed did not appear. Two groups of samples can be distinguished, however: those with contents lower than 1.44 mg/kg dw (mean ± SD = 0.345 ± 0.346 mg/kg dw), which correspond to 86% of the samples analysed, and those with contents greater than 40 mg/kg dw (76.4 ± 20.7 mg/kg dw), which represent 14% of the total and they all correspond to the species *H. fusiforme*. For this species, inorganic As represents from 47% to 80% of the total As of the sample, a percentage which decreases in the other types of seaweed (0.2–16%). The data for inorganic As in seaweed reported in the literature are very scanty (Yasui et al., 1983; Morita and Shibata, 1990; Shibata et al., 1990; Kuehnelt et al., 2001; Almela et al., 2002; Laparra et al., 2003; Laparra et al., 2004; FSA, 2004) and for *H. fusiforme* they show the same pattern as that described in the present work. There are no previous references in the literature concerning foods of vegetable or animal origin with such alarming levels of carcinogenic inorganic As as those found in *H. fusiforme* (41.6–117 mg/kg dw).

The Pb contents ranged between <0.05 mg/kg dw, the limit of detection of the methodology, and 2.44 mg/kg dw, found in *U. pinnatifida* brown seaweed. The differences in Pb content were not very pronounced, and it cannot be said that particular types of algae had higher Pb contents than others (mean ± SD: red, 0.554 ± 0.409 mg/kg dw; brown, 0.598 ± 0.663 mg/kg dw). The Pb contents in edible seaweed reported in the literature are scanty, with contents given for only 32 samples, and those contents are very variable. Some authors detect much higher contents in red and brown seaweed than those found in our study (2.2–14.2 mg/kg dw) (Ortega-Calvo et al., 1993; Munilla et al., 1995), while van Netten et al. (2000) found contents below 0.57 mg/kg dw.

The Cd contents ranged between 0.020 mg/kg dw, detected in *Enteromorpha* sp. green seaweed, and 3.19 mg/kg dw, found in *Porphyra* sp. red seaweed. There were no differences between the mean contents found in red seaweed (mean ± SD: 0.843 ± 1.053 mg/kg dw) and those found in brown seaweed (mean ± SD: 0.846 ± 0.604 mg/kg dw). The contents reported in the literature are scanty, with values given for only 32 samples, and they are less than 2.8 mg/kg dw, with the highest concentrations found in *Undaria* sp., *Laminaria* sp. and *H. fusiforme* (Ortega-Calvo et al., 1993; Munilla et al., 1995; van Netten et al., 2000).

3.2. Seaweed tablets and concentrates

We analysed ten samples of tablets of two species, *Spirulina* and *Fucus*, and a soluble concentrate of *Fucus vesiculosus* (Table 2). The total As concentrations varied between 0.231 and 37.4 mg/kg dw. The concentrations in the *Fucus* sp. tablets were much higher than in the *Spirulina* tablets

Table 1
Contents of total arsenic (t-As), inorganic arsenic (i-As), lead and cadmium in edible seaweed. Results expressed in mg/kg dry weight

