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Detection of Cylindrospermopsin and its decomposition products in raw and cooked fish (*Oreochromis niloticus*) by analytical pyrolysis (Py-GC/MS)

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*Highlights (3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

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Highlights

- Analytical pyrolysis has potential for CYN detection in fish samples
- CYN decomposition products (*m/z* 290.1, 169.1 and 336.2) can be detected
- Cooking modified the abundance of CYN and its degradation products in fish
- Boiling decreased the relative abundance of CYN in fish

Abstract

The presence of the toxin cylindrospermopsin is increasingly frequent in samples from different ecosystems and it is a serious problem both at environmental level and for animal and human health. To be able to prevent CYN exposure risk, it is important to have suitable analytical methods, but also quick and economical ones. Analytical pyrolysis coupled to GC/MS (Py-GC/MS) represents an important alternative for the rapid detection, characterization or "fingerprinting" of different materials. However, it has been less studied with cyanotoxins up to date. The present work aims to investigate: 1) the suitability of Py-GC/MS for detection of CYN and its decomposition products in raw and cooked fish samples before consumption and 2) the influence of the different cooking methods on the presence of different CYN degradation products detected by Py-GC/MS. For first time, these results present that Py-GC/MS could be a rapid and economical alternative for the detection and monitoring of CYN and its degradation products (DP. *m/z* 290.1, 169.1 and 336.2) in raw or cooked fish. Moreover, the changes induced in CYN and DP by cooking could be amenable and detected by Py-GC/MS.

Keywords: analytical pyrolysis, cylindrospermopsin, decomposition products, raw fish, cooked fish.

1. Introduction

The presence of cyanobacterial blooms is increasingly frequent in samples from different ecosystems (Sotton et al., 2015). Many of these cyanobacterial species are spread worldwide and they are capable of producing toxins such as cylindrospermopsin (CYN) (*Aphanizomenon*, *Anabaena*, *Umezakia*, *Lyngbya* and *Rhaphidiopsis*) (Kokociński et al., 2017; Pichardo et al., 2017). CYN is a tricyclic alkaloid of 415 Da that possesses a tricyclic guanidine moiety combined with hydroxymethyluracil (Chiswell et al., 1999; Sotton et al., 2015). CYN has demonstrated to be stable to a range of light intensities, in the dark and temperatures and pH under laboratory conditions (van Apeldoorn et al., 2007). Moreover, this toxin presents highly water solublility due to its zwitterionic nature (Shaw et al., 2000) and it is mainly produced (90% of the total) as extracellular toxin. All these characteristics increase the possibility of exposure to CYN, mainly in aquatic animals.

In 1979 a serious incident associated with CYN in Palm Island (Australia) demonstrated for the first time the harmful effects of this toxin in humans. People exposed to CYN developed symptoms such as headache, fever, abdominal pain, hepatomegaly, anorexia, vomiting, dehydration and initial constipation followed by bloody diarrhea (Hawkins et al., 1985; Griffiths and Saker, 2003). There are different ways of exposure to the toxin such as the intake of contaminated drinking water or food (especially fish) and the practice of recreational water activities (Gutiérrez-Praena et al., 2013). CYN has been classified as a cytotoxin and, although its main target is the liver, other organs such as kidneys, heart, gastrointestinal tract, lungs, adrenal gland, thymus, marrow bone, immune and nervous system have been reported as potential targets as well (Falconer et al., 1999; Hawkins et al., 1985; Hinojosa et al., 2019a,b; Humpage et al., 2000; Smith et al., 2008; Terao et al., 1994). The main mechanism of action of CYN is the inhibition of protein and GSH synthesis (Froscio et al., 2003; Runnegar et al.,

1995; Terao et al., 1994). However, different studies have demonstrated that this cyanotoxin also produces oxidative stress (Gutiérrez-Praena et al., 2011, 2012; Guzmán-Guillén et al., 2013; Puerto et al., 2011) resulting in cell death processes (apoptosis) or DNA damage and presents pro-genotoxic properties (Humpage et al., 2005; Žegura et al., 2011; Puerto et al., 2018).

At present, different analytical methods such as immunological assays and different chromatographic separation methods using different detectors (LC-DAD, LC-MS, LC-MS/MS) have been developed for the detection and quantification of cyanotoxins in different matrices such as water (Bogialli et al., 2006; Eaglesham et al., 1999; Guzmán-Guillén et al., 2012a), lyophilized cyanobacterial cells (Cameán et al., 2004; Guzmán-Guillén et al., 2012b), vegetables (Díez-Quijada et al., 2018; Prieto et al., 2011, 2018) and fish (Gallo et al., 2009; Guzmán-Guillén et al., 2015). Most of these methods require a previous phase of toxin extraction from the matrix, such as food, to purify the analyte and improve the yield, although it implies the use of chemicals and it is time-consuming.

Analytical pyrolysis (Py) has the ability to characterize a material or a chemical process in an inert atmosphere, by a chemical degradation induced by thermal energy (Uden, 1993). It deals with the structural identification and quantitation of pyrolysis products to identify the original material and the mechanisms of its thermal decomposition (Kusch, 2018). One important advantage is that it requires minimum sample handling steps, very small sample amounts and it eliminates pretreatment by performing analyses directly on the sample, thus reducing the possible losses of analytes, length of analysis and the manpower in the laboratory (Kusch, 2018). Because of this, analytical pyrolysis coupled to GC/MS (Py-GC/MS) represents an important alternative for the rapid detection, characterization or “fingerprinting” of different

materials such as synthetic organic polymers and copolymers, biopolymers including polylactic acid, natural resins, bio-films, bacteria, algal mats, additives and veterinary and human pharmaceuticals (de Oliveira et al., 2011; Kusch, 2018; Llana-Ruíz-Cabello et al., 2016a,b, 2017; Melucci et al., 2013; Tian et al., 2017; Voorhees et al., 1997). Although this destructive technique has been thoroughly used from the 90's for a wide variety of compounds, it has been less studied with cyanotoxins, such as microcystins (MCs) (Cameán et al., 2005) or CYN (Ríos et al., 2014). The latter successfully demonstrated the presence of specific molecular fragments (*m/z* 194 and 336) related to the molecular structure of CYN in cultures of the CYN-producer *Aphanizomenon ovalisporum*.

