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PII: S0003-2670(18)30698-6

DOI: [10.1016/j.aca.2018.05.066](https://doi.org/10.1016/j.aca.2018.05.066)

Reference: ACA 236003

To appear in: *Analytica Chimica Acta*

Received Date: 29 March 2018

Revised Date: 23 May 2018

Accepted Date: 25 May 2018

Please cite this article as: L. Zhang, E. Gionfriddo, V. Acquaro Jr., J. Pawliszyn, Direct Immersion Solid-Phase Microextraction Analysis of Multi-class Contaminants in Edible Seaweeds by Gas Chromatography-Mass Spectrometry, *Analytica Chimica Acta* (2018), doi: 10.1016/j.aca.2018.05.066.

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ACCEPTED MANUSCRIPT

## Direct Immersion Solid-Phase Microextraction Analysis of Multi-class Contaminants in Edible Seaweeds by Gas Chromatography-Mass Spectrometry

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### Abstract

The present work aimed at the development of a simple and accurate direct immersion-solidphase microextraction-gas-chromatography-mass spectrometry (DI-SPME-GC-MS) method for simultaneous determination of PAHs, PCBs, and pesticide residues in edible seaweeds. As the target contaminants possess a wide range of physical-chemical properties, multivariate experimental design was used for method optimization. In particular, two different methods were optimized and validated: one that allows for simultaneous determination of all targets, and an ad hoc method for determination of hydrophobic analytes, a class that often poses a challenge for extraction from food matrices. Optimum conditions suitable for simultaneous quantitation of all targeted compounds, namely buffer at pH=7.0, 20% acetone (v/v), 10% NaCl (w/w), 0.02% NaN<sub>3</sub>, 60 min DI extraction at 55 °C, and 20 min desorption at 270 °C, afforded limits of quantitation (LOQs) in the range of 1-30 µg kg<sup>-1</sup>, a wide linear range of 5-2000 µg kg<sup>-1</sup>, the attainment of satisfactory determination coefficients ( $R^2 > 0.99$ ) with no significant lack of fit ( $p > 0.05$ ) at the 5% level, and satisfactory accuracy and precision values. By modifying the extraction conditions to favor extraction of the most hydrophobic analytes (e.g. higher amount

29 of organic modifier and pH, and lower salt content) lower LOQs were obtained for these  
30 compounds ranging from 0.2-13.3  $\mu\text{g kg}^{-1}$ . The established methods were then used for screening  
31 of commercial, edible dry seaweeds, with PCBs ( $\leq 16.0 \text{ ng g}^{-1}$ ) and PAHs ( $\leq 15.5 \text{ ng g}^{-1}$ ) detected  
32 in some samples. This method overcomes most challenges commonly encountered in dry sample  
33 analysis applications, and represents the first report of a DI-SPME method employing the matrix-  
34 compatible fiber for simultaneous multiclass and multiresidue analysis of seaweeds.

35 Keywords: Matrix-compatible SPME; Pesticides; Polycyclic aromatic hydrocarbons (PAHs);  
36 Polychlorinated biphenyls (PCBs); Multi-residue analysis; Dry seaweed

37

## 38 1. Introduction

39 Edible seaweeds, such as brown, green, and red seaweeds, among other varieties, represent  
40 a well-known source of sustenance, and are often considered a staple in many cuisines of Asian  
41 origin [1]. From a nutritional point of view, seaweeds are a low-calorie food, containing  
42 significant quantities of proteins, vitamins (A, E, C, and K), essential unsaturated fatty acids, and  
43 minerals, as well as bioactive compounds with known antioxidant, antimutagenic, and  
44 anticoagulant properties [2, 3]. Furthermore, seaweeds are a valuable source of dietary fiber;  
45 according to a previous study, an 8 g serving of dry seaweed can provide up to 12.5% of a  
46 person's daily fiber needs [4]. Indeed, the dietary value of edible seaweeds has prompted a large  
47 increase in their consumption as a healthy food worldwide in recent years, as well as led to the  
48 development of various seaweed-based industries [5].

49 However, seaweeds are inevitably exposed to the ubiquitous presence of organic pollutants  
50 such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and various  
51 pesticides, compounds that derive from both natural and anthropogenic sources, and are known  
52 to pose a health hazard [6, 7]. PAHs, which include a large group of over 200 different  
53 compounds, are categorized as compounds containing two or more fused benzene rings. Some  
54 of these compounds, such as benzo[a]pyrene, are known carcinogens, while others have been  
55 indicated as suspected carcinogens [6-9]. The U.S. Environmental Protection Agency (EPA-USA)  
56 considers 16 PAHs as priority organic pollutants; as such, these compounds have been  
57 extensively monitored [6, 10, 11]. PCBs are a family of compounds comprised of 209 chemically

58 related congeners that were widely used more than 25 years ago. Owing to their insulating and  
59 fire-retardant properties, PCBs were used in a variety of industrial applications, such as  
60 microscope oils, electrical insulators, capacitors, and electrical appliances [12]. They were also  
61 widely sprayed on dirt roads as a dust-control measure until some of the unintended  
62 consequences from their widespread use were unearthed, prompting their decreased use  
63 worldwide, as well as the establishment of CBC production bans in many countries. Indeed,  
64 exposure to PCBs has been implicated as a risk factor for “endocrine (hormone) disruption”,  
65 which can lead to infertility, the development of certain types of cancer, and other hormone-  
66 related disorders [13]. Pesticides such as certain organophosphates (OP), carbamates (CAR), and  
67 pyrethroids (PYR) are widely employed in the agricultural and aquaculture industries as pest and  
68 disease control measures [14, 15]. Due to their widespread use, the potential human health  
69 hazards (neurotoxicity, among others) posed by these compounds have increasingly become a  
70 focus of public attention [16]. Owing to their hydrophobicity, PAHs, PCBs, and pesticides tend  
71 to associate to particulate matters; in this regard, within the context of our discussion, all three  
72 groups of compounds have been previously detected in seaweeds [16, 17]. Regulation (EC) No  
73 396/2005 of the European Parliament only sets maximum residue levels (MRL) for some  
74 pesticides, ranging from  $10 \mu\text{g kg}^{-1}$  to  $50 \mu\text{g kg}^{-1}$  in edible seaweeds [18]. Alternatively, only  
75 scarce information is available on the limitation levels of PAH and PCB contaminants in  
76 seaweed. In view of the above, the development of effective extraction and enrichment  
77 techniques to determine the levels of the above pollutants present in edible seaweeds is of great  
78 interest.

79 Currently, most reports available in the literature addressing seaweed analysis have the  
80 detection of heavy metals as their focus. For determination of organic residues in seaweeds,  
81 liquid-liquid extraction, such as Soxhlet [19], pressured liquid extraction (PLE) [20], microwave-  
82 assisted extraction (MAE) [21], or matrix solid-phase dispersion (MSPD) [5] have been reported  
83 as methods of choice – approaches that unavoidably employ large volumes of organic solvents,  
84 and which require pre-concentration and clean-up steps. As a result, such methods are tedious,  
85 time-consuming, and not environmentally friendly. Conversely, solid-phase microextraction  
86 (SPME), which has been successfully applied towards analyses of organic contaminants in  
87 various matrices [22, 23], integrates sampling, extraction, concentration, and sample introduction  
88 into a single, low-solvent consuming and automatable step. Nonetheless, very few reports

89 mention the use of SPME for analysis of organic contaminants in dry seaweeds, especially for  
90 poorly volatile compounds, such as PAHs, PCBs, and pesticides.

91 Despite the numerous advantages presented by SPME, including its simplicity of operation,  
92 development of SPME methods for complex matrices, such as seaweeds, requires careful method  
93 optimization, including as a first step, the selection of mode of extraction. Since the majority of  
94 compounds studied in this work bear poor volatility, direct-immersion SPME (DI-SPME), in  
95 which the extraction phase is placed directly into contact with the sample, was selected as mode  
96 of extraction with aims to attain higher method sensitivity as well as better representativeness of  
97 analytes extracted from the seaweed matrix. The ideal features of a fiber coating for DI-SPME  
98 should be matrix compatibility, robustness, and good affinity towards the analytes of interest.  
99 Within this context, the analytes targeted in this work belong to three different chemical classes,  
100 and are characterized by widely different physiochemical properties, certainly posing a challenge  
101 for SPME analysis. A matrix-compatible coating (namely PDMS/DVB/PDMS) was developed  
102 with the purpose of enabling DI-SPME in complex matrices such as food commodities [24]. The  
103 robustness and endurance of this new coating were evaluated in various food samples, such as  
104 fruits [25-27] (i.e. grape, strawberries and avocado pulp) and raw blended vegetables[28] (i.e.  
105 spinach, tomato, and carrot), that present different analytical challenges such as water content,  
106 pigmentation, interfering matrix compounds, and vegetable texture. In all tested matrices, the  
107 PDMS/DVB/PDMS fiber showed excellent durability and robustness, allowing for over 100  
108 consecutive extractions. Based on the above development, the currently presented work aimed at  
109 the optimization and development of a DI-SPME-GC-MS method for simultaneous analysis of  
110 multiresidue PAHs, PCBs, and pesticides in edible seaweeds. In order to achieve an accurate and  
111 robust analytical method by DI-SPME, multivariate approaches such as Plackett-Burman and  
112 Central Composite Design (CCD) were employed to screen and optimize the most relevant  
113 parameters affecting extraction efficiency (such as pH, ionic strength, organic solvent content,  
114 sample temperature, and extraction time). As the studied compounds have a wide range of  
115 polarities, with most bearing high hydrophobicity, two optimized DI-SPME protocols — one  
116 aimed at broad-spectrum detection, and one targeting more hydrophobic compounds — were  
117 evaluated for their suitability towards the currently discussed application. An evaluation of the  
118 abovementioned DI-SPME conditions was carried out as a means to provide information to  
119 future users on how to tune their SPME method based on the physiochemical properties of the

120 targeted compounds. Despite the challenges encountered by this method, to the best of these  
121 authors' knowledge, this is the first report of a DI-SPME method using matrix-compatible fibers  
122 for multiclass and multiresidue analysis of edible seaweed. Further, the established method was  
123 successfully applied to the analysis of commercial samples.

