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Supercritical Fluid Extraction of Polycyclic Aromatic Hydrocarbons from Seaweed Samples Before and After the *Prestige* Oil Spill

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Abstract Samples of seaweed which are used for human consumption were collected along the Galician coast (NW Spain), in order to determine the level of contamination from polycyclic aromatic hydrocarbons, by supercritical fluid extraction and liquid chromatographic analysis. No detection was made of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene and dibenzo [*ah*]anthracene. PAHs were found and quantified in only two samples. The PAHs found were the following: anthracene, chrysene, fluoranthene, fluorene and pyrene. The levels found were below maximum limits established by the Spanish Food Safety authority (<200 mg/kg dry weight). Here we show that no relevant effects were detected in terms of PAHs contamination in seaweed.

Keywords Seaweed · Polycyclic aromatic hydrocarbons · Prestige · SFE–HPLC–FL

Polycyclic aromatic hydrocarbons (PAHs) contaminate the aquatic environment and, consequently, aquatic organisms that may be consumed by humans. PAHs are formed by the incomplete burning of organic matter and fossil fuels or by the spill of petroleum substances. In marine environments, organisms with low metabolic activity, such as seaweed, are ideal candidates for the assessment of this type of contamination, especially since ingested PAHs undergo no significant transformation (Okay et al. 2000; Soriano et al. 2006; Varela et al. 2006). IARC (2007) classifies substances according to their cancerigenic action. Of the substances analysed in this study, benzo[*a*]pyrene is classified as carcinogenic to humans (1); dibenzo[*a,h*]anthracene is classified as probably carcinogenic to humans (2A); benzo[*a*]anthracene, benzo[*b*]fluoranthene, and indene[*1,2,3-cd*]pyrene as possibly carcinogenic to humans (2B); while anthracene, benzo[*ghi*]perylene, chrysene, fluoranthene, fluorene and pyrene are not classifiable as to their carcinogenicity to humans (3). Residual fuel oil is classified as a 2B.

Supercritical fluid extraction (SFE) applied to solid environmental matrices containing traces of pollutants has become a widespread analytical technique in both systematic applications and the development of new processes. Analytical determination has been carried out by HPLC with a fluorescence detector (González Amigo et al. 2000). The aim of this article was to determine PAHs in seaweed samples produced and manufactured in Galicia (NW Spain) before and after the Prestige oil spill (November 2002), in order to ascertain if they are fit for use as human food, according to Council Regulation (258/97/EC, 1997), AESA (2003) and Commission Regulation (1881/2006/EC, 2006).

Materials and Methods

Edible seaweeds were collected during the years 2001–2003. Nine samples were collected along the Galician coast (Spain), and one on the coast of Portugal (Fig. 1 and Table 1). These samples were supplied dehydrated to the laboratory, except sample number 1 and sample number 2 that were sent fresh. The fresh samples were dried in an oven until constant weight was obtained. The dried samples were powdered, homogenized and packed in glass flasks.

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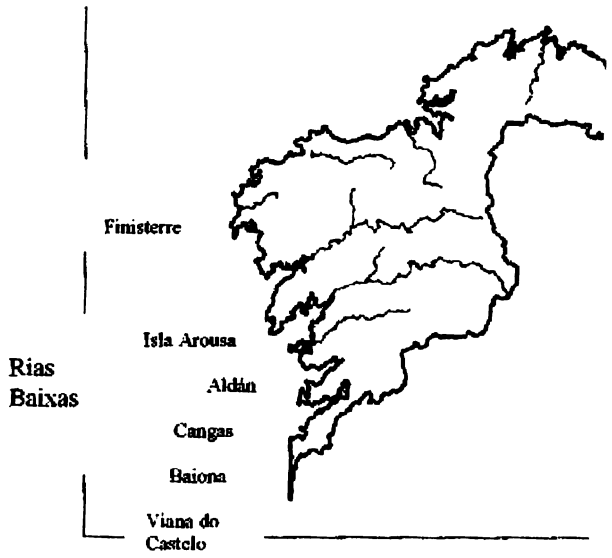

Fig. 1 Map of Galician coast showing location of samples

To perform extraction and clean-up, a Hewlett Packard 7680 A Supercritical fluid extractor controlled by a personal computer running SFE software, was used. For analysis we used a Spectra-Physics liquid chromatograph equipped with a P100 isocratic pump, a 50 μL injection loop (Rheodyne, Cotati, CA), Tecknokroma reversed-phase Tracer Tr-C-160 C18 precolumn and a Hypersil Green PAH column (5 mm particle size; 10 cm × 4.6 mm I.D.). For HPLC, a FL2000 fluorescence detector connected via Labnet to a PC with Borwin processing software was used.