Fish can be frequently exposed to this toxin in the environment, its accumulation in different organs of tilapia has been reported (Buratti et al., 2017; Mohamed and Bakr, 2018), and they can be consumed either raw or cooked; cooking processes could vary its composition and generate decomposition products (DP) (Domingo et al., 2011). Recently, our research group has shown changes in the concentration of CYN and the formation of their DP in contaminated fish muscle subjected to different cooking techniques such as microwaving, broiling, boiling or steaming by UPLC-MS/MS (Guzmán-Guillén et al., 2017; Prieto et al., 2017). Taking this into account, the aim of the present work was to investigate the suitability of Py-GC/MS as a simple, fast and economical analytical technique for CYN and its DP detections in raw and cooked fish samples before consumption.

2. Experimental

2.1 Chemicals

Pure cylindrospermopsin standard (purity ≥ 95%) was supplied by Enzo Life Sciences, Inc. (Lausen, Switzerland) and standard solutions (100 µg/mL) were prepared in Milli-Q water for the following experiment. Milli-Q water (18 MΩ.cm resistivity) was obtained from a Milli-Q water purification system (NANOpure Diamond^{TM}, Millipore, Bedford, USA).

2.2 Experimental setup

Tilapia fish (*Oreochromis niloticus*) were supplied by Valenciana de Acuicultura (fish hatchery, Valencia, Spain) and transferred to the laboratory for acclimation for 15 days in two 96-L of tap-water aquaria (8 individuals/aquarium), with constant temperature (21 ± 2°C), being fed daily (0.3 g/day) with commercial fish food only (Dibaq S.L., Segovia, Spain). After acclimation, they were sacrificed, dissected and the muscles divided into approximately 4 g (4±0.2) portions. One CYN-spiked muscle sample per condition: positive control C+ or raw, microwaving, broiling, boiling and steaming was assayed. Therefore, 5 samples were spiked with 500 µL of a pure CYN stock solution (100 µg CYN/L, equivalent to 50 ng CYN/g dry weight -d.w.-), by injection of the toxin directly into the muscle. Moreover, one raw muscle sample without toxin was selected as negative control (C-). CYN concentration was selected taking into account naturally environmental data in aquatic organisms reported by other studies (Freitas et al., 2016; Gutiérrez-Praena et al., 2013; Guzmán-Guillén et al., 2017; Prieto et al., 2017).

2.3 Cooking of fish samples

Four cooking methods were applied to the fish fillets: microwaving, broiling, boiling and steaming, for 2 min (Guzmán-Guillén et al., 2017; Prieto et al., 2017). A conventional household microwave oven (Samsung M17-13, 300W, 2450 MHz) was used for microwaving, and for broiling, samples were cooked in Teflon pans for both sides of the fillet. The fish muscle was placed in cool water into a pot or onto a food steamer, for the boiling and steaming methods, respectively, and cooked for 2 min when water began to boil. All samples were frozen at -80 ºC and lyophilized (Cryodos 80 model, Telstar, Tarrasa, Spain) before pyrolysis.

2.4 Analytical pyrolysis

The Py-GC/MS was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i, Fukushima, Japan) attached to a GC system (Agilent Technologies, Palo Alto, CA, USA, model 6890N). The muscle samples (approximately 2 mg d.w. for the negative control and the cooked samples, and 3 mg d.w. for the positive control –raw muscle-) were placed in crucible deactivated steel pyrolysis capsules and introduced into a preheated micro-furnace at (350°C) for 1 min. The volatile pyrolysates were then directly injected into the GC/MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5%-phenyl-methylpolysiloxane) capillary column (Agilent J&W HP-5ms Ultra Inert, of 30 m × 250 μm × 0.25 μm film thickness). The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 30 °C min^{-1}, from 100 °C to 300 °C at 10 °C min^{-1}, and stabilized at 300 °C for 10 min, with a total analysis time of 32 min. The carrier gas was He at a controlled flow of 1 mL min^{-1}. The detector consisted of a mass selective detector (Agilent Technologies, Palo Alto, CA. USA, model 5973N) and mass spectra were acquired at 70 eV ionizing energy. Compound assignment was achieved by single-

ion monitoring (SIM) for the major homologous series and by comparison with published data reported in the literature or stored in digital NIST 14 (Maryland, USA) and Wiley 7 (Weinheim, Germany) libraries. In a first step, the pyrolysis conditions were optimized by producing a detailed pyrolysis fingerprint of raw fish muscle without CYN and by studying the effect of the pyrolysis temperature from 150 to 550 ºC in 100 ºC-increments by both 1) applying each temperature to a different sample (single-shot) or 2) sequentially applying each temperature to the same sample (multi-shot). In a second phase, the ability of pyrolysis to detect CYN and its DP was tested in single-shot mode at 250 and 350 ºC in raw samples spiked with CYN. Finally, in a third step, after stablishing the optimum pyrolysis temperature, the effect of the different cooking methods in the fish muscle pyrolysates with CYN was studied by single-shot pyrolysis.