## 124 **2 Experimental section**

### 125 2.1 Materials and reagents

126 All employed solvents were of HPLC grade. Acetonitrile (ACN) and acetone were  
127 purchased from Fisher Scientific (Ottawa, ON, Canada). Sodium chloride and sodium azide were  
128 purchased from Sigma Aldrich (Oakville, Ontario, Canada). PDMS/DVB/PDMS (SPME-OC  
129 fiber assembly) 75  $\mu\text{m}$  (Coating thickness includes 65  $\mu\text{m}$  coating + 10  $\mu\text{m}$  OC (overcoating))  
130 fibers were purchased from Supelco (Bellefonte, PA, USA). Dry edible seaweeds (Wakame and  
131 Nori) were purchased at local markets in Waterloo(ON, Canada).

132 All standards were purchased from Sigma Aldrich (Oakville, ON, Canada), Accustandard  
133 (New Haven, CT, USA), and Cambridge Isotope Laboratories (Montreal, Quebec, Canada). With  
134 the exception of PCBs (Congeners Mix 3, 10  $\mu\text{g mL}^{-1}$  in Isooctane), PAHs (Calibration Mix, 10  
135  $\mu\text{g mL}^{-1}$  in acetonitrile), lamda-cyhalothrin (100  $\mu\text{g mL}^{-1}$  in acetonitrile), and (phenoxy- $^{13}\text{C}_6$ )-cis-  
136 permethrin, all employed standards were of a purity higher than 94%. Detailed compound  
137 information for all analytes used in this work can be found in Table S1of Supporting  
138 ISInformation.

139 Individual solutions of pesticide standards were prepared in acetonitrile at 10 mg mL<sup>-1</sup>.  
140 Internal standard (naphthalene-d8, benzo[a]anthracene-d12, Acenaphthene-d10, Phenanthrene-  
141 d10, Chrysene-d12, Benzo[a]pyrene-d12, PCB 30, PCB 103, PCB 169, and (diethyl-D10)-  
142 chlorpyrifos) solutions were prepared at 1 mg mL<sup>-1</sup> in acetonitrile. All standard solutions were  
143 stored in a freezer at -30 °C.

144 For method development steps, a series of mixed calibration solutions, ranging from 0.01  
145 to 10 ng  $\mu\text{L}^{-1}$  (for PAHs and PCBs; seven levels) and 0.1 to 100 ng  $\mu\text{L}^{-1}$  (for pesticides; seven  
146 levels), were prepared during method development to calculate amounts (in ng) extracted by  
147 SPME for each analyte. Liquid injections of calibration solutions were carried out in triplicate.

148 During the method validation steps, spiking standard mixtures were firstly prepared  
149 containing target analytes at 1000  $\mu\text{g mL}^{-1}$  (for pesticides), 10, 1 and 0.1  $\mu\text{g mL}^{-1}$  (for all  
150 analytes), and internal standards were prepared at 5  $\mu\text{g mL}^{-1}$ . Aliquots of the above mentioned  
151 mixtures were spiked into blank seaweed samples to obtain related concentration levels required  
152 for each validation part.

## 153 2.2 Instrumentation

154 Seaweed samples were grinded with the use of a Salton grinder CG1451 (Montreal,  
155 Quebec, Canada). Sample pH was measured with a Mettler Toledo MP220 (Schwerzenbach,  
156 Switzerland) pH meter. For homogenization of samples, a Benchmark BenchMixer<sup>TM</sup> (Edison,  
157 NJ, USA) was employed.

158 GC-MS analysis was performed with an Agilent 7890B gas chromatography coupled to a  
159 5977A mass spectrometer (Agilent Technologies, Mississauga, ON, Canada), equipped with a  
160 Gerstel MultiPurpose Sampler (GERSTEL, Linthicum, MD, USA). Chromatographic separation  
161 was performed in an HP-5 MS column (30 m  $\times$  250  $\mu\text{m}$   $\times$  0.25  $\mu\text{m}$ ). Helium was employed as  
162 carrier gas, with a column flow at 1.0  $\text{mL min}^{-1}$ . The oven temperature program was initially set  
163 at 70  $^{\circ}\text{C}$  for 10 min, then ramped at 20  $^{\circ}\text{C min}^{-1}$  to 200  $^{\circ}\text{C}$  for 8 min. Next, it was ramped at  
164 10  $^{\circ}\text{C min}^{-1}$  to 260  $^{\circ}\text{C}$  for 8 min, and then ramped at 10  $^{\circ}\text{C min}^{-1}$  to 300  $^{\circ}\text{C}$ , at which point it was  
165 held for 8 min, resulting in a total run time of 38 min. The injector (equipped with a deactivated  
166 glass liner for SPME, 0.75mm i.d.) was maintained at 270  $^{\circ}\text{C}$  in splitless mode (the split valve  
167 was opened for after 20 min in case of SPME desorption and after 1.0 min for liquid injections).  
168 For the single quadrupole MS, the operational conditions were as follows: the transfer line, ion  
169 source, and MS Quad temperature were 280  $^{\circ}\text{C}$ , 230  $^{\circ}\text{C}$ , and 150  $^{\circ}\text{C}$ , respectively; the fixed  
170 electron energy (EI), 70 eV; the mass range, m/z 50-400; acquisition rate, 50 Hz; detector  
171 voltage, 1338 V. Retention times, as well as the selected quantifier ions obtained in the above  
172 GC-MS conditions are presented in SI Table S2.

173

## 174 2.3 Preparation of spiked seaweed samples

175 Dry organic edible seaweed samples (previously analyzed for the absence of target  
176 analytes) were grinded into powder and transferred to a glass container. In order to demonstrate  
177 the suitability of the proposed method for different types of edible seaweed, a mixture of Nori

178 and Wakame (1:1, w/w) was used as matrix during SPME method optimization. 30 g of mixed  
179 seaweed powder was weighed into a 250 mL glass jar, after which 40 mL of acetonitrile spiked  
180 with the multi-class analytes were added. The mixture was then left to homogenize overnight  
181 under agitation conditions so as to allow for sufficient analyte-matrix binding to occur. Spiked  
182 samples were dried under N<sub>2</sub> flow in a fume hood and stored at +4 °C in refrigerator for 2 days  
183 prior to extraction to simulate typical interaction conditions between seaweed and the target  
184 compounds.

185 Spiking concentrations were carefully selected to guarantee enough sensitivity for all  
186 analytes during the optimization processes (see SI Table S3). Blank seaweed samples were  
187 prepared in the same manner as described above, with the exception that no standard was added.  
188 All the samples needed for matrix matched calibration were prepared at according to the  
189 abovementioned protocol properly adjusting the amount of spiked analytes and internal standards.

190

#### 191 2.4 SPME procedure

192 Preliminary tests in spiked seaweed sample were performed by adjusting desorption  
193 temperature and time. As the data indicated (not shown), desorption temperature at 270 °C and  
194 desorption time of 15 min yielded no significant carryover.

195 0.25 g spiked seaweed sample and 8 mL dispersive solution were placed into a 10 mL glass  
196 vial, and thoroughly vortexed for 1h prior to extraction. For initial SPME method development,  
197 deionized water was added as dispersive solution. After 1 h mixing, a 1 min pre-extraction  
198 incubation of the sample was performed at 40 °C in the agitation unit at 500 rpm, followed by a  
199 30 min direct immersion extraction. Following extraction, the fiber was rinsed for 15 s in  
200 deionized water, and then desorbed for 15 min at 270 °C.

201 For Plackett-Burman and CCD experiments, all SPME parameters were set as described  
202 above, with the exception of the composition of the employed dispersive solutions, which was  
203 varied to correspond to each experimental condition, in accordance with the experimental matrix.