Samples were analyzed in duplicate, for acenaphthene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*] fluoranthene, benzo[*ghi*]perylene, chrysene, dibenzo[*ah*] anthracene, fluoranthene, fluorene and pyrene by LC analysis following SFE.

The SFE method was carried out as described by González Amigo et al. (2000). The seaweed sample (0.5 g dried) was preadsorbed onto 15% deactived silicagel (2 g), and placed in a 7-mL extraction thimble, which was then filled with silicagel (1 g) and methanol (200 μL, as modifier). Two layers of Whatman 541 filter papers cut to the diameter of the thimble were placed just above and below the thimble cap.

Carbon dioxide was used as a cryogenic coolant gas to cool different parts of the SFE apparatus and as the extraction medium. An initial 10-min static equilibration period at 100°C and 256 bar was followed by 50-min extraction in the dynamic or continuous-flow mode at a rate of 1.5 mL/min of supercritical CO_2 at a density of 0.6 g/mL. The variable diameter nozzle was heated at 75°C; analyte collection was performed with an ODS trap at 75°C and the PAHs were eluted with four 1.5 mL portions of acetonitrile. During extraction, the trap and nozzle were kept at 75°C. The acetonitrile in the fourth vial was discarded. Acetonitrile extracts (5 mL) were collected.

An aliquot (50 μL) of the acetonitrile solution was injected into the HPLC system and eluted with acetonitrile–water (78–22, v/v) at a constant flow-rate of 0.5 mL/min. For detection and quantification, two excitation (Ex) and emission (Em) wavelength programs were used (Table 2). The analysis was performed with the column thermostated at 31°C.

Results and Discussion

PAH peaks were identified by comparing sample chromatograms to PAH standards. Quantification was carried out by external standard method.

The silicagel used as adsorbent in supercritical fluid extraction presented small interferences: in program 1 there was a small peak at 9.5 min (retention time of the F); and in program 2 there was a small peak at 8 min (retention time of the A). Both values were subtracted.

Table 1 Species, date and collection area of the samples

	Scientific name	Common name	Date	Collection area
1	*Laminaria ochroleuca*	Kombu	July/2001	Baiona
2	*Himanthalia elongata*	Thongweed	July/2001	Finisterre
3	*Porphyra sp.*	Nori	August/2001	Viana do Castelo (Portugal)
4	*Palmaria sp.*	Dulse	September/2001	Rinlo
5	*Undaria pinnatifida*	Wakame	February/2003	Aldán
6	*Undaria pinnatifida*	Wakame	March/2003	Cangas
7	*Undaria pinnatifida*	Wakame	April/2003	Galicia (Rías Baixas)
8	*Laminaria ochroleuca* e *hyperborea*	Kombu	July/2003	Galicia (Rías Baixas)
9	*Himanthalia elongata*	Thongweed	July/2003	Isla de Arousa
10	*Laminaria ochroleuca*	Kombu	July/2003	Isla de Arousa

Table 2 Wavelength of excitation (λexc) and emission (λem) and retention times for identification of PAHs

	λexc (nm)	λem (nm)	RT
PAH (program 1)			
F	286	456	9.5
BaA	270	390	16.2
BbF	296	426	26.7
BaP	296	406	38.0
DBaA	296	406	50.0
Bghi	296	406	60.0
PAH (program 2)			
Fl	266	302	6.5
A	250	400	8.5
Py	266	380	12.0
Chry	266	380	20.1

The identification of acenaphthene in the mixture was not possible, since its retention time in program 1 is too close to those for fluorene and anthracene. The latter two analytes are identified under the conditions of program 2.

The calibration line was constructed by regressing the mean peak area (n = 3) on standard concentration. Working standard solutions were prepared in acetonitrile in the concentrations ranges of 1–10 μg/L. Calibration curves were linear. The analytes identified by LC under the conditions described with Ex and Em wavelength program 1 were confirmed, when possible, by LC under the conditions of program 2 and viceversa. Under these conditions, different emission band intensities were detected. Limits of detection (LOD), the signal that is three times the height of the level of noise, were established in accordance with the American Chemical Society (Table 3).