3. Results and Discussion

3.1 Optimization of pyrolysis conditions

In the first approach, the pyrolysis of raw fish muscle without toxin served for obtaining the negative control to which the different positive samples were compared, and for determining the optimal analytical pyrolysis temperature. Temperature ranges of 150-550 °C in single-shot and multi-shot on a raw fish was tested (Fig. 1 A & B). Almost all fatty acids were thermally desorbed from the fish muscle at sub-pyrolysis temperatures (<350 ºC), whereas the N-protein derived-compounds only appeared in the pyrograms obtained at pyrolysis temperature (≥350 ºC), probably when the energy to disrupt amide bonds in polypeptide structures is reached. Moreover, more complete pyrograms were obtained when using single-shot mode (Fig. 1 A). Therefore, a temperature of 350 °C (1 min) in single-shot was established as optimal pyrolysis conditions.

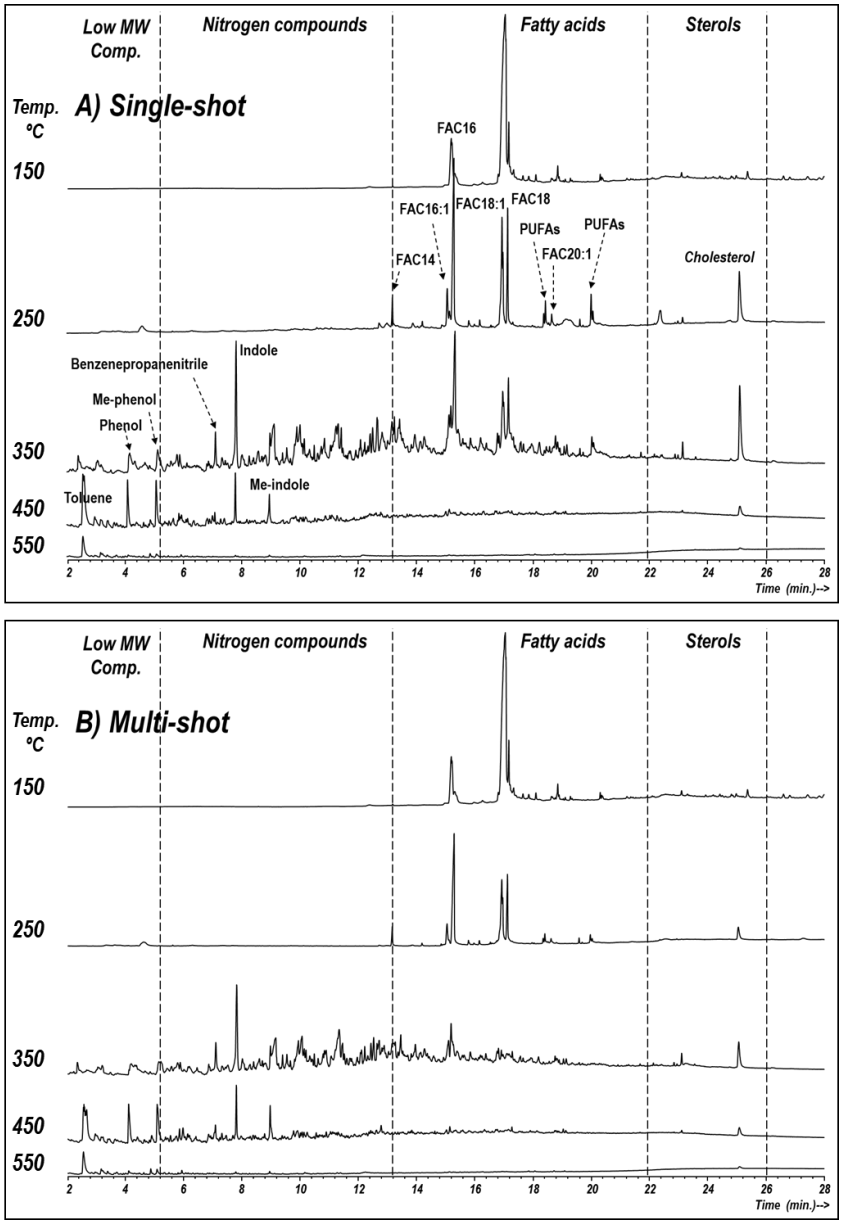
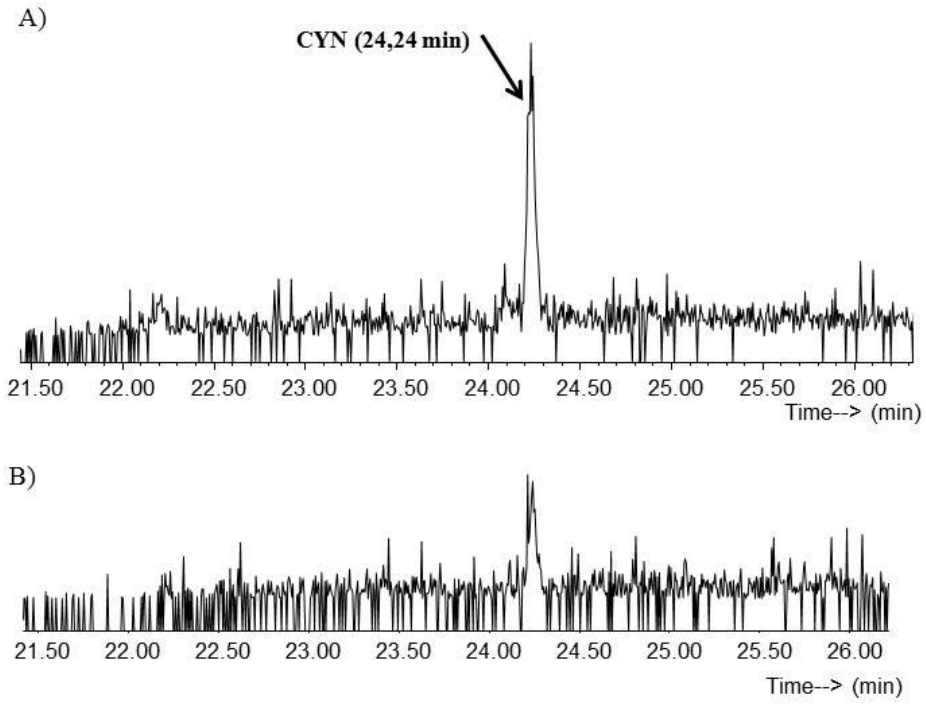