204 Two SPME methods were optimized, one for simultaneous determination of all targeted  
205 analytes (method#1), and a second one (method#2) optimized ad hoc for the most hydrophobic

206 analytes ( $\text{LogP} > 5.2$ ). For method #1, optimized sample preparation conditions were: buffer at  
207  $\text{pH} = 7.0$ , containing 20% acetone (v/v), 10% NaCl (w/w) and 0.02%  $\text{NaN}_3$ . Method #2 utilized a  
208 buffer with  $\text{pH} = 10.0$ , containing 40% acetone (v/v) and 0.02%  $\text{NaN}_3$ , aiming at enhanced  
209 extraction of the more hydrophobic compounds. For both protocols the following procedure was  
210 followed: after 1 h mixing, samples were incubated for 5 min at  $55\text{ }^\circ\text{C}$  in the agitation unit at 600  
211 rpm, followed by 60 min and 30 min direct immersion extractions, for method #1 and method #2  
212 respectively. Following extraction, fibers were rinsed for 25 s in an acetone-water (2:8, v/v)  
213 solution, and then desorbed for 20 min at  $270\text{ }^\circ\text{C}$ .

## 214 2.5 Optimization by experimental design

215 Aiming to optimize the analysis of 41 contaminants in seaweeds, a Plackett-Burman  
216 experimental design (PBD) was selected to screen significant independent variables (factors)  
217 impacting analysis. Following the identification of the most significant factors ( $p\text{-value} < 0.05$ ), a  
218 response surface methodology (RSM), namely central composite design (CCD)  $2^4$ , was used for  
219 optimization. Data were processed through the software Statistica 13.0 (TIBCO® Statistica™,  
220 CA, USA).

221 For PBD screening, evaluated factors and respective ranges included the following:  
222 Extraction time (15 – 45 min), Salt content (0 – 20 %), Organic solvent (0 – 20 %, w/w),  
223 Temperature (30 –  $60\text{ }^\circ\text{C}$ ), Stirring rate (250 – 600 rpm), Incubation time (5 – 15 min) and  $\text{pH}$  (4  
224 – 10), as shown in SI Table S4. Factor ranges were selected according to preliminary tests.

225 For CCD experiments, extraction temperature ( $^\circ\text{C}$ ), organic solvent (mL), salt content (%),  
226 and  $\text{pH}$  were evaluated. A total of 30 experiments were performed: eighteen in the factorial  
227 points (-1, 1), eight in the axial points (-2, 2), and four in the central point (0). The studied  
228 factors and their associated ranges can be found in SI Table S5.

229

## 230 **3 Results and discussion**

231 3.1 Evaluation of binding time as a factor in the extraction of target analytes from spiked  
232 seaweeds

233 Significant differences in extraction results were observed between spiked seaweed  
234 samples submitted to extraction at the same experimental conditions, but at different time  
235 intervals after preparation of samples. According to Burford [29], “freshly” spiked analytes have  
236 little time to interact with the sample matrix. As such, sufficient equilibration (binding) time  
237 between spiked analyte and matrix should be allocated so as to attain samples that can be  
238 considered representative of real contamination scenarios. To investigate optimum binding time  
239 for analytes spiked on seaweeds, SPME was carried out at different binding periods (at +4 °C in  
240 refrigerator) of the spiked sample, namely at 24, 48, and 72 hours following spiking of analytes.  
241 The results are shown in Supporting Information Figure S1. For most target analytes, extraction  
242 amounts were observed to decrease from 24 h to 48 h, but remain relatively unchanged as  
243 binding time surpassed the 48 hour mark. Therefore, in order not to introduce errors associated  
244 with insufficient binding time, “freshly” prepared spiked samples were allowed to equilibrate  
245 with the spiked analytes at least 48 h prior to extraction.

## 246 3.2 Sample preparation optimization

### 247 3.2.1 Evaluation of sample to water ratio

248 Unlike most fresh vegetable and fruit matrices, small amounts of dry seaweed can expand  
249 to large volumes once introduced to water. In DI-SPME analysis, when 10 mL vials are used for  
250 sampling, care should be exercised to prepare total sample volumes. The volumes should be  
251 large enough to enable the full immersion of the SPME fiber, but do not exceed 9 mL so as to  
252 avoid contamination of the fiber holder due to spilling, that can result into contamination of the  
253 GC injector. Moreover, samples should not be so dense as to incur SPME fiber breakage during  
254 DI extraction in agitation conditions. As such, careful optimization of the seaweed-to-water ratio  
255 was carried out, taking into account the abovementioned requirements, prior to SPME method  
256 development. Results showed that when 0.25 g seaweed and 8 mL water were used, the sample  
257 solution enabled suitable conditions for DI-SPME.

### 258 3.2.2 Degradation study of analytes in sample solution at room temperature

259 Envisioning the application of the proposed SPME to routine analysis, and thus foreseeing  
260 the preparation of large amounts of samples that will inevitably be queued for analysis on an  
261 autosampler rack, the currently presented work included an evaluation of whether any

262 degradation of analytes in the sample matrix occurred at room temperature. This assessment  
263 constituted an important step in method development, as it allowed for proper tuning of the  
264 sample preparation strategy, and thus circumvented the production of unreproducible and  
265 unreliable data sets. For this assessment, after carrying out sample preparation as per procedures  
266 previously outlined in this work (Sections 2.3 and 2.4), samples were placed in an autosampler  
267 tray at room temperature. DI-SPME was then carried out under the following conditions:  
268 immediately after vortexing (0 h), and after 6, 12, 24, and 48 h (n=3 for each set). Any potential  
269 loss and/or degradation of analytes was assessed by evaluating variations in their extracted  
270 amounts. According to the obtained results, a decreasing trend was observed for most analytes  
271 from 12 h to 48 h (data not shown). Of note, pressure build up into the vial was noticed since the  
272 septum of the sample vial was observed to 'bloat' after being placed at room temperature for 24  
273 h; thus, the authors presume that biodegradation may have taken place during the investigated  
274 waiting time.

275 Based on previous reports [30, 31], the dispersion solution was enriched with 0.02%  
276 sodium azide ( $\text{NaN}_3$ ) so as to prevent bacterial growth from occurring in the matrix media. The  
277 abovementioned set of experiments was repeated to verify the efficacy of the bacterial growth  
278 inhibitor added to the dispersion solution. Related results regarding the target analytes, namely  
279 pesticides, PCBs, and PAHs, are shown in Figure 1(a-c). For almost all analytes, extraction  
280 amounts remained relatively stable within 48 h, indicating that addition of 0.02%  $\text{NaN}_3$   
281 effectively prevented any form of analyte degradation/loss. Therefore, the prepared seaweed  
282 samples that included addition of 0.02%  $\text{NaN}_3$  were able to stand as long as 48 h at room  
283 temperature prior to be submitted to extraction. Moreover, a comparison of extracted amounts of  
284 each targeted analyte by DI-SPME, with or without addition of 0.02%  $\text{NaN}_3$ , was also carried out  
285 (immediately after vortexing) so as to investigate the effect of addition of  $\text{NaN}_3$  on the extraction  
286 of target analytes. The attained results revealed comparable analyte extraction amounts for both  
287 extraction conditions, confirming that addition of  $\text{NaN}_3$  does not significantly affect analyte  
288 extraction (Supporting information Figure S2).

### 289 3.2.3 Optimization of pre-desorption rinsing of fiber coatings

290 As optimization of a pre-desorption rinsing step constitutes a crucial step in the  
291 development of a reliable DI-SPME protocol, preliminary studies were performed using spiked

292 seaweeds in order to select the most suitable rinsing conditions. According to a previous study,  
293 acetone showed good capability in removing oily residues from coating surfaces [27].  
294 Considering that seaweed, as a complex matrix, contains abundant unsaturated fatty acids,  
295 proteins, and pigments, among other possible interfering constituents, acetone was selected as  
296 organic solvent for the rinsing step. Moreover, five different ratios of acetone to water were  
297 tested as rinsing solutions, namely water, acetone-water (1:9, v/v), acetone-water (2:8, v/v),  
298 acetone-water (1:1, v/v) and acetone-water (9:1, v/v). For each rinsing solution, three different  
299 rinsing times, 5 s, 15 s and 25 s, were tested. The tested procedures are summarized in Figure S3  
300 (Supporting Information). Results were compared in terms of average extraction amounts of  
301 analytes, as well as in terms of SPME fiber and glass liner cleanliness. Although longer rinsing  
302 times may afford cleaner fibers, extended fiber exposure to the rinsing solution may also lead to  
303 loss of analytes extracted onto the fiber. Thus, when optimizing this parameter, a suitable  
304 compromise must be made between extraction sensitivity and effective cleaning of the coating  
305 surface.

306 In the current work, best results were achieved when rinsing time was increased to 25 s for  
307 all rinsing solutions, with the exception of acetone-water (9:1, v/v), for which a rinsing time of  
308 15 s was deemed as most suitable. Detailed comparisons of data pertaining to these experiments  
309 can be found in Supporting Information Figure S4 (a-e).