Precision was determined by applying the full procedure to six replicates of seaweed *Himanthalia elongata* (sample number 2). To calculate the recovery, six replicates of dry sample (0.5 g) were spiked with 2 μg/L of each PAH (Table 3). The recoveries found for most of the PAHs were satisfactory. The lowest recoveries were observed for the "light" PAH (up to four aromatic rings).

PAHs were not detectable in all samples (less than LOD reported in Table 3), with the exception of fluoranthene (10 mg/kg dw) and chrysene (6.7 mg/kg dw) in sample number 2 and fluorene (33 mg/kg dw), anthracene (4.7 mg/kg dw), pyrene (73 mg/kg dw) and chrysene (16 mg/kg dw) in sample number 5. The levels of these substances were below maximum limits established by the Spanish Food Safety Authority (<200 mg/kg dry weight) (AESA 2003). Seaweed sample number 5 was collected in February 2003, three months after the Prestige oil spill. The samples collected at later dates do not contain PAHs.

Benzo[a]pyrene was not detected in any sample. Recently, the EU (Regulation 1881/2006) have established a limit between 2 and 10 mg BaP/kg wet weight. This is the maximum level for a variety of seafood other than smoked.

In the Venice lagoon, Pavoni et al. (2003) found concentrations below 10 mg/g dry weight (average concentrations) of PAHs (mainly tri-, tetra-aromatic compounds) in seaweed. Gianguzza and Orecchio (2006) examine PAHs in different Sicilian coastal environments. The total concentration of PAHs investigated in seaweed varied from 23 to 36 mg/kg of dry sample. The presence of low molecular weight compounds is typically from spill-associated hydrocarbons.

PAH levels found in fish and molluscs in Galicia after the Prestige oil spill maintain values similar to samples obtained in previous years and to samples from other areas of Spain. With exception of the coastal areas directly oiled by the spill, it is difficult to identify major toxicological impact in the continental shelf (Albaigés et al. 2006; Gónzalez et al. 2006). These values were below the Spanish Food Safety Authority limits (AESA 2003). Denton et al. (2006) have studied PAHs in algae; the absence of detectable 2-and 3-ring PAHs indicated that these sites had not been affected by significant fuel spills in the recent past.

Table 3 Regression equation, limit of detection, precision and recovery for PAHs

	Intercept	Slope	Determination coefficient	LOD (μg/L)	Precision (RSD %)	Recovery (%)
F (program 1)	6787615	21963010	0.98	0.55	3.9	59
BaA (program 1)	6265346	47301949	0.98	0.26	2.8	67
BbF (program 1)	10211447	32202005	0.99	0.47	6.8	98
BaP (program 1)	49707438	124994808	0.99	0.13	3.4	91
DBaA (program 1)	54956074	146323685	0.98	0.16	1.1	111
Bghi (program 1)	1817569	21592037	0.95	0.60	5.4	133
Fl (program 2)	23673593	192710795	0.98	0.08	6.8	63
A (program 2)	18950872	540415076	0.96	0.03	8.0	53
Py (program 2)	16897568	21344353	0.97	0.75	3.3	104
Chry (program 2)	24167366	132499142	0.98	0.15	0.6	103

In the opinion of Alvarez-Salgado et al. (2006), the impact of the oceanographic conditions during the spring of 2003 on the fuel spilled by the prestige produced the dissolution/dispersion of PAHs in the surface layer. The dispersion by the surface currents in the Northern Iberian basin, together with the low solubility of the Prestige oil and the biodegradation from natural bacterial populations in the area (readily able to degrade crude oil) could explain the low PAH concentrations recorded. These results are in line with the data found in our studies. Varela et al. (2006) found no relevant effects on the pelagic system. In addition, the phytoplankton and zooplankton community structure exhibited no significant differences before and after the oil spill.

The method proposed is precise and may be considered suitable for determining PAH in seaweed for safety evaluation. PAHs were not detected in the majority of our samples, and when PAHs were detected they were usually non-carcinogenic hydrocarbons and below 200 mg/kg dry weight (AESA 2003). BaP was not found (Regulation 1881/2006). Therefore, the seaweed analysed can be considered acceptable for human consumption.

Overall, we conclude that the Galician coast, which is far from pollution sources, is not contaminated.

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