Fig. 1: Analytical pyrolysis of tilapia muscle. Total current ion chromatograms (TIC) performed at (A) single-shot mode i.e. different sample at different temperatures and (B) multi-shot mode i.e. same sample at increasing temperatures. Chromatograms are divided in sections of preferential compound classes and major compounds detected are labeled on the corresponding peaks. FA: fatty acid; PUFA: polyunsaturated fatty acid; Me: methyl.

188 3.2 Pyrolytic products in raw fish muscle spiked with CYN

189 For a more accurate optimization of the process in the matrix spiked with CYN, two
190 different temperatures (350 ºC and 250 ºC) were tested in single-shot. Fig. 2 shows the
191 single current ion chromatograms for ion at *m/z* 416.00 (415.70 to 416.70) of a positive
192 control (50 ng CYN/g d.w.) of raw fish.



193

194 Fig. 2: Single current ion chromatograms (SIN) for ion 416.00 (415.70 to 416.70) of a positive
195 control (50 ng CYN/g d.w.) of raw fish (*Oreochromis niloticus*) (A) at 350 ºC and (B) at 250 ºC.

196 Taking into account the higher resolution of the peak for CYN at 350 ºC (where
197 nitrogen compounds as CYN appear), compared to the desorption temperature of 250
198 ºC, together with the previous preliminary results in negative samples, an optimum
199 pyrolysis temperature of 350 ºC (1 min) was selected to continue with the experiment.
200 Based on known DP and potential degradation compounds related to CYN, the ions
201 presented in Table 1 were searched in the CYN-spiked muscle sample (C+) compared to
202 the negative one (C-).

203

Table 1: Possible decomposition products and potential degradation compounds related to CYN structure detected in C- and C+ by pyrolysis. D: detected; ND: no detected.

Molecular weight	**C-**	**C+**	**Molecular weight**	**C-**	**C+**
434.1	ND	ND	214.1	ND	ND
416.1	**ND**	**D**	212.1	D	D
414.1	ND	ND	210.1	ND	ND
338.2	ND	ND	194.1	D	D
336.2	**ND**	**D**	192.1	ND	ND
334.1	ND	ND	178.1	ND	ND
318.2	ND	ND	176.1	ND	ND
292.1	ND	ND	**169.1**	**ND**	**D**
290.1	**ND**	**D**	151.1	ND	ND
274.1	ND	ND	142.1	ND	ND
272.1	ND	ND	137.1	ND	ND
249.1	ND	ND	110.1	ND	ND

Only the fragments detected in C+ but not in C- were selected and taken into account as distinctive. In this sense, CYN (*m/z* 416.1, 24.24 min) and three of its DP were detected in the samples, at *m/z* 290.1 (15.92 min), *m/z* 169.1 (22.45 min) and *m/z* 336.2 (25.25 min) were characteristic of the positive samples. Table 2 presents their retention times, putative chemical formulae and the specific fragmentation ions for each one of them.

213 Table 2. CYN decomposition products (DP) and its fragmentation ions detected by direct
214 (single-shot) pyrolysis (350 ºC) in uncooked and cooked muscle of tilapia fish (*Oreochromis*
215 *niloticus*).

Decomposition products	Retention Time (min)	Putative Chemical formula	$[M+H]^+$ ion *m/z*	Fragmentation ions
CYN	24.24	$C_{15}H_{21}N_5O_7S$	416.1	58.2, 99.1, 130.1, 151.2, 176.1, 192.1, 281.1, 416.1
DP-1	15.92	$C_{10}H_{15}N_3O_5S$	290.1	56.2, 72.2, 91.1, 97.1, 159.1, 187.1, 200.2, 207.2, 221.2, 230.2, 290.1
DP-2	22.45	$C_9H_{20}N_2O$	169.1	83.2, 91.1, 117.1, 169.1, 199.2, 276.1
DP-3	25.25	$C_{15}H_{21}N_5O_4$	336.2	70.2, 130.1, 170.1, 208.1, 267.1, 299.1, 336.3, 390.0, 440.3

216

217 Cylindrospermopsin (*m/z* 416.1) was detected in the positive fish muscle at 24.24 min
218 and was not identified in the negative control muscle, as the fragmentation ions
219 obtained from a peak in the negative muscle at the same retention time did not agree
220 with those from the positive sample (Fig. 3, Table 2). Previously, Ríos et al. (2014)
221 were able to detect CYN presence in cyanobacterial algal blooms using analytical
222 pyrolysis and thermochemolysis. A diagnostic fragment (*m/z* 194) was selected by
223 analytical pyrolysis at 500 ºC by the visible peaks at 25.0 and 28.9 min only in the pure
224 CYN and CYN+ culture samples, but these authors did not detect CYN (*m/z* 416.1).
225 However, in our experiments, the fragment at *m/z* 194 was not selected as characteristic
226 as no differences were found between the spiked and non-spiked samples (Table 1).
227 These discrepancies could be due to the different matrices and temperatures employed.

