

# Effects of proteolytic digestion on the cyanotoxins microcystin-LR and cylindrospermopsin: the importance in integrating the bioaccessibility in human health risk assessment

Freitas M.<sup>1,2,3\*</sup>, Azevedo J.<sup>1</sup>, Carvalho A.P.<sup>1,2</sup>, Mendes V.M.<sup>4</sup>, Manadas B.<sup>4</sup>, Campos A.<sup>1</sup>, Vasconcelos V.<sup>1,2</sup>

(1) CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal

(2) Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal

(3) Department of Environmental Health, School of Allied Health Technologies. Polytechnic Institute of Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, 4400-330 Gaia, Portugal

(4) Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

\* marisaalexandrafreitas@gmail.com

The occurrence and proliferation of toxic cyanobacterial blooms are an emergent environmental concern worldwide. Microcystin-LR (MC-LR), a potent hepatotoxin, is the most documented and studied cyanotoxin. The cytotoxin cylindrospermopsin (CYN) has been recognized of increased concern due to the invasive nature of its main producer, *Cylindrospermopsis raciborskii*. Previous studies have shown that edible aquatic organisms, especially bivalves, can accumulate high levels of these cyanotoxins. MC-LR and CYN are stable at a wide range of temperatures and pHs, thus the knowledge of the influence of human digestion on its concentration in food is required to achieve a more accurate health risk assessment. The aim of this study was to assess the MC-LR and CYN bioaccessibility in edible bivalves.

Clams (*C. fluminea*) fed MC-LR-producing *M. aeruginosa* and mussels (*M. galloprovincialis*) fed CYN-producing *C. raciborskii* were subjected to an *in vitro* digestion model adapted from Maulvault et al. (2011) and Versantvoort et al. (2005). Bioaccessibility of MC-LR and CYN were then assessed by LC-MS/MS.

The bioaccessibility of MC-LR after proteolytic digestion was reduced to 83%, potentially because of its degradation by pancreatic enzymes. The *in vitro* digestion with salivary and gastrointestinal juices considerably decreased the CYN availability in uncooked and steamed mussels. Our results suggest that risk assessment based on MC-LR and CYN concentration in raw products might not be representative of true human exposure, once bioaccessibility strongly reduces the potential toxicological risks. Thus, the incorporation of the bioaccessibility of these cyanotoxins in the human exposure estimation would be of particular relevance to the application of more forceful management measures.

Palavras-Chave/Palabras Clave: Bioaccessibility, bivalves, cylindrospermopsin, microcystin-LR, risk assessment