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Bioaccessibility and changes on cylindrospermopsin concentration in edible mussels over storage and processing time

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Introduction

The cyanobacterial alkaloid cylindrospermopsin (CYN) has been recognized of increased concern due to the global expansion of its main producer, *Cylindrospermopsis raciborskii*. Previous studies have shown that aquatic organisms, especially bivalves, can accumulate high levels of CYN without lethal effect. Based on the potential for human health risks, a provisional tolerable daily intake of 0.03 µg/kg-body weight has been recommended. However, the human exposure assessment has been based on the CYN concentration in raw food items. Thus, this study aimed to assess the changes on CYN concentration in edible mussels over storage and processing time as well as CYN bioaccessibility.

Materials & Methods

Mussels storage and processing

Storage

Table 1. Details of food storage practices applied in the experiment.

25° C	4° C	- 20° C
0h	24h	48h
24h	48h	1 week
		1 month

Heat Processing

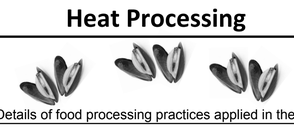

Table 2. Details of food processing practices applied in the experiment.

Microwaving (550W)	Boiling (100°C)	Steaming
0.5 min	5 min	10 min
1min	10 min	15 min
	15 min	

After CYN extraction, all analyses were performed by LC-MS/MS.

Mytilus galloprovincialis

↓

Exposure of 4 days

↓

C. raciborskii

Mussels *in vitro* digestion

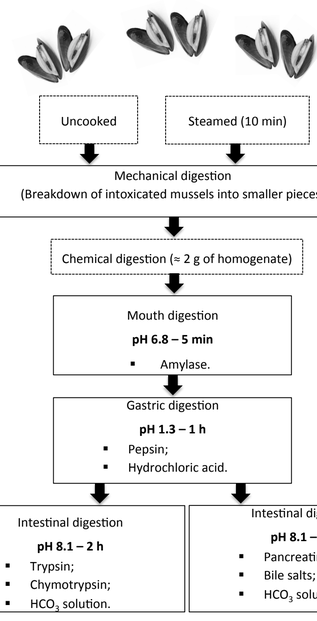

Fig. 1. Schematic representation of the *in vitro* digestion model used in the experiment.

Results & Discussion

Mussels storage and processing

The conservation of mussels at unrefrigerated and refrigerated conditions did not alter the concentration of CYN in comparison to the control group (41.6 ± 5.7 ng/g). Contrarily, in mussels stored frozen the recovery of CYN increased significantly ($p < 0.05$) (Fig.2). These results can be associated to the stability of CYN and a more efficient extraction from mussel tissues due to the potential cell disruption and protein denaturation caused by freezing/thawing.

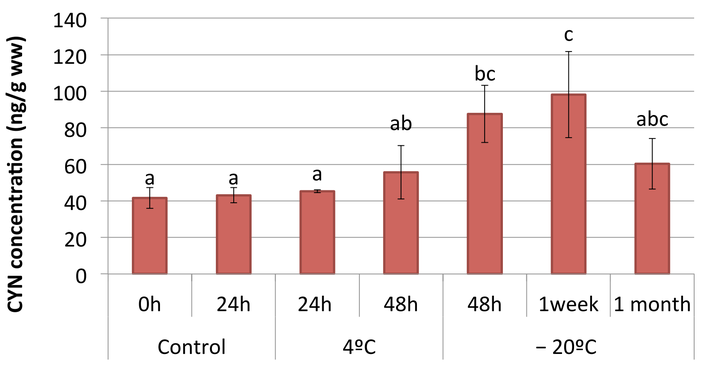

Fig. 2. The concentration of CYN (ng/g) in mussel matrices submitted to different storage treatments at different periods of time. Values are expressed as the mean ± SD (n = 3). Different letters (a, b and c) indicate significant differences ($p<0.05$). Columns that share the same letter are not significantly different.