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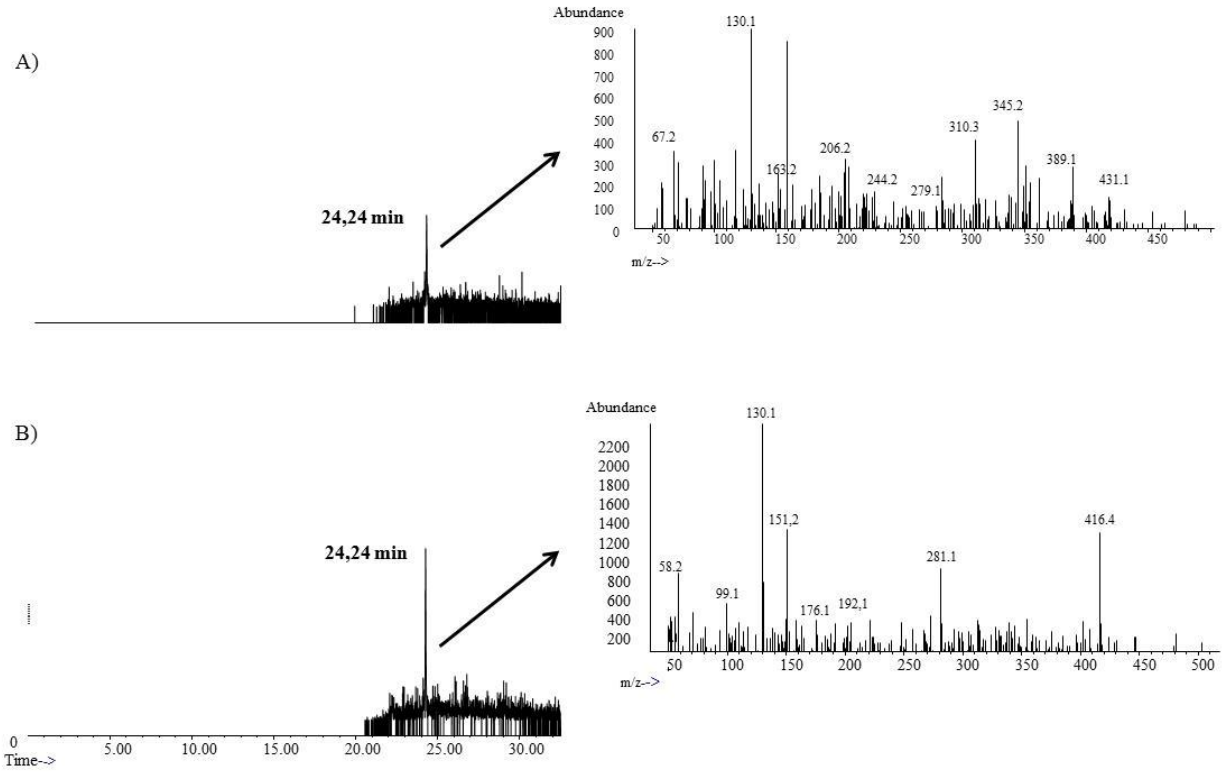


Fig. 3: Single current ion chromatograms (SIN) for ion 416.00 (415.70 to 416.70) from the direct pyrolysis (350 ºC) of (A) a negative control and (B) a positive control (50 ng CYN/g d.w.) of raw fish (*Oreochromis niloticus*).

Regarding the ion at *m/z* 290.1, Adamski et al. (2016a) detected it by UPLC-MS/MS in *Cylindrospermopsis raciborskii* CYN extracts under the influence of UV-B treatment in alkaline conditions. This DP may be formed by cleavage of the uracil ring from the tricyclic guanidine moiety followed by insertion of oxygen. In our experiment, this ion is the first of the DP detected (lower t_R), in agreement with Adamski et al. (2016a). The second DP detected in our study was at *m/z* 169.1; by its molecular weight, it could be expected to observe it at a lower retention time. The higher retention time (22.45 min) could be explained if it were not a direct DP of CYN, but an indirect one, this is, a fragment from another intermediate DP, such as the ion at *m/z* 434.1 (Adamski et al., 2016b). These authors suggested that generation of the ion at *m/z* 434.1 may result from

the hydrolysis of the guanidine moiety to a urea derivative, and then it could suffer a breakage to a bicyclic moiety (*m/z* 292.1) with the loss of cyanic acid (*m/z* 249.1), sulphate (*m/z* 169.1) and H_2O (*m/z* 151.1). This last ion also appears as a fragmentation ion of CYN (Table 2). The ion detected in our experiment with higher retention time (*m/z* 336.2) agrees with the diagnostic fragment detected by thermochemolysis by Ríos et al. (2014) at 22.23 min. Moreover, it was detected as DP of CYN in *C. raciborskii* CYN extracts under the influence pH and different temperatures (Adamski et al., 2016b) and as ion fragment of CYN and 7-epi-CYN (Adamski et al., 2016a, b).