	Seaweed	Origin	t-As	i-As	Pb	Cd
Green	*Enteromorpha* sp.	Unknown	2.15	0.346	0.205	0.020
	Ulva pertusa	Unknown	3.24	0.268	<LD	0.190
Red	*Porphyra tenera*	Japan	24.1	0.280	0.123	0.089
		Japan	23.2	0.167	0.126	0.235
	Porphyra umbilicalis	Spain	34.5	0.239	0.817	0.126
	Porphyra sp.	Japan	32.7	0.189	0.444	0.319
		Spain	24.3	0.383	0.371	0.219
		Corea	20.8	0.176	0.546	2.51
		South Korea	18.4	0.131	0.664	2.91
		South Korea	23.5	0.116	0.280	3.19
		China	41.7	0.402	1.24	1.05
		China	58.3	0.223	0.730	0.408
	Porphyra tenera	Japan	24.1	0.280	0.123	0.089
		Japan	23.2	0.167	0.126	0.235
	Porphyra umbilicalis	Spain	34.5	0.239	0.817	0.126
	Palmaria sp.	Spain	13.0	0.466	<LD	0.147
	Palmaria palmata	Japan	12.6	0.595	1.52	0.877
	Rhodymenia palmata	Unknown	8.80	0.153	0.237	0.079
		Unknown	7.68	0.152	0.150	0.181
	Chondrus crispus	Spain	12.7	0.357	0.348	0.722
		Spain	16.1	0.842	0.720	0.418
Brown	*Laminaria* sp.	Spain	39.6	0.473	<LD	0.327
		Japan	48.3	0.145	0.260	0.385
	Laminaria japonica	Japan	116	1.44	<LD	0.908
		Japan	104	0.238	<LD	0.074
	Laminaria digitata	Japan	65.7	0.251	0.106	0.343
	Eisenia bicyclis	Japan	22.4	0.167	0.169	0.549
		Japan	25.2	1.35	0.218	0.383
		Japan	26.3	0.135	0.239	0.559
		Japan	4.1	0.292	<LD	0.571
		Japan	26.6	0.206	<LD	0.549
	Undaria pinnatifida	Japan	41.4	<LD	0.113	1.55
		Japan	45.2	<LD	<LD	1.02
		Spain	46.2	1.12	1.10	1.90
		Spain	28.0	0.268	0.941	0.227
		Spain	32.3	0.371	2.44	0.323
		Korea	46.0	1.06	0.648	2.15
		Japan	41.5	0.610	0.795	1.22
	Hizikia fusiforme	Japan	111	75.4	0.885	0.621
		Japan	89.2	41.6	<LD	1.07
		Japan	114	91.2	<LD	1.16
		Japan	131	81.1	0.537	0.511
		Japan	93.9	61.6	0.060	1.16
		Japan	124	80.3	<LD	0.811
		Japan	149	117	0.063	0.948
		Japan	68.3	43.7	2.06	1.53
		Japan	106	69.4	n.a.	1.52
	Fucus vesiculosus	Unknown	40.4	0.291	0.898	0.412
	Himanthalia elongata	Spain	23.6	<LD	0.198	0.389
		Spain	31.2	0.202	0.126	0.222
		Spain	21.3	<LD	0.115	0.395
	Durvillaea antartica	Chile	15.2	0.318	<LD	2.46

LD: limit of detection.
n.a.: not analysed.

(mean ± SD: 26.7 ± 6.4 mg/kg dw and 0.5 ± 0.2 mg/kg dw, respectively). The soluble concentrate of *Fucus* had an intermediate value (4.75 mg/kg dw). With regard to inorganic As content, apart from certain *Fucus* tablets (1.80 mg/kg dw), the other samples had concentrations less than 1 mg/kg dw. The percentage of inorganic As with respect to total As was higher in the *Spirulina* tablets (27%–60%) than in the *Fucus* tablets (1–5%). The scanty previous reports of total As in *Spirulina* tablets ($n = 2$) indicate contents below 2 mg/kg dw (Hsu et al., 2001), while no

Table 2
Contents of total arsenic (t-As), inorganic arsenic (i-As), lead and cadmium in seaweed tablets and capsules. Results expressed in mg/kg dry weight

Product	Seaweed	Origin	t-As	i-As	Pb	Cd
Tablets	*Spirulina*	Spain	0.706	0.414	3.31	0.564
		Spain	0.686	0.411	12.1	0.455
		Spain	0.394	0.139	9.20	0.170
		Spain	0.502	0.138	2.71	0.037
		Spain	0.231	0.108	0.081	3.55
	Fucus sp.	Spain	24.2	0.267	<LD	0.247
		Spain	37.4	1.80	0.250	0.331
		Spain	20.7	0.571	0.162	0.252
		Spain	24.4	0.646	0.064	0.356
		Spain	27.0	0.439	<LD	0.318
Soluble concentrate	*Fucus vesiculosus*	Spain	4.75	0.253	0.147	0.011

LD: limit of detection.

data were found concerning inorganic As contents in those samples.

