

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

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### Summary

The cyanotoxin cylindrospermopsin 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 cylindrospermopsin. Based on the potential for human health risks, a provisional tolerable daily intake of 0.03 µg/kg body-weight has been recommended. However, human exposure assessment has been based on the cylindrospermopsin concentration in raw food items. This study aimed to assess the changes on cylindrospermopsin concentration in edible mussels over storage and processing time as well as cylindrospermopsin bioaccessibility. Mussels, (*Mytilus galloprovincialis*) fed cylindrospermopsin-producing *C. raciborskii*, were subjected to the treatments and then analyzed by LC-MS/MS. Mussels stored frozen allowed a significantly higher recovery of cylindrospermopsin (52.5%/48 h and 57.7%/one week). The cooking treatments did not produce significant differences in cylindrospermopsin concentration in mussel matrices (flesh), however, cylindrospermopsin was found in the cooking water, suggesting that heat processing can be used to reduce the availability of cylindrospermopsin in this food item. The *in vitro* digestion with salivary and gastrointestinal juices considerably decreased the cylindrospermopsin availability in uncooked and steamed mussels, highlighting the importance in integrating the bioaccessibility in the human health risk assessment.

### Introduction

The cyanotoxin cylindrospermopsin (CYN), an alkaloid, consisting of a hydroxymethyluracil moiety linked to a tricyclic guanidine, was firstly isolated from a culture of *Cylindrospermopsis raciborskii* (Ohtani et al., 1992). The high levels and the persistence of CYN in water can potentiate its accumulation in a wide range of aquatic organisms, such as bivalves (Ibelings and Chorus, 2007). Based on the potential for human health risks a provisional tolerable daily intake (TDI) of 0.03 µg/kg of body weight has been proposed by Humpage and Falconer (2003). The human exposure assessment has been based on the direct comparison between CYN concentration in raw edible organisms and TDI (Ibelings and Chorus, 2007). However, edible organisms are usually stored and processed before consumption and these practices may change the concentration of CYN available in food. Furthermore, the oral bioavailability of CYN is an important parameter to consider, once it can vary from the CYN contained in food matrices. The bioaccessibility corresponds to the fraction of a contaminant that is released from food matrix by digestive juices and can be seen as an appropriate indicator of the maximal oral bioavailability (Versantvoort et al., 2005). The aim of this study was to assess the changes on CYN concentration in edible mussels over storage and processing time as well as to assess the bioaccessibility of CYN in raw and processed (steamed) edible mussels.

## Materials and Methods

Mussels, (*Mytilus galloprovincialis*) fed cylindrospermopsin-producing *C. raciborskii* for four days, were subjected to the common food storage and processing practices applied according to Freitas et al. (2014). The *in vitro* model used to simulate the human digestion was adapted from Versantvoort et al. (2005). After each treatment, the CYN content in mussel matrices and soluble fractions was analyzed by LC-MS/MS.

## Results and Discussion

The conservation of mussels at unrefrigerated and refrigerated conditions did not alter the concentration of CYN in comparison to the control group ( $41.6 \pm 5.7$  ng/g). Contrarily, in mussels stored frozen the recovery of CYN increased significantly ( $p < 0.05$ ). 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. The heat processing had no effect on the CYN concentration in mussel tissues (flesh), 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. Thus, food-processing 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, inclusive for other industrial processes applied in mussels, such as canning and brine. The sequential digestion of mussels with salivary, gastric and intestinal juices reduces the concentration of CYN to levels below limits of quantification (50 ng/g). However, the peak area of the chromatograms obtained suggests that the *in vitro* digestion progressively decrease the amount of CYN in both fractions. The incubation of CYN in its free form in solution with salivary juice promotes a significant reduction of its concentration (90%,  $p < 0.05$ ). Then, there was a progressive decrease in CYN availability after digestion with gastric juice and intestinal proteolytic juice (98 and 99%, respectively,  $p < 0.05$ ). These results suggest 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. 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 can be applied to reduce its concentration 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.

## References

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