To the best of our knowledge there are no many studies about the possible toxicity of CYN DPs. Nonetheless, it has been suggested that, at last some CYN induced disorders are due to structural molecular changes. In this respect, the hepatotoxic action of CYN has been related to the presence of a hydroxyl group on the uracil bridge or to the keto-enol status of the uracil moiety (Norris et al., 1999; Masten and Carson, 2000); Cartmell et al. (2017) revealed that the −OH group at C-7 of the toxin was responsible of toxic effects induced on human white blood cells (neutrophils). Based on the chemical structure of the CYN degradation products found, it is unlikely that DP-1 and DP-2 (theoretical m/z 290.1 and 169.1, respectively), both without the cited characteristics, might contribute to the potential toxicity of CYN in fish muscles. On the other hand, DP-3 (m/z 336.2) contains the uracil but no the sulphate group, although the latter is not considered to be relevant for CYN biological activity (Runnegar et al., 2002). Therefore, it would not be expected neither DP-3 to bear a higher toxicity than CYN. Nevertheless, we believe that more studies are necessary on CYN degradation and toxicity characterization of the decomposition products to achieve a more realistic risk evaluation of CYN-contaminated food.

3.3 Pyrolytic products in fish muscle spiked with CYN and cooked by different methods

The relative percentages of CYN and its DP detected by direct (single-shot) pyrolysis (350 ºC) in contaminated raw fish muscle (positive control) and muscle cooked for 2 min by microwaving, broiling, boiling or steaming are shown in Fig. 4.

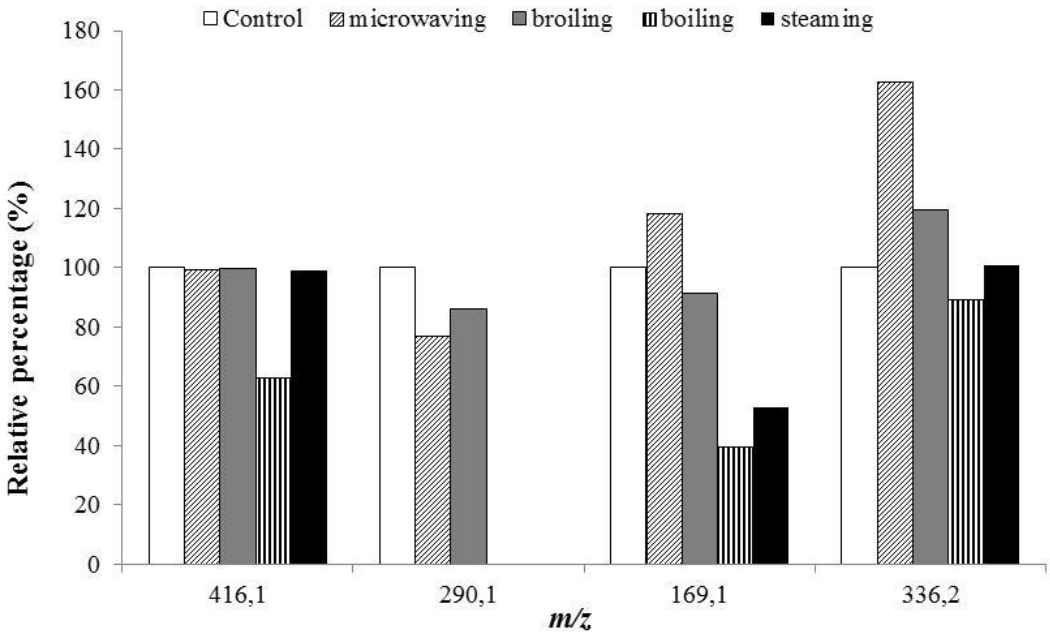
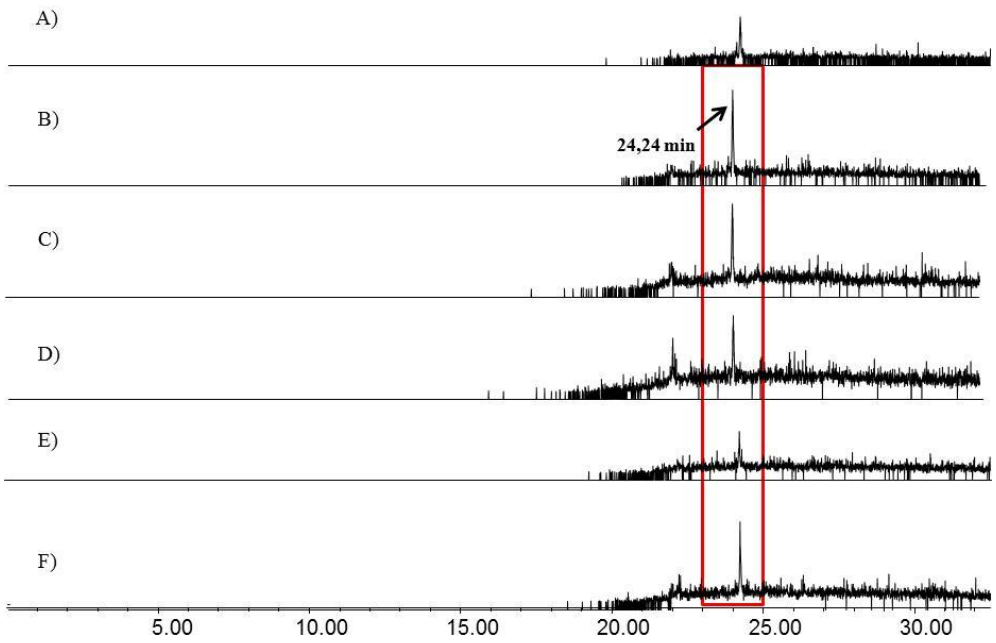


Fig. 4: Relative percentage of CYN decomposition fragments detected by direct (single-shot) pyrolysis (350 ºC) in contaminated samples of raw fish (*Oreochromis niloticus*) and cooked for 2 min by different cooking processes (microwaving, broiling, boiling or steaming).