310 While addition of acetone in the rinsing solution can aid in the removal of co-extracted  
311 matrix macro-components, it may also act as an additional phase, competing with the SPME  
312 coating for the partition of analytes by inducing their back-extraction from the coating and into  
313 the rinsing solution [32]. In view of this, extraction results pertaining to the three rinsing times  
314 tested for each rinsing solution were compared in terms of analyte loss, with results shown in  
315 Figure 2. As part of the optimization of rinsing time for each solution, SPME coatings and GC  
316 injection port glass liner inserts were also inspected in terms of cleanliness, either visually or by  
317 microscope, after every 9 extractions and injections. Accumulation of matrix components onto  
318 the coating surface could be clearly observed in microscope photos of fibers that were rinsed  
319 with solutions containing water; in this regard, the cleanliness of the fiber coatings was observed  
320 to be positively correlated with the amount of acetone added to the solution, with higher  
321 percentages of acetone yielding cleaner fibers (Supporting Information Figure S5).

322 In summary, a mixture of acetone-water (2:8, v/v) for the pre-desorption rinsing step and  
323 25 s as rinsing time were chosen as optimal parameters to minimize the deposition of matrix  
324 residues on the coating, while also enabling suitable extraction efficiencies for the studied  
325 analytes.

### 326 3.3 Optimization of SPME parameters

327 The Plackett-Burman (PBD) experimental design is generally employed in method  
328 development to identify the most important factors affecting a given process without  
329 consideration given to the interaction effects between and among the evaluated factors. As such,  
330 PBD designs are often used as a screening approach in cases where the analysis of a given matrix  
331 may involve a high number of factors. As part of method development, once significant factors  
332 are identified via PBD, a response surface methodology should be subsequently employed in  
333 order to fully optimize the process [33].

334 Results were evaluated by taking into account the extracted mass of each analyte under  
335 study. Due to the substantial chemical diversity of the targeted analytes, Pareto charts obtained  
336 from the design were evaluated for each chemical class (pesticides, PAHs and PCBs), while  
337 analyte response was divided within each class according to compound hydrophobicity ( $\text{LogP} <$   
338  $4$ ,  $4 < \text{logP} < 5$ ,  $\text{logP} > 5$ ). An examination of the obtained Pareto charts, which can be found  
339 under Supporting Information Figures S6-S8, demonstrated that extraction time and stirring rate  
340 were positive significant factors for all classes and subgroups of analytes considered. In light of  
341 these results, the stirring rate was set at the maximum tested value, 600 rpm (chosen to enable  
342 fast agitation and preserve SPME coatings from mechanical damage), with further investigations  
343 of the entire extraction time profile for each analyte carried out under this optimized condition  
344 (Section 3.4). While incubation time, namely the period of time that samples spend in the  
345 heater/agitator prior to extraction, is generally a more important factor to consider for headspace  
346 SPME applications — as it is needed to establish equilibrium between the sample and its  
347 headspace — in DI-SPME, the incubation period can be employed to control the temperature of  
348 samples prior to extraction. The results obtained from the Pareto charts (Figures S6-S8) revealed  
349 that for most analyte groups and classes, incubation time did not significantly affect the  
350 extraction performances; as for analytes for which incubation significantly affected extraction,  
351 the highest responses were obtained at short incubation periods. As such, incubation time was set

352 at 5 min (the lowest level tested in the PBD) for further experiments. While addition of organic  
353 solvent as a matrix modifier can promote the release of analytes originally bound to the sample  
354 matrix — and thus not available for extraction via SPME — on the other hand, any added  
355 organic solvent can act as a competing extraction phase with the SPME coating, diminishing the  
356 extractable amount of certain analytes. As evident in Figure S6, addition of organic solvent did  
357 not positively affect the recovery of the least hydrophobic analytes ( $\log P < 4$ ), which are less  
358 likely to bind to matrix constituents, and could thus be directly affected by addition of a  
359 competing partition phase. Conversely, for compounds with  $\log P > 4$ , addition of organic solvent  
360 was shown to yield a significant positive effect on extracted amounts. This effect can be related  
361 to two possible phenomena: the shifting of the binding equilibria established within the matrix  
362 toward the free forms of analytes belonging to this class ( $\log P > 4$ ), and the stabilization of these  
363 analytes in the dispersive solution, which avoids loss of analytes due to poor solubility in pure  
364 aqueous media. Extraction temperature does affect the diffusivity of analytes into the sample  
365 media, and may have an effect on the partition coefficient of the analytes into the SPME coating.  
366 In analyses of complex matrices, temperature may also play a role in the binding equilibria  
367 established with matrix components; thus, an attentive optimization of this parameter is required  
368 so as to ensure temperature has a positive effect on extraction. In the current work, temperature  
369 was revealed to be positively significant for all classes and subgroups, with the exception of  
370 pesticides with  $4 < \log P < 5$ . As such, optimization of this parameter was further carried out by  
371 CCD. Varying the ionic strength of the sample by addition of salts can promote the extraction of  
372 certain analytes via the salting-out effect; however, in the presence of binding media, variation of  
373 the ionic strength can shift binding equilibria toward the bound form of analytes, reducing  
374 recoveries by SPME. Considering that an interaction effect exists between ionic strength  
375 adjustments and media temperature, this parameter was further optimized by CCD. Sample pH  
376 adjustments play a significant role in SPME analysis, as certain SPME coatings are only capable  
377 of extracting molecular species in their neutral form. Moreover, the sorption of pyrene into some  
378 dissolved hydrophobic organic matters commonly found in the marine environment, such as  
379 humic acid (HA) and fulvic acid (FA), among others, has been previously shown to be strongly  
380 pH-dependent. As pH increases, an obvious decreasing trend can be observed for the partition  
381 coefficients of pyrene binding to HA and FA [34]. An examination of the Pareto Charts (Figures  
382 S6-S8) revealed that pH played a significant role in the recovery of different chemical classes,

383 yielding both a positive and negative significant effect; as such, this parameter was also further  
384 optimized via CCD.

385 Following the successful identification of parameters significantly influencing targeted  
386 analyte response, a multivariate optimization of these variables was carried out with aims of  
387 selecting parameters capable of yielding maximum signal response.

388 Response surface methodology (RSM) has been widely employed in the optimization of  
389 analytical chemistry processes [35-38]. Its widespread application in analytical chemistry lies in  
390 the ability of RSM to allow for optimization of various parameters via few experiments, as well  
391 as in its capability of not only providing information regarding the individual influences of  
392 significant factors, but information pertaining to the interactions occurring among these  
393 parameters as well. In addition, a suitable prediction mathematic model can be applied to  
394 determine the response inside the range studied for each factor, and it is worth highlighting that  
395 this can be achieved by only using experimental design approaches [37, 39].

396 For this purpose, a Central Composite Design (CCD) was employed for optimization of  
397 extraction temperature ( $^{\circ}\text{C}$ ), acetone addition (% v/v), pH, and salt addition (% of NaCl). All  
398 other parameters were kept constant, as follows: stirring rate set at 600 rpm; pre-incubation time  
399 of 5 min; extraction time set at 45 min.

400 The 41 contaminants evaluated were distributed in classes of hydrophilic and lipophilic  
401 compounds due to their diverse affinities for the SPME coating, as already described in previous  
402 work [40]. However, in contrast to the abovementioned work [40], the currently presented work  
403 included an evaluation of the response of each compound, as opposed to an evaluation of the  
404 sum of signals pertaining to the targeted analytes. When only the sums of analyte signals are  
405 considered, individual information regarding each compound can be missed, especially in cases  
406 where there is high intensity variability among the targeted analytes. As an example, an increase  
407 in the total response (sum) does not always correspond to an increased response for a given  
408 analyte, as variations in independent variables (factors) can significantly enhance responses  
409 analytes while negatively affecting the responses of others. Moreover, the effect of a given  
410 independent variable on the response of a given analyte is strictly related to the chemical  
411 properties of said analyte. Thus, in cases where many compounds are being studied, dividing  
412 them into groups based on their chemical properties may constitute a feasible firsthand approach

413 to analysis. However, when grouping compounds in such a manner, one should not interpret the  
414 sum of their response as a single response corresponding to the whole group. When such an  
415 approach is carried out, individual information regarding each compound will likely be missed,  
416 enforcing the necessity of individually evaluating compounds, even if they are firstly divided  
417 into groups for convenience of analysis.

418 In view of the above, as a firsthand approach to an evaluation of the influences of  
419 individual factors on analyte response, the 41 contaminants under study were separated into three  
420 groups based on their Log P: highly lipophilic ( $\text{Log P} \geq 5.6$ ), lipophilic ( $3.46 \leq \text{Log P} < 5.6$ ), and  
421 hydrophilic compounds ( $\text{Log P} < 3.46$ ). Log P ranges were selected based on the similarity of  
422 significant factors among compounds ( $p < 0.05$ ); in other words, categorization of compounds  
423 into groups was based on the similarity of their behavior in relation to the evaluated factors. A  
424 detailed discussion regarding the effect of the individual variables for each group of analytes will  
425 be carried out next.