With regard to Pb contents, the *Spirulina* tablets (0.081–12.1 mg/kg dw) had higher concentrations than the *Fucus* tablets (<LD–0.25 mg/kg dw), unlike what was observed for total As. For Cd, with the exception of the high content found in one of the *Spirulina* samples (3.55 mg/kg dw), the concentrations were similar in the two species (*Fucus*: 0.011–0.356 mg/kg dw; *Spirulina*: 0.037–0.564 mg/kg dw). In *Spirulina* tablets or capsules the literature reports Pb contents (2.1–15.2 mg/kg dw) similar to those in the present samples (Ortega-Calvo et al., 1993; Hsu et al., 2001). But in *Fucus* tablets the Pb contents found by Ortega-Calvo et al. (1993) (11.3–14 mg/kg dw) are much higher than those of the samples analysed here. With regard to Cd, the contents reported in the literature for 14 samples of *Spirulina* and *Fucus* tablets are less than 1 mg/kg dw (Ortega-Calvo et al., 1993; Hsu et al., 2001).

3.3. Foods containing seaweed

Twenty-eight products were analysed (Table 3). There are no previous data in the literature concerning inorganic contaminants in products of this kind. The total As contents found varied between 0.031 and 50.3 mg/kg dw. Most of the samples (64%) had total As concentrations less than 1 mg/kg dw, although there were higher concentrations (1.25–2.38 mg/kg dw) in products which incorporated brown seaweed of the *Hizikia*, *Himanthalia* and *Undaria* genera in their composition. The concentrations were much higher in one sample of *Himanthalia* toasted and sold as an instant soluble powder (15.6 mg/kg dw) and in a tisane made with *Fucus* (50.3 mg/kg dw). With regard to inorganic As, only three of the samples had concentrations greater than 1 mg/kg dw, all corresponding to products that contained a quantity of *H. fusiforme* not specified by the manufacturer (Bio tofu burger and Bio pizza). For those samples, between 54% and 85% of the total As was found as inorganic As.

Table 3
Contents of total arsenic (t-As), inorganic arsenic (i-As), lead and cadmium in foods which contain seaweed. Results expressed in mg/kg dry weight

Seaweed	Product	Origin	t-As	i-As	Pb	Cd
Spirulina	Noodles	Spain	0.673	<LD	0.160	0.016
Porphyra sp.	Pinchitos	Spain	0.052	0.047	<LD	0.020
	Instant soup	France	0.486	0.055	<LD	0.190
H. fusiforme	Bio tofu burger	Spain	2.38	1.32	0.144	0.038
		Spain	2.31	1.70	0.203	0.049
	Bio pizza	Spain	0.853	0.240	0.147	0.036
		Spain	0.077	0.072	0.136	0.025
		Spain	1.25	1.13	0.114	0.045
		Spain	1.42	0.527	n.a.	0.048
		Spain	0.056	0.017	n.a.	0.027
Laminaria sp.	Vegetarian hamburger	Spain	0.150	<LD	0.234	0.035
		Spain	0.137	0.061	0.201	0.038
		Spain	0.121	0.056	0.174	0.041
	Seitan	Spain	0.031	0.034	0.087	0.020
		Spain	0.128	0.047	<LD	0.059
		Spain	0.218	0.197	0.228	0.034
		Spain	0.448	0.462	0.106	0.026
		Spain	0.059	0.067	0.058	0.027
Himanthalia sp.	Pâté	Spain	1.67	0.078	<LD	0.035
	Instant toasted seaweed	Spain	15.6	0.222	n.a.	0.537
E. bicyclis	Tahini sauce	Italy	0.894	0.041	<LD	0.027
U. pinnatifida	Spring tofu	Spain	0.091	0.058	<LD	0.009
	Red miso soup	Spain	0.174	0.050	n.a.	0.067
	Semolina soup	Spain	2.30	0.553	0.233	0.118
	Sesame biscuits	Spain	1.40	<LD	0.440	0.012
	Sesame snack	Spain	1.62	0.107	0.314	<LD
Laminaria sp.	Gomasio with seaweed	Italy	0.424	0.051	<LD	0.030
Fucus sp.	Tisane	Spain	50.3	0.610	1.21	0.505

LD: limit of detection.
n.a.: not analysed.