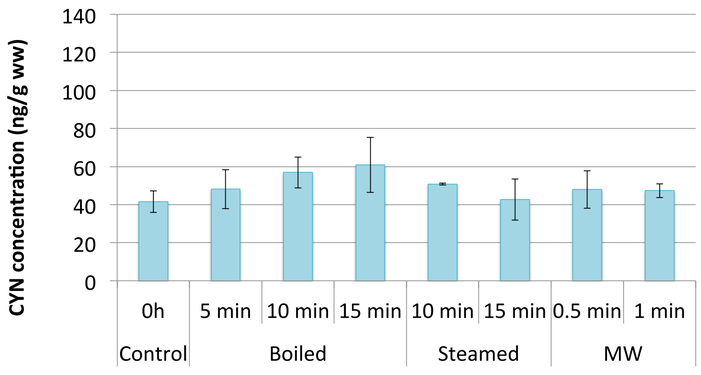

Fig. 3. The concentration of CYN (ng/g) in mussel matrices submitted to different processing practices at different periods of time. Values are expressed as the mean ± SD (n = 3).

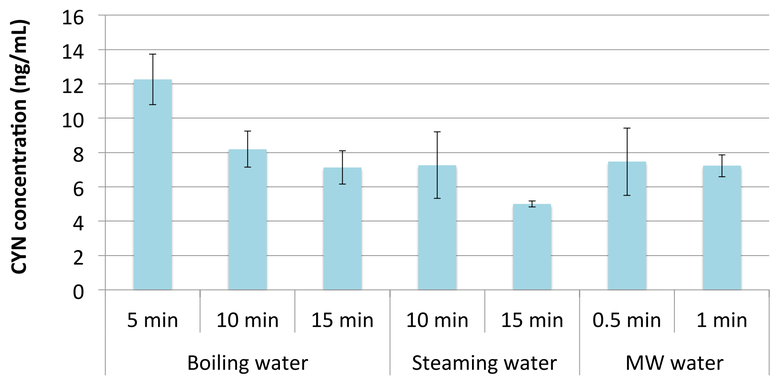

Fig. 4. The concentration of CYN (ng/mL) in the water in which mussels were cooked. Values are expressed as the mean ± SD (n = 3).

The heat processing had no effect on the CYN concentration in mussel tissues (flesh) (Fig. 3), however, an amount of the toxin was removed with this procedure, most likely due to the release of the intervalval fluid of mussels into cooking water (Fig. 4). Cooking practices could be applied to reduce CYN availability in this food item, by simply rejecting the cooking water. This can be relevant for implementation of the Hazard Analysis Critical Control Points (HACCP) system, inclusively for other industrial processes applied in mussels, such as canning and brine.

Mussels *in vitro* digestion

The digestion of mussels with salivary, gastric and intestinal juices sequentially reduce the concentration of CYN to levels below limits of quantification (50 ng/g) (Figs. 5 and 6).

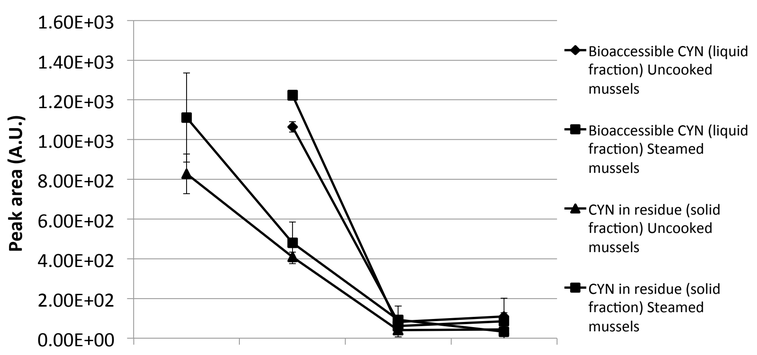

Fig. 5. Chromatographic peak area of CYN detected from uncooked and steamed mussels before (CYN C) and after digestion (for liquid and solid fractions). Letters represent each digestive step: mouth (M), gastric (G) and gastrointestinal (GI) with proteolytic enzymes (PE). Values are expressed as the mean ± SD (n = 3). A.U., Arbitrary Units.