426 Using the Derringer & Suich's desirability function approach, which can maximize the  
427 overall desirability (multiple response) based on controllable factors, optimized conditions for  
428 each specific group of analytes [41, 42] based on their Log P were attained, as well as a suitable  
429 compromise of all conditions for simultaneous analysis of all targeted analytes. Seeing as most  
430 targeted analytes under study are characterized by a lipophilic nature, the conditions optimized  
431 for simultaneous analysis of all compounds mainly favored an enhancement of response for  
432 lipophilic compounds. As overall desirability is obtained by combining individual desirabilities  
433 via their geometric mean, general method response decreases significantly for hydrophilic  
434 analytes for which best conditions differ from the desired conditions for analysis of lipophilic  
435 compounds (which represent the majority of the targeted analytes) [37, 41, 42]. To solve this  
436 issue, the final conditions for analysis of all targeted analytes were selected by slightly displacing  
437 the values of the independent variables to also favor hydrophilic compounds.

438 Experimental values attained using the selected conditions were then compared to values  
439 obtained through a mathematical model of predicted values. The relative standard deviation  
440 (RSD) obtained for all compounds presented less than 5% variation, indicating that the equation  
441 was well adjusted. The determination coefficient ( $R^2$ ) ranged from 0.668 to 0.925, and the lack-

442 of-fit test yielded non-significant results ( $p > 0.05$ ), confirming a good fit for all compounds [37,  
443 41, 42].

444 In sum, optimal conditions were obtained for the simultaneous analysis of all targeted  
445 compounds, as well as for each of the three groups described above. The development of  
446 different optimization processes, including a process for the simultaneous analysis of all studied  
447 compounds as well as optimizations targeted at specific groups, can be very useful in a variety of  
448 applications. As the analyst can easily select conditions that lead to optimal method performance  
449 for a specific group of contaminants of interest, or select conditions that allow for the  
450 simultaneous analysis of different classes of pollutants characterized by a wide range of  
451 physicochemical properties. Thus, the currently presented methods can be easily tailored to  
452 various analytical goals.

453 Optimum conditions for analysis of highly lipophilic compounds were determined as 80 °C  
454 extraction temperature, 40 % organic solvent, 5 % salt content, and pH 10 (Figure S9).  
455 Conditions for analysis of medium lipophilicity compounds included 30 °C extraction  
456 temperature, 20 % organic solvent, 0 % salt content, and pH 4 (Figure S10). For hydrophilic  
457 compounds, optimum conditions were 30 °C extraction temperature, 0 % organic solvent, 20 %  
458 salt content, and pH 4 (Figure S11). Lastly, optimized conditions for the simultaneous  
459 determination of all targeted analytes were 55 °C extraction temperature, 20% organic solvent,  
460 pH 7, and 10 % salt content (Figure S12).

461 This approach provided a powerful analytical tool, as based on the focus of the study,  
462 applying optimized conditions for a specific group of analytes can enable the attainment of lower  
463 limits of quantification in compliance with MRLs imposed by regulatory agencies. However, it is  
464 important to highlight here that the focus of this work centered on the simultaneous evaluation of  
465 all studied contaminants in a single analytical run; consequently, only optimized conditions  
466 pertaining to the whole group of contaminants were employed for further method validation  
467 within the scope of this work.

468 3.4 Evaluation of the extraction time

469 Based on the CCD results, extraction times varying from 5 min to 120 min were studied  
470 under the optimized conditions for all analytes (method #1), and for hydrophobic analytes  
471 (method#2).

472 The results obtained by method#1 are shown in Figure 3. As can be seen, for most analytes  
473 with  $\text{LogP} < 4.5$ , equilibrium was reached within 30 min, while equilibrium of analytes with  
474  $4.5 < \text{LogP} < 5.2$ , necessitated an extraction period of 60 min, Lastly, analytes with  $\text{LogP} > 5.2$   
475 necessitated an equilibration time equal to or longer than 90 min. Thus, an extraction time of 60  
476 min was selected as a compromise between method sensitivity and practicality of method  
477 throughput.

478 As discussed in section 3.3.2, a higher extraction temperature of 80 °C was deemed as most  
479 suitable for most hydrophobic compounds. However, a decrease in the extracted amounts for  
480 certain pesticides, such as cerpermethrin and cyfluthrin, was observed for extractions carried out  
481 at 80 °C. To investigate this phenomenon, spiked seaweed samples were extracted at three  
482 temperatures, 55 °C, 67 °C, and 80 °C, The extracted peaks in the above three conditions were  
483 compared with those attained from spiked water. As shown in Figure 4, peak shapes for the two  
484 isomers of cerpermethrin and cyfluthrin started to change at 67 °C, with new peaks with different  
485 retention times observed at 80 °C. Such a phenomenon can be assumed to occur due to the  
486 degradation of these compounds at a high temperature. A similar result was obtained in past  
487 research[43], where it was assessed that the hydrolysis of cypermethrin in aqueous solutions was  
488 accelerated by high temperatures.

489 Therefore, the extraction time profile for hydrophobic analytes with  $\text{LogP} > 5.2$  was set at a  
490 lower temperature of 55 °C, while all other parameters obtained from the CCD (pH=10, 40%  
491 acetone (v/v)) remained as previously stated. As can be seen from results shown in Figure 5,  
492 equilibrium was reached at 60 min for almost all PAHs, pesticides, and some PCBs, such as PCB  
493 18, PCB 28, etc., although equilibration for most hydrophobic PCBs could only be reached after  
494 90 min.

495

### 496 3.4 Method validation

#### 497 3.4.1 Selection of internal standard

498 For method validation, quantitation of multi-class analytes was carried out with the use of  
499 fortified internal standards (IS) in seaweed samples so as to compensate for matrix effects, drifts  
500 in instrumental responses, as well as losses during sample preparation steps. As commonly  
501 established, a suitable IS should be characterized by physicochemical properties that are very  
502 similar to that of the analyte under study, while also allowing for sufficient separation from said  
503 analyte via chromatography. In SPME, a suitable IS should also be able to mimic the partition of  
504 the analyte toward the extraction phase as well as its partition to any other competing phase [26].  
505 To date, the most accurate and simplest method for quantification is the use of an isotopically  
506 labeled internal standard. However, due to the prohibitive cost of some isotopically labeled  
507 analytes, congeners of analytes or compounds meeting the above requirements are often selected  
508 as alternative IS.

509 Based on previous reports, (phenoxy-<sup>13</sup>C<sub>6</sub>)-cis-permethrin was used as IS for PYR, while  
510 (diethyl-D<sub>10</sub>)-chlorpyrifos was used as IS for the remaining pesticides [44]. Naphthalene-d<sub>8</sub>,  
511 benzo[a]anthracene-d<sub>12</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub>, chrysene-d<sub>12</sub>, and benzo[a]pyrene-  
512 d<sub>12</sub> were used as IS for PAHs [45, 46]. However, for PCBs, due to the existence of chlorine  
513 isotopes in PCB compounds, perdeuterated PCBs do not show enough mass shift to overcome  
514 the chlorine isotope patterns, while <sup>13</sup>C labeled PCB congeners are prohibitively expensive [47].  
515 Therefore, PCB congeners (isomers), which are not reported as main contaminants in aquatic  
516 products and can be fully separated from all other PCB congeners in the GC conditions used for  
517 this work, were selected as internal standards for PCBs (e.g PCB 30, PCB 105, and PCB 169).

#### 518 3.4.2 Linearity and Limits of Quantitation

519 Linearity was evaluated by matrix-matched calibration curves, using relative area versus IS  
520 area. Preparation of the spiked samples at different calibration levels was performed as reported  
521 in section 2.1. Calibration levels were set at 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng  
522 g<sup>-1</sup>, while the concentration of the internal standards was 100 ng g<sup>-1</sup>. Limits of quantification  
523 (LOQ) were calculated using the signal-to-noise method. For these calculations, peak-to-peak  
524 noise values around each analyte retention time were measured, and concentrations of a given  
525 analyte that would yield a signal equal to ten times that of the signal-to-noise ratio (10 S/N) were  
526 established as the LOQ value for said analyte. Results obtained for method #1 in DI-SPME mode  
527 for all analytes are shown in Table 1.

528 Generally, the coefficient of determination ( $R^2$ ) of a calibration equation is used to evaluate  
529 linearity of calibration curves. However, the intercept term of the calibration equation might be  
530 influenced by the baseline of the spectrometer and the nature of the reference sample. As extra  
531 terms in an equation will always improve the fit to a straight line, simply determining how well a  
532 straight line is fitted to the data does not always provide reliable information regarding the  
533 linearity of the calibration curve over the entire calibration range studied. Therefore, in addition  
534 to evaluating the coefficient of determination, a lack-of-fit statistical test was also employed in  
535 the present work to confirm linearity for all analytes. The overall lack-of-fit of the calibration  
536 equation, excluding the intercept term, was then compared with the replicate error (analysis of  
537 variance, ANOVA), with effects deemed statistically significant at a 95% confidence level ( $p <$   
538  $0.05$ ). The attained results revealed no significant effects for all analytes, meaning that the  
539 intercept term was not important. Thus,  $R^2$  could be used to evaluate linearity without  
540 consideration given to other interference factors. As can be seen from Table 1, the method  
541 showed good linearity for all analytes in the entire calibration range selected for each analyte,  
542 with  $R^2 > 0.995$ .