The Pb contents can be considered low (<LD–0.440 mg/kg dw), with the exception of the *Fucus* tisane, which had 1.21 mg/kg dw. A similar pattern was observed for Cd, with the products made solely out of seaweed (instant soup, instant toasted seaweed and tisane) having the highest concentrations of the contaminant (0.190–0.537 mg/kg dw).

3.4. Canned seaweed

At the time when this study was being performed, the only canned seaweed products commercially available contained brown seaweed of the *Himanthalia* or *Undaria* genera. The 21 samples included canned seaweed and mixtures of seaweed with another product of vegetable origin (garlic or mushrooms) or animal origin (cockles). Only the total As and inorganic As contents were analysed (Table 4), there being no previous references in the literature in this respect. The total As contents were higher in the products consisting solely of seaweed (15.9–45.2 mg/kg dw) than in

Table 4
Contents of total arsenic (t-As) and inorganic arsenic (i-As) in canned seaweed. Results expressed in mg/kg dry weight

Seaweed	Product	Origin	t-As	i-As
Undaria pinnatifida	In brine	Spain	45.2	0.185
Himanthalia elongata	In brine	Spain	29.4	0.134
		Spain	21.0	0.146
		Spain	17.2	0.081
		Spain	15.9	0.086
		Spain	16.5	0.081
		Spain	17.0	0.087
		Spain	17.3	0.083
		Spain	16.6	0.087
		Spain	18.1	0.092
		Spain	16.3	0.080
		Spain	16.2	0.100
		Spain	16.5	0.070
	With cockles	Spain	12.6	0.230
		Spain	3.7	0.087
		Spain	4.8	0.100
		Spain	13.8	0.257
	With mushrooms	Spain	10.9	0.483
		Spain	9.5	0.487
		Spain	9.1	0.457
	With garlic	Spain	10.8	0.138

the mixtures (3.70–13.8 mg/kg dw), with canned *Undaria pinnatifida* in brine having the highest content (45.2 mg/kg dw). With regard to inorganic As, the contents were less than 0.5 mg/kg dw (0.070–0.487), with the highest values (0.457–0.487 mg/kg dw) appearing in the seaweed and mushroom mixtures, which might indicate a contribution of inorganic arsenic from the mushrooms used to make the product. In canned seaweed, inorganic As represented 0.4–5% of total As.

4. Discussion

A system of continuous surveillance of contaminant content in food is crucial for consumer protection and facilitates international trade (Kuhnlein and Chan, 2000). Risk assessment is a continually evolving process since information on contaminants, the health effects involved and their occurrence in food are all factors that should be continuously studied and monitored (Kuhnlein and Chan, 2000).

In Spanish legislation there are no specific regulations for seaweed and derived products. For the purposes of controlling the maximum limit of contaminants they have been included in the canned vegetables group, for which the maximum established limit of total As is 1 mg/kg of product (Real Decreto, 2420/78). This value is exceeded by all the samples of seaweed and canned seaweed analysed. Only the *Spirulina* tablets and some of the foods which contain seaweed do not exceed this value. It should be noted that such restrictive legislation for total As is not appropriate for seaweed, in which the presence of As in concentrations greater than 1 mg/kg dw is customary but does not indicate contamination of the product or a risk for the consumer. Seaweed is a primary accumulator of arsenic in the marine environment and is an important stage of arsenic metabolism through the food chain.

From the viewpoint of risk evaluation, total As content in foods has no toxicological value. It is necessary to quantify inorganic As, a term that embraces the most toxic carcinogenic arsenic species so far quantified in food, As(III) + As(V). Specific legislation for seaweed, limiting inorganic As, would make the sale of seaweed compatible with protection of the consumer. In this regard, it would be desirable to follow the example of the most advanced legislation which specifically regulates levels of inorganic As in seaweed, as in the cases of France, the USA, Australia and New Zealand (Mabeau and Fleurence, 1993; ANZFA, 1997).