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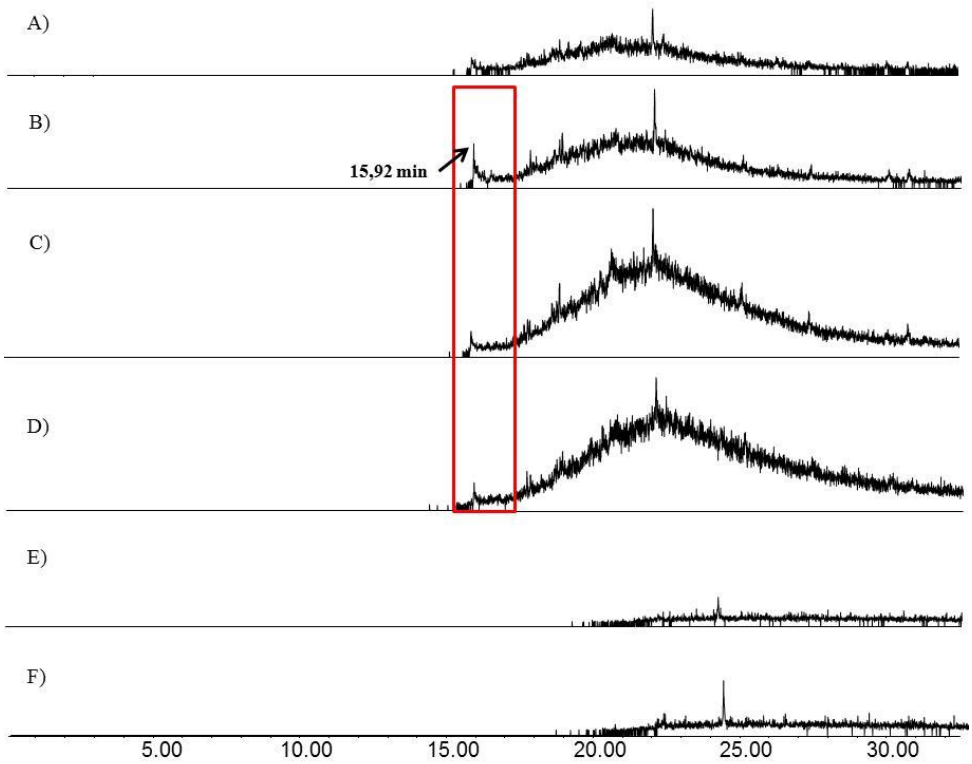
278 Fig. 5: Single current ion chromatograms (SIN) for ion 416.00 (415.70 to 416.70) from the
279 direct pyrolysis (350 ºC) of (A) a negative control and (B) a positive control (50 ng CYN/g
280 d.w.) of raw fish (*Oreochromis niloticus*) and contaminated cooked samples for 2 min by C)
281 microwaving, D) broiling, E) boiling or F) steaming.

282

283 The only cooking technique able to decrease the relative percentage of CYN was
284 boiling (Figs. 4 and 5). Moreover, the other characteristic DP also decreased their
285 relative abundance when fish muscle is cooked by boiling, especially the ion at *m/z*
286 169.1. In fact, the DP at *m/z* 290.1 could not be detected in muscles cooked by boiling
287 or steaming (Figs. 4 and 5).



288

289 Fig. 6: Single current ion chromatograms (SIN) for ion 290.10 (289.80 to 290.80) from the
290 direct pyrolysis (350 ºC) of (A) a negative control and (B) a positive control (50 ng CYN/g
291 d.w.) of raw fish (*Oreochromis niloticus*) and contaminated cooked samples for 2 min by C)
292 microwaving, D) broiling, E) boiling or F) steaming.

293 These results could indicate that the ion at *m/z* 290.1 should not be one of the
294 characteristic fragments to look for in fish cooked by these techniques involving water
295 when searching for CYN (Table 3). On the contrary, Guzmán-Guillén et al. (2017)
296 observed this DP in boiled and steamed fish muscle by UPLC-MS/MS. Moreover, this
297 fragment was detected by Adamski et al. (2016a) in *C. racibosrkii* cultures under UV-B
298 irradiation and alkaline conditions. Therefore, if CYN is decreased by boiling but its
299 characteristic DP detected in this study have not shown any increase by this cooking
300 technique, there might be other unknown routes by which it is being degraded which
301 have not been detected by pyrolysis.

302

303 Table 3. CYN decomposition products (DP) detected by direct (single-shot) pyrolysis (350 ºC)
304 in contaminated samples of fish (*Oreochromis niloticus*) cooked for 2 min by different cooking
305 processes (microwaving, broiling, boiling or steaming). ND: not detected.

Decomposition products	$[M+H]^+$ ion *m/z*	**Cooking process**			
		Microwaving	Broiling	Boiling	Steaming
DP-1	290.1	X	X	ND	ND
DP-2	169.1	X	X	X	X
DP-3	336.2	X	X	X	X

306

307 Moreover, cooking in the microwave seemed to slightly increase the relative percentage
308 of the ion at *m/z* 169.1 (Figs. 5 and 8), but the most outstanding increase was shown for
309 the product at *m/z* 336.2 (Figs. 4 and 8). This is in agreement with previous results
310 found in fish muscle spiked with CYN where six diastereoisomers of this DP were
311 detected by UPLC-MS/MS in samples cooked by microwaving (Prieto et al., 2017), and
312 in *C. racibosrkii* cultures under pH and temperature influence where four DP at *m/z*
313 336.1 were observed (Adamski et al. 2016b). The increase in the relative abundance
314 found in this study could be explained by the co-occurrence of different
315 diastereoisomers that have been observed in those experiments by UPLC-MS/MS, but
316 that are not differentiated when using Py-GC/MS. The abundance of ion at *m/z* 336.2
317 also increased by broiling, but to a lesser extent compared to microwaving, and this
318 behavior is in agreement with Prieto et al. (2017).