543 Excellent LOQs were achieved with the proposed method. For all PCBs, LOQs lower than  
544 or equal to  $13.3 \text{ ng g}^{-1}$  were achieved, while LOQs lower than or equal to  $10.0 \text{ ng g}^{-1}$  were  
545 achieved for all PAHs. LOQs of  $20.0 \text{ ng g}^{-1}$  or lower were achieved for most pesticides, with the  
546 exceptions of LOQs corresponding to more hydrophilic pesticides (such as propoxur and  
547 carbaryl), owing to the low affinity of these compounds toward the fiber coating, as well as to  
548 their impaired GC responses and of LOQs of some highly hydrophobic pesticides, such as  
549 resmethrin, cyfluthrin, cypermethrin, and deltamethrin, among others ( $20.0 < \text{LOQs} \leq 33.0 \text{ ng g}^{-1}$ ).  
550 Despite the toxicity of the above contaminants, which may pose a hazard to both human health  
551 and the environment at high enough quantities, regulation for these contaminants in edible  
552 seaweed is still very limited. While the EU Pesticide Database [48] regulated the maximum  
553 residue levels (MRLs) for some pesticides in seaweeds, no clear limit information was found for  
554 PCBs and PAHs in seaweed matrix. The attained LOQs of the currently discussed method  
555 (method #1) for all pesticides were lower than the MRL set for seaweeds in the EU Pesticide  
556 Database, with the exceptions of carbaryl, resmethrin and flucythrinate, for which the LOQ was  
557 a bit higher than the MRL (Table 1).

558 The analytes studied in the present work belong to three different classes of compounds  
559 encompassing a wide range of polarities, with the majority of studied compounds characterized  
560 by hydrophobicity. In order to evaluate the sensitivity of the DI-SPME method for detection of  
561 these hydrophobic compounds in seaweeds, as well as provide additional information regarding  
562 method selection based on the physiochemical properties of compounds of interest, linearity and  
563 LOQ were also evaluated for the optimized DI-SPME conditions (method #2) targeted at the  
564 analysis of hydrophobic compounds ( $\text{Log } P > 5.2$ ). Results shown in Table 2. Excellent linearities  
565 for all hydrophobic analytes were achieved in their own linear range. Furthermore, LOQs for all  
566 pesticides obtained in this optimized condition were much lower than those attained via method #1  
567 ( $\text{LOQs} \leq 13.3 \text{ ng g}^{-1}$ ), satisfying the LOQ requirements set by EU regulation [48].

568 Since MRLs for PCBs and PAHs in seaweed have yet to be established by regulatory  
569 agencies, the LOQs achieved in the proposed work were compared with recent publications  
570 reported for GC-based residue analyses in seaweeds. To the best of our knowledge, no reports  
571 on the simultaneous detection of all PCBs and PAHs studied in the current work have been  
572 published to date; thus, different papers, focusing on either PCBs or PAHs, were used for  
573 comparison [49, 50] (Table 3). As can be clearly surmised by comparing results in Table 3, the  
574 majority of LOQs for PCBs and PAHs achievable via either one of the two optimized DI-SPME  
575 conditions were similar or lower than those previously reported. Although LOQs for some PAHs,  
576 such as naphthalene and acenaphthylene, were slightly higher than previously reported LOQs, the  
577 attained values are nonetheless still satisfactory, particularly in view of the wide spectrum  
578 analytical capabilities of the method herein discussed.

#### 579 3.4.3 Accuracy and precision

580 The spiking standard mixtures, as well as the spiked seaweed samples used in during steps  
581 were prepared according to the procedure described in section 2.3. Precision was studied by  
582 performing repeatability (intra-day precision) and reproducibility (inter-day precision) studies.  
583 Repeatability was determined via analysis of seaweed samples spiked at four concentrations: 15,  
584 50, 100, and  $500 \text{ ng g}^{-1}$ . Data from five analyses for each concentration level performed in the  
585 same day were used for calculations ( $n=5$ ), with the data expressed as relative standard  
586 deviations (RSD %). For reproducibility measurements, all seaweed samples were spiked at the  
587 same concentration levels as abovementioned and submitted to analysis. Data from three  
588 analyses for each concentration level performed in three nonconsecutive days were calculated,

589 and the reproducibility was expressed as RSD %. As presented in Table 4, good results were  
590 obtained for analytes studied in this work, with attained RSDs for repeatability and  
591 reproducibility lower than 22.3% and 25.5%, respectively.

592 To evaluate the accuracy of present method, the mean relative recovery of the analyte was  
593 assessed by fortifying blank seaweed samples at the four concentration levels above mentioned,  
594 considering both inter- and intra-day measurements. The spiked samples were quantified using  
595 the matrix matched calibration curves. For most analytes, accuracy ranged from 60 to 125%  
596 (Table 4).

597 Since the present work had as central focus the simultaneous analysis of multiresidues  
598 characterized by a wide range of polarities, precision and accuracy for DI-SPME method #2,  
599 which was developed to specifically target hydrophobic analytes, were not investigated in this  
600 work.

#### 601 **4 Analysis of real samples**

602 Five different commercially available edible seaweed sample types, including natural dry  
603 nori and wakame, as well as different flavored seaweed snacks, were bought from a local  
604 supermarket in Waterloo, ON, Canada. The validated DI-SPME-GC-MS (method #1) was used  
605 for analysis of the above real samples. The attained results are shown in Table 5. Analysis  
606 showed that concentrations of the three classes of residues were below the LOQs in four samples.  
607 However, one pesticide (tetramethrin, 8.3 ng g<sup>-1</sup>), one PCB (PCB 18, 16.0 ng g<sup>-1</sup>), and two PAHs  
608 (phenanthrene, 15.5 ng g<sup>-1</sup> and fluoranthene, 5.2 ng g<sup>-1</sup>) were detected in a dry Wakame sample.  
609 As a matter of concern, it should be noted that the above detected compounds, which are widely  
610 employed in household or industrial applications, are well-known to have toxic effects on both  
611 humans and the environment; as such, their presence in edible foodstuffs should be given careful  
612 attention.

#### 613 **5 Conclusion**

614 For the first time, a matrix-compatible PDMS/DVB/PDMS coating was applied to DI-  
615 SPME of multiclass residue analysis in dry seaweeds, enabling the simultaneous quantitation of  
616 41 analytes belonging to three different chemical classes. The validated method yielded good  
617 accuracy, precision, and sensitivity. In view of the satisfactory performance of the method, as

618 well as its green and automated nature, the proposed method can be considered as appropriate for  
619 the detection of pesticides, PCBs, and PAHs in seaweeds. Furthermore, the method was  
620 successfully applied to the determination of multiresidues in commercial dry seaweeds.

621 Although the DI-SPME method developed for wide spectrum coverage of analytes  
622 (method#1) failed to yield a satisfactory enough performance in the detection of some highly  
623 hydrophobic pesticides (e.g., cyfluthrin, cypermethrin, etc.), better results were achieved via the  
624 optimized condition focused on hydrophobic analytes with  $\text{LogP} > 5.2$ . Therefore, using this work  
625 as reference, analysts can select the appropriate DI-SPME conditions for a given application  
626 based on the physiochemical characteristics of the compounds of interest, as well as the  
627 regulated requirements of detection of said analyte(s). A limitation of the currently presented  
628 method pertains to the relatively poor LOQs obtained for hydrophilic pesticides (e.g., carbaryl  
629 and propoxur). In this regard, further investigations will be carried out in the future, particularly  
630 in view of the development of alternative coating chemistries with enhanced affinity toward  
631 polar analytes.

632 The currently presented method overcomes most of the common challenges associated with  
633 dry sample analysis. Compared with previous reports, which have mainly employed liquid-liquid  
634 extraction, the proposed SPME method integrates sampling, extraction, concentration, and  
635 sample introduction into a single, low-solvent consuming and automatable step, presenting a  
636 much simpler and greener approach to analysis. Moreover, by using the matrix-compatible  
637 PDMS/DVB/PDMS coating and thoroughly optimizing the DI-SPME conditions, higher  
638 sensitivity and better representativeness of analytes were achieved, affording a especially  
639 suitable method for the analysis of hydrophobic compounds in dry seaweed matrix. The current  
640 work represents a first report of a DI-SPME method utilizing matrix-compatible fibers for  
641 simultaneous multiclass and multiresidue analysis of seaweeds.

642

### 643 **Acknowledgements**

644 This work was supported by the Natural Sciences and Engineering Research Council of  
645 Canada. L.Z. thanks the Chinese Scholarship Council for her scholarship. V.A.J. thanks FAPESP,  
646 process 2016/16180-6 for his scholarship. The authors would also like to express their gratitude

647 to Dominika Sylwia Gruszecka, Mohammad Maududul Huq, and Prof. Zengyuan Niu for their  
648 kind assistance.