If the inorganic As results obtained are compared with the values established by legislation, 27% of the edible seaweed samples analysed, one sample of *Laminaria japonica*, one of *Eisenia bicyclis* and all the samples of *H. fusiforme*, all brown seaweed, exceed the maximum limit of 1 mg/kg dw admitted by the regulations in Australia and New Zealand (ANZFA, 1997). Only the samples of *H. fusiforme* exceed 3 mg/kg dw of inorganic As, the maximum limit admitted by France and the USA (Mabeau and Fleurence, 1993). For the other products analysed, one sample of *Fucus* tablets and three samples of foods containing *H. fusiforme* exceed the legislation of Australia and New Zealand.

From the viewpoint of the evaluation of the food safety of these products, the estimate of intake of inorganic As from consumption of seaweed has aroused growing interest. Previous references in this respect are scanty. The work carried out by Norman et al. (1987) was a first contribution in this respect, although by analysing reducible arsenic, which includes not only inorganic As but also the species monomethylarsonic acid and dimethylarsinic acid, they made an overestimate of the inorganic As content. They reported that, with the exception of one sample which contributed 700 μg/day, the consumption of kelp seaweed did not exceed the Tolerable Daily Intake (TDI) established as 150 μg inorganic arsenic/day for an adult weighing 70 kg (WHO, 1989). For the samples analysed in the present study the situation is different. No data are available concerning consumption of seaweed in Spain, and therefore the estimate of intake has been made on the basis of the consumption reported for the population of Japan: daily average consumption of 2–3 g dw of brown algae and a maximum daily average consumption of 12 g (Sakurai et al., 1997). For *H. fusiforme*, assuming the mean consumption (3 g/day) and the inorganic As content range reported in the literature, which includes the values found in the present study (36 and 135 mg/kg dw) (Kuehnelt et al., 2001; Hanaoka et al., 2001; Almela et al., 2002; Laparra et al., 2003; Laparra et al., 2004; FSA, 2004), for an adult weighing 70 kg the intake of inorganic As would vary between 108 and 405 μg/day, values close to or considerably above the Tolerable Daily Intake (150 μg inorganic arsenic) established by the WHO (1989). Consequently such a food could represents a risk.

To assess the exposure to arsenic via *H. fusiforme*, in addition to the analytic work of speciation and quantification of inorganic As one should consider the effect of the treatment applied to the seaweed for its consumption over the toxic. The cooking treatment applied by the consumer produces a striking change in the inorganic As content. In previous studies it has been shown that about 28–60% of inorganic As was removed from *H. fusiforme* after washing, soaking or boiling (FSA, 2004; Hanaoka et al., 2001; Laparra et al., 2003; Laparra et al., 2004), but the inorganic As content remained very high, as much as 49 mg/kg dw (Hanaoka et al., 2001). The bioaccessibility (maximum soluble content in simulated gastrointestinal media) of inorganic As should also be considered in order to make an accurate estimate of the biological effects associated with intake of this seaweed. Work done recently by our group (Laparra et al., 2003; Laparra et al., 2004) shows that between 78% and 88% of the inorganic As present in cooked *H. fusiforme* is solubilized after in vitro gastrointestinal digestion and remains available for absorption into the intestinal mucosa. These previous references reported for cooking and bioaccessibility show that the inorganic As content in *H. fusiforme* as sold does not reflect the quantity of contaminant available for the organism, and this alters the evaluation of its food safety. Therefore, considering the toxicological implications of the alarming inorganic As content in *H. fusiforme*, a study of the bioavailability of the inorganic As in this seaweed should be carried out. At present, the authorities in the UK and Canada have advised consumers to avoid consumption of this seaweed (FSA, 2004).