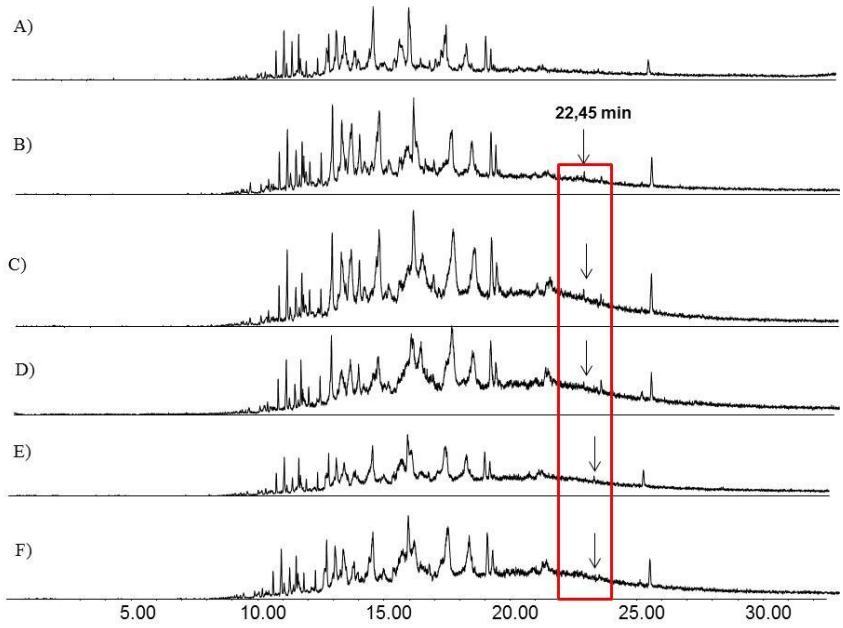


Fig. 7: Single current ion chromatograms (SIN) for ion 169.10 (168.80 to 169.80) from the direct pyrolysis (350 ºC) of (A) a negative control and (B) a positive control (50 ng CYN/g d.w.) of raw fish (*Oreochromis niloticus*) and contaminated cooked samples for 2 min by C) microwaving, D) broiling, E) boiling or F) steaming.

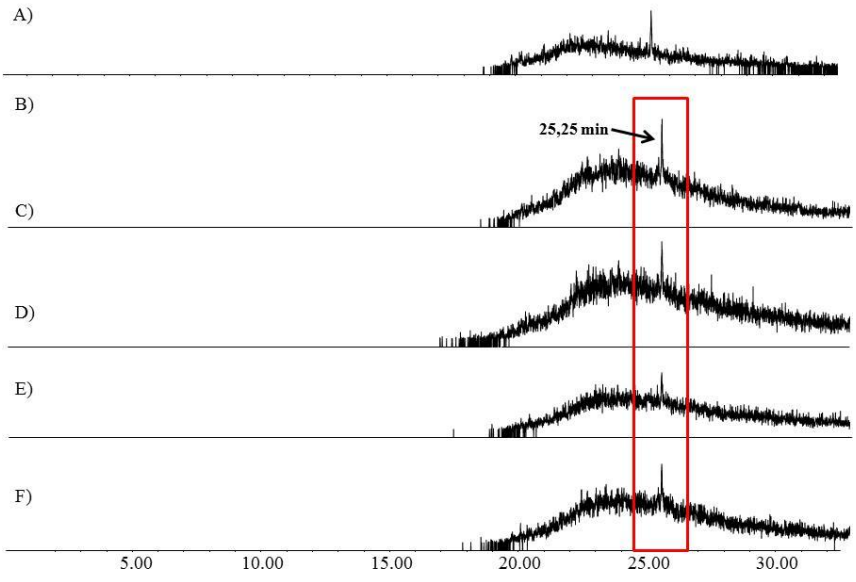


Fig. 8: Single current ion chromatograms (SIN) for ion 336.00 (335.70 to 336.70) from the direct pyrolisis (350 ºC) of (A) a negative control and (B) a positive control (50 ng CYN/g d.w.) of raw fish (*Oreochromis niloticus*) and contaminated cooked samples for 2 min by C) microwaving, D) broiling, E) boiling or F) steaming.

4. Conclusions

In the present work, it is demonstrated for the first time that Py-GC/MS is an adequate technique to detect CYN (*m/z* 416.1) in fish muscle samples. This technique could be a rapid and economical alternative (less use of chemical products and laboratory material and labor) for the detection and monitoring of CYN in raw or cooked fish. In addition to CYN, Py-GC/MS allows to detect the presence of its direct (*m/z* 290.1 and *m/z* 336.2) or indirect (*m/z* 169.1) degradation products in an effective way. Depending on the cooking process, the relative abundance of these products is different; indeed, ion at *m/z* 290.1 is only present in samples cooked by microwaving and broiling, while ions at *m/z* 169.1 and *m/z* 336.2 are present in all cooking process assayed (microwaving, broiling, boiling and steaming). Finally, the boiling is the only cooking technique that showed to decrease the relative percentage of CYN compared to the control group (uncooked fish C+). Therefore, it could be concluded that the peaks detected in this work by Py-GC/MS may be considered as diagnostic ions with a potential use for the direct detection of CYN in fish contaminated with this toxin, and consequently, in the exposure evaluation for risk assessment.

Conflicts of interest

The authors have no conflict of interest to declare.

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