649

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789

## 790 **Figure Captions**

791

792 Fig.1 Extracted amount of (a) pesticides, (b) PAHs and (c) PCBs from spiked samples,  
793 containing 0.02% of NaN<sub>3</sub>, held in the autosampler rack at r.t. at different waiting times prior  
794 SPME extraction.

795 Fig. 2 Comparison of DI-SPME results for extractions carried out under the optimal rinsing  
796 times for each rinsing solution.

797 Fig. 3 Extraction time profiles of a) PAHs, b) PCBs and c) pesticides for DI-SPME method  
798 carried out under optimized conditions for simultaneous analysis of all targeted compounds

799 Fig. 4 Chromatographic profiles showing the variations in extraction amounts of cyfluthrin and  
800 cypermethrin for extractions carried out at different temperatures

801 Fig. 5 Extraction time profile of a) PAHs, b) PCBs and c) pesticides, obtained under optimized  
802 conditions for highly hydrophobic compounds

Table 1. Linearity,  $R^2$ , and LOQs for all targets extracted under optimized DI-SPME conditions for simultaneous analysis of all analytes under study

Compound	Linear range/ng g <sup>-1</sup>	$R^2$	LOQ/ ng g <sup>-1</sup>	MRL <sup>a</sup> / ng g <sup>-1</sup>
2-phenylphenol	20-2000	0.997	16.7	50
Propoxur	50-2000	0.994	26.2	
Chlorpyrifos-methyl	1-2000	0.996	1.0	
Carbaryl	20-2000	0.995	29.2	10
Chlorpyrifos-ethyl	5-2000	0.995	5.0	
Piperonyl butoxide	2-2000	0.998	1.4	
Resmethrin	50-2000	0.997	25.4	10*
Tetramethrin	10-2000	0.995	6.2	
Lamda-cyhalothrin	20-2000	0.994	13.3	20*
Permethrin	10-2000	1.000	8.5	50*
Cyfluthrin	50-2000	0.993	30.0	20*
Cypermethrin	50-2000	0.995	30.0	50*
Flucythrinate	20-2000	0.994	20.0	10*
Fenvalerate	20-2000	0.998	13.4	20*
Deltamethrin	50-2000	0.993	33.0	50 <sup>W</sup>
PCB 18	10-2000	0.997	10.0	
PCB 28	5-2000	0.999	5.0	
PCB 52	5-2000	0.999	4.0	
PCB 44	2-2000	0.999	1.7	
PCB 101	5-2000	0.999	5.0	
PCB 149	5-2000	0.993	5.0	
PCB 118	5-2000	0.999	2.5	
PCB 153	10-2000	0.995	7.8	
PCB 138	5-2000	0.995	5.0	
PCB 180	10-2000	0.998	6.0	
PCB 170	10-2000	0.998	6.0	

Table 1 (Continued)

Compound	Linear range/ng g <sup>-1</sup>	R <sup>2</sup>	LOQ/ ng g <sup>-1</sup>	MRL <sup>a</sup> / ng g <sup>-1</sup>
Naphthalene	10-2000	0.999	10.0	
Acenaphthylene	10-2000	0.999	7.8	
Acenaphthene	20-2000	1.000	13.3	
Fluorene	10-2000	1.000	10.0	
Phenanthrene	5-2000	0.998	3.4	
Anthracene	5-1000	0.999	2.6	
Fluoranthene	2-2000	1.000	2.0	
Pyrene	5-2000	0.999	2.2	
Benzo(a)anthracene	2-2000	1.000	1.8	
Chrysene	5-2000	1.000	3.3	
Benzo(b)fluoranthene	1-2000	1.000	1.0	
Benzo(k)fluoranthene	1-2000	1.000	1.0	
Benzo(a)pyrene	1-2000	1.000	1.0	
Indeno(1,2,3-cd)pyrene	1-2000	0.999	1.0	
Dibenz(ah)anthrene	1-2000	0.999	1.0	
Benzo(ghi)perylene	1-2000	0.997	1.0	

Note: “\*”- sum of all isomers; “ψ”- sum of cis-isomers

“a”- EU Pesticide Database. <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN>

Table 2. Linearity,  $R^2$ , and LOQs for hydrophobic compounds with  $\log P > 5.2$ 

Compound	Linear range/ng g <sup>-1</sup>	$R^2$	LOQ/ ng g <sup>-1</sup>
Benzo(a)anthracene	1-2000	0.999	0.7
Chrysene	1-2000	0.999	1.0
Benzo(b)fluoranthene	1-2000	0.999	0.4
Benzo(k)fluoranthene	1-2000	0.998	0.2
Benzo(a)pyrene	1-2000	0.999	0.6
Indeno(1,2,3-cd)pyrene	1-2000	0.999	0.7
Dibenz(ah)anthrene	1-2000	0.995	0.5
Benzo(ghi)perylene	1-2000	0.999	0.6
PCB 18	1-2000	0.999	0.4
PCB 28	2-2000	1.000	1.6
PCB 52	1-2000	1.000	0.8
PCB 44	1-2000	1.000	1.0
PCB 101	2-2000	1.000	1.5
PCB 149	1-2000	0.999	0.6
PCB 118	1-2000	1.000	0.8
PCB 153	1-2000	1.000	0.9
PCB 138	1-2000	0.999	0.8
PCB 180	1-2000	0.999	1.0
PCB 170	1-2000	0.999	0.8
Resmethrin	5-2000	0.998	4.0
Lamda-cyhalothrin	10-2000	0.998	6.0
Permethrin	5-2000	0.999	4.6
Cyfluthrin	20-2000	0.997	19.2
Cypermethrin	20-2000	0.998	13.3
Flucythrinate	10-2000	0.999	7.8
Fenvalerate	5-2000	0.999	4.0
Deltamethrin	10-2000	0.998	5.4

Table 3. Comparison of LOQs obtained in present work versus previously reported LOQs for PAHs and PCBs

Compound	LOQ/ ng g <sup>-1</sup>			Compound	LOQ/ ng g <sup>-1</sup>		
	Present work (a)	Present work (b)	Paper [37]		Present work (a)	Present work (b)	Paper [36]
Naphthalene	10.0		0.6	PCB 18	10.0	1.4	
Aceaphthylene	7.8		0.1	PCB 28	5.0	1.6	8
Acenaphthene	13.3		0.5	PCB 52	4.0	0.8	6.3
Fluorene	10.0		0.2	PCB 44	1.7	1.0	
Phenanthrene	3.4		0.4	PCB 101	5.4	1.5	5.7
Anthracene	2.6		0.5	PCB 149	5.0	0.6	
Fluoranthene	2.0		0.4	PCB 118	2.5	0.8	0.62
Pyrene	2.2		0.6	PCB 153	7.8	0.9	10
Benzo (a) anthracene	1.8	0.7	0.9	PCB 138	5.0	0.8	
Chrysene	3.3	1.0	0.7	PCB 180	6.0	1.0	6.9
Benzo (b) fluoranthene	1.0	0.4	1.0	PCB 170	6.0	0.8	
Benzo (k) fluoranthene	1.0	0.2	0.5				
Benzo (a) pyrene	1.0	0.6	0.8				
Indeno (1,2,3-cd)pyrene	1.0	0.7	0.2				
Dibenz (ah) anthracene	1.0	0.5	0.8				
Benzo (ghi) perylene	1.0	0.6	0.7				

Note: "a" means optimized DI-SPME conditions for simultaneous extraction of all targeted analytes;

"b" means optimized DI-SPME conditions for hydrophobic analytes with logP>5.