With regard to Pb, from a legislative viewpoint, five samples of edible seaweed, the sample of tisane made with *Fucus* and all the *Spirulina* tablets exceed the maximum limit of 1 mg/kg of Pb established in Real Decreto 2420/78. This legislation for Pb is stricter than in other countries such as France, where the maximum limit (5 mg/kg of Pb) is exceeded by only two samples of *Spirulina* tablets. Taiwanese and Japanese manufacturers allow up to 20 mg/kg for *Spirulina* products, a value not attained by the samples analysed (Ortega-Calvo et al., 1993; Hsu et al., 2001). With regard to estimation of intake, if one assumes a mean consumption of 3 g/day and the range of Pb contents found in the present study (<0.05–12.1 μg/g), these seaweeds would contribute between 0.15 and 52.3 μg/day, which is 0.06% to 15% of the TDI recommended by the WHO for an adult weighing 70 kg (0.250 mg/day) (WHO, 1993). Hsu et al. (2001) have also shown a contribution of 25% of the TDI from *Spirulina*. This contribution of Pb through consumption of a single product, *Spirulina*, can be considered high, and in the case of extreme consumers it could substantially increase the Pb dietary intakes found for some European countries, which vary between 17% and 131% of the TDI (Nasreddine and Parent-Massin, 2002). Many consumers of seaweed are vegetarians, and Pb contents are higher in plant foodstuffs than in foods of animal origin with the exception of offal.

With regard to Cd, in Spain there is no legislation for these products, and therefore, in order to evaluate the food safety of the samples analysed, it is necessary to refer to regulations in other countries. In 51% of the samples of red and brown edible seaweed analysed the concentrations of Cd exceed the maximum values permitted by France (0.5 mg/kg dw) (Mabeau and Fleurence, 1993). The situation is worse if one takes the regulations in Australia and New Zealand as a reference (0.2 mg/kg dw; ANZFA, 1997), as 84% of the samples of seaweed analysed (Table 1) exceed that limit. Other products which exceed the limits mentioned are 72% of the tablets (Table 2), the tisane made with *Fucus* and the toasted *Himanthalia* (Table 3). Cd is, therefore, a contaminant for which greater vigilance should be exercised. Assuming a mean consumption of 3 g/day (Sakurai et al., 1997) and the mean Cd content found (0.555 μg/g dw), these seaweeds would contribute a mean of 1.67 μg/day, which is 2.4% of the TDI recommended by the WHO for an adult weighing 70 kg (70 μg/day) (WHO, 2003). In the samples with Cd contents below the limit of detection, the content was taken as being equal to the limit of detection (0.003 μg/g) for the purpose of calculating intake, which would represent 0.013% of the TDI. The maximum content detected (3.6 μg/g dw) would increase the intake to 15% of the TDI, which would be a high contribution from a single product. The Cd dietary intakes for some European countries have been estimated as being between 7% and 50% of the TDI, with plant products contributing two thirds of Cd intake (Nasreddine and Parent-Massin, 2002).

5. Conclusions

The seaweeds analysed provide an example of how the current globalization of trade brings new foods to market for which national food control systems often do not provide a rapid response. The work done shows situations that require measures to be taken. In terms of legislation, for all the contaminants analysed there are breaches of the values established in legislation: (a) the limit of total As established by Spanish regulations is exceeded by practically all the samples; (b) the limit of inorganic As established by certain countries is exceeded by all the samples of *H. fusiforme*; (c) most of the samples of *Spirulina* and some samples of red and brown seaweed do not comply with Spanish regulations for Pb; (d) a considerable number of samples exceed the Cd limits set by international regulations. With regard to food safety, the situation is worrying for the intake of inorganic As from *H. fusiforme*, which is three times the established TDI.

In future, inorganic As, Pb and Cd should be constantly monitored in seaweed preparations commercially available as part of a programme to keep watch on their chemical safety. Also, the effect of cooking and studies of bioavailability (i.e., the fraction of absorbed contaminant that reaches the systemic circulation) are aspects to be taken into account with a view to making a more realistic evaluation of the food safety of seaweed.

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