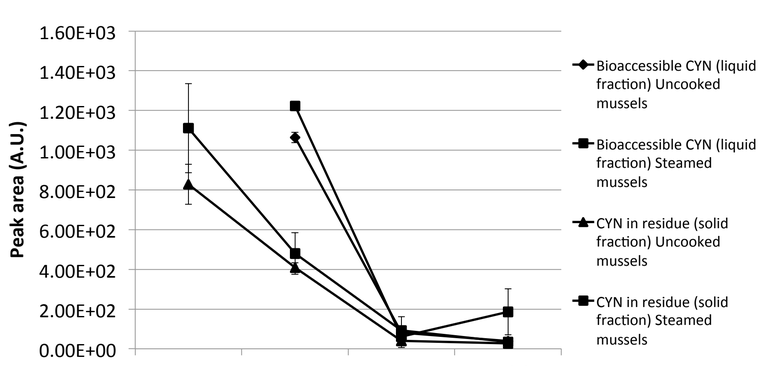

Fig. 6. Chromatographic peak area of CYN detected from uncooked and steamed mussels before (CYN C) and after digestion (for liquid and solid fractions). Letters represent each digestive step: mouth (M), gastric (G) and gastrointestinal (GI) with pancreatic juice (P). Values are expressed as the mean ± SD (n = 3). A.U., Arbitrary Units.

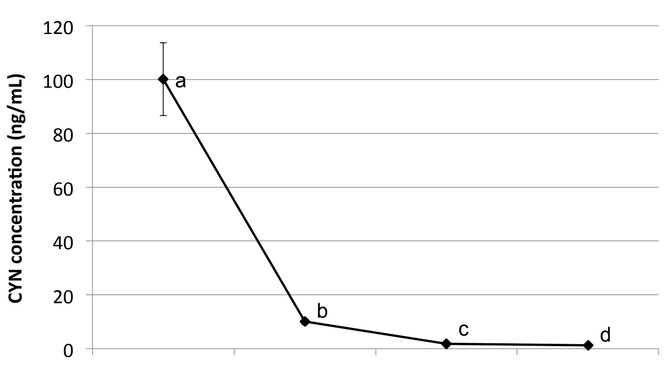

Fig. 7. The concentration of CYN (ng/mL) before (CYN C) and after digestion of its free form (purified CYN) in solution. Letters represent each digestive step: mouth (M), gastric (G) and gastrointestinal (GI) with proteolytic enzymes (PE). Values are expressed as the mean ± SD (n = 3). Different letters (a, b, c and d) indicate significant differences ($p<0.05$). Columns that share the same letter are not significantly different.

The incubation of CYN in its free form in solution with salivary juice promotes a significant reduction on its concentration (90%, $p < 0.05$). Then, there was a progressive decrease in CYN availability after digestion with gastric juice (P) and intestinal proteolytic juice (PE) (98 and 99%, respectively, $p < 0.05$) (Fig. 7). This suggests that the oral bioavailability of CYN is not similar to the levels quantified in raw and cooked mussels, thus the comparison of TDI with CYN extracted from food items is not recommended.

Conclusions

In conclusion, this study fills a gap on the knowledge of the influence of food storage and cooking practices on the levels of the CYN in edible organisms. Our results show that the recovery of CYN can be enhanced with frozen storage and cooking could reduce the concentration of CYN in mussels. At the concentrations tested, the digestive juices seem to be effective in the removal of CYN, either if it is present in solution or in raw and steamed mussel matrices. Further studies should be developed to identify the contributing factors of CYN removal, particularly the role of salivary juice.