Table 4. Precision and accuracy of the proposed method

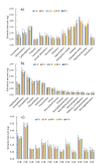
Compound	Intra-day accuracy (%)				Intra-day precision (RSD %)				Inter-day accuracy (%)				Inter-day precision (RSD %)			
	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>
2-phenylphenol	/	113.7	105.5	96.4	/	5.8	7.2	13.5	/	113.5	101.4	88.9	/	8.8	14.4	2.2
Propoxur	/	44.8	77.2	102.4	/	17.0	3.7	2.7	/	52.9	92.5	122.7	/	14.1	1.8	3.3
Chlorpyrifos-methyl	63.3	119.8	87.2	95.6	22.3	12.0	7.1	7.2	63.7	92.9	79.4	128.0	20.2	19.5	16.4	7.4
Carbaryl	/	107.9	113.7	93.7	/	13.5	8.6	6.9	/	96.7	96.5	96.4	/	13.2	16.4	10.9
Chlorpyrifos-ethyl	105.0	143.3	75.2	95.6	2.7	20.8	6.7	7.2	137.9	95.9	100.0	95.6	21.7	17.0	8.9	6.4
Piperonyl butoxide	117.5	53.4	95.0	109.1	20.0	14.5	1.0	7.0	140.8	76.2	86.5	97.8	16.7	21.7	18.2	5.6
Resmethrin	/	97.3	98.7	93.1	/	16.1	6.6	6.9	/	74.8	102.9	95.7	/	3.2	1.1	1.7
Tetramethrin	83.2	101.6	109.6	125.5	11.9	8.8	3.7	10.2	91.9	88.1	107.9	99.7	11.9	24.7	4.3	17.0
Lamda-cyhalothrin	107.8	103.1	96.3	103.0	8.6	3.2	4.0	4.7	100.9	106.0	92.6	100.1	12.5	12.5	9.3	2.3
Permethrin	78.0	101.7	101.2	103.1	15.8	4.5	4.6	4.6	79.2	100.9	99.2	99.9	16.9	9.0	6.4	1.2
Cyfluthrin	/	87.9	103.1	113.5	/	10.2	8.5	8.8	/	93.3	101.2	100.0	/	14.1	7.8	5.7
Cypermethrin	/	100.4	110.3	114.2	/	10.8	8.2	11.3	/	110.3	97.0	106.3	/	21.2	18.1	2.1
Flucythrinate	/	106.9	106.9	109.8	/	6.0	6.6	3.7	/	96.1	102.0	99.9	/	15.9	8.9	5.6
Fenvalerate	87.4	109.7	112.6	110.6	16.9	13.9	11.5	3.8	84.6	99.2	100.8	99.9	22.8	12.3	9.5	6.2
Deltamethrin	/	96.5	95.2	112.3	/	14.3	8.0	4.6	/	91.4	84.9	100.8	/	15.2	15.9	5.6
PCB 18	106.0	102.6	98.2	88.1	6.2	4.9	1.0	1.6	99.8	107.1	98.3	100.0	11.6	8.6	3.3	7.7
PCB 28	79.7	99.8	93.6	83.2	14.6	1.8	2.9	3.1	77.5	104.7	93.3	100.2	20.6	14.1	6.2	11.0
PCB 52	75.1	102.4	98.2	93.6	6.7	2.9	1.7	3.3	72.4	105.4	100.2	99.9	7.3	14.4	4.9	5.5
PCB 44	64.2	103.6	100.4	97.4	12.3	2.1	2.1	4.2	65.4	104.1	102.2	99.8	13.4	12.7	4.6	4.3
PCB 101	103.0	99.6	89.2	84.4	7.2	4.7	5.9	5.2	91.2	97.3	88.7	100.7	25.5	7.2	5.4	11.3
PCB 149	71.0	101.0	114.6	106.2	6.1	7.9	4.3	4.5	61.2	97.1	113.4	99.5	20.2	13.7	5.7	5.8
PCB 118	106.8	101.3	90.5	82.5	10.4	0.9	5.6	5.2	96.3	110.9	91.5	100.2	16.1	17.6	8.8	11.9
PCB 153	73.1	104.0	108.9	97.1	8.9	3.5	3.2	2.8	70.5	99.3	108.1	99.7	17.8	12.8	4.9	3.5

Table 4 (Continued)

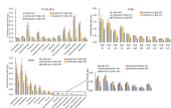
Compound	Intra-day accuracy (%)				Intra-day precision (RSD %)				Inter-day accuracy (%)				Inter-day precision (RSD %)			
	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>	15 ng g <sup>-1</sup>	50 ng g <sup>-1</sup>	100 ng g <sup>-1</sup>	500 ng g <sup>-1</sup>
PCB 138	65.2	104.0	110.3	100.4	4.9	5.8	3.2	3.0	61.7	100.0	109.6	99.6	18.7	15.2	4.9	3.1
PCB 180	75.0	98.6	99.3	86.4	11.7	2.1	0.7	2.2	74.0	104.2	99.3	100.0	10.4	20.5	4.7	8.9
PCB 170	87.0	103.8	100.6	90.3	10.7	0.5	0.6	1.9	80.5	106.2	100.6	99.9	21.1	13.4	3.4	6.5
Naphthalene	85.6	103.5	89.8	99.7	21.8	5.3	8.3	10.1	86.1	108.5	88.2	102.6	16.7	9.8	12.8	9.9
Acenaphthylene	110.9	111.4	91.7	101.4	4.3	0.7	0.9	1.1	109.9	116.7	97.7	106.1	5.3	14.0	9.4	5.0
Acenaphthene	87.6	105.6	95.8	100.4	5.6	1.8	1.4	3.9	87.3	101.9	96.2	104.8	18.5	8.0	5.3	6.7
Fluorene	88.9	104.6	97.7	100.0	3.2	4.6	2.2	0.8	89.1	109.3	98.8	102.0	7.6	15.8	4.1	9.2
Phenanthrene	93.4	105.5	97.0	100.9	2.4	3.7	1.1	0.7	94.1	102.9	98.6	106.5	20.5	15.3	1.1	3.5
Anthracene	96.5	102.9	97.9	100.7	5.8	1.4	1.8	0.9	96.7	103.7	100.0	105.4	7.5	13.7	2.3	3.9
Fluoranthene	99.7	109.7	94.0	101.9	2.8	1.1	3.7	3.7	98.9	109.2	96.8	105.9	13.5	18.8	3.4	4.4
Pyrene	94.8	109.3	94.4	101.6	3.4	1.7	4.0	4.0	95.0	110.0	97.1	105.8	6.7	14.6	3.7	5.4
Benzo(a)anthracene	95.9	108.8	94.1	100.3	6.4	2.6	1.2	0.7	96.2	107.6	96.0	103.1	7.1	10.9	3.7	1.6
Chrysene	104.8	110.7	92.7	99.8	2.7	2.4	1.6	1.9	104.8	110.8	95.4	103.3	2.7	12.8	3.5	2.7
Benzo(b)fluoranthene	80.6	114.5	91.4	100.7	4.0	2.4	1.3	1.4	80.6	118.1	94.1	104.2	6.7	18.5	5.5	3.4
Benzo(k)fluoranthene	69.4	109.3	96.1	100.5	6.7	5.9	1.8	0.9	70.1	116.6	98.0	103.2	5.1	17.3	3.6	5.0
Benzo(a)pyrene	79.1	110.6	94.0	100.3	4.4	0.9	1.3	1.0	77.6	116.9	96.4	103.1	12.2	17.6	1.4	1.6
Indeno(1,2,3-cd)pyrene	58.6	113.2	92.9	101.6	14.0	3.0	1.0	3.4	58.1	123.7	96.9	103.8	20.4	20.1	2.8	4.3
Dibenz(ah)anthrene	58.8	110.1	97.2	101.5	14.4	3.4	3.7	2.4	57.9	118.2	100.0	105.0	23.1	20.0	2.9	6.8
Benzo(ghi)perylene	88.0	122.6	84.7	102.5	11.7	1.0	1.2	1.7	86.6	113.7	90.0	105.2	18.4	20.4	10.5	5.9

Table 5. Detection results for commercial edible seaweed samples

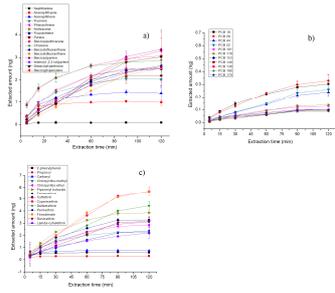
Compound	Detection amount (ng g <sup>-1</sup> )				
	Dry Nori-W	Dry Wakame-Guanqun	Dry Wakame-WE1PAC	Seaweed snack-Paido	Seaweed snack-Paido
PCB 18	<LOQ	<LOQ	16.0	<LOQ	<LOQ
PCB 52	n.d	<LOQ	<LOQ	<LOQ	<LOQ
PCB 44	n.d	<LOQ	n.d	n.d	n.d
PCB 149	n.d	<LOQ	n.d	n.d	n.d
Naphthalene	n.d	n.d	n.d	<LOQ	n.d
Acenaphthene	<LOQ	<LOQ	<LOQ	n.d	n.d
Fluorene	n.d	n.d	<LOQ	n.d	n.d
Phenanthrene	n.d	<LOQ	15.5	n.d	n.d
Anthracene	<LOQ	<LOQ	<LOQ	n.d	n.d
Fluoranthene	n.d	n.d	5.2	n.d	n.d
Pyrene	<LOQ	<LOQ	n.d	n.d	n.d
Chrysene	n.d	<LOQ	<LOQ	n.d	n.d
2-phenylphenol	n.d	n.d	n.d	<LOQ	<LOQ
Chlorpyrifos-methyl	n.d	<LOQ	<LOQ	n.d	n.d
Resmethrin	n.d	<LOQ	n.d	n.d	n.d
Tetramethrin	n.d	n.d	8.3	n.d	n.d
Permethrin	<LOQ	n.d	<LOQ	n.d	n.d
Cyfluthrin	n.d	n.d	<LOQ	n.d	n.d
Fenvalerate	n.d	n.d	n.d	n.d	n.d



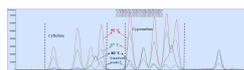
ACCEPTED MANUSCRIPT



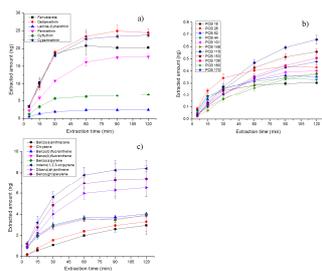
ACCEPTED MANUSCRIPT



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