



Abstract

Effects of Refrigeration and Freezing in Cylindrospermopsin and Microcystin Concentrations on Leaves of Lettuce (*Lactuca sativa*) and Spinach (*Spinacia oleracea*)[†]

Antonio Casas-Rodríguez, Leticia Diez-Quijada , Ana Isabel Prieto ^{*,‡} , Angeles Jos and Ana María Cameán

Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, 41012 Seville, Spain; acasasr@us.es (A.C.-R.); ldiezquijada@us.es (L.D.-Q.); angelesjos@us.es (A.J.); camean@us.es (A.M.C.)

* Correspondence: anaprieto@us.es

† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.

‡ Presenting author (poster).

Abstract: As a consequence of climate change, an increase in the occurrence of cyanotoxins is happening. These toxins are a large group of secondary metabolites with different chemical structures, mechanisms of action and worldwide distribution, and can cause harmful effects in humans and animals. Humans can come in contact with these toxins through the oral route through the consumption of contaminated water and food. Frequently, in order to improve their shelf life, many foods, such as vegetables, are refrigerated or frozen before being consumed. Therefore, the aim of this work was to assess the potential effect of refrigeration (4 °C for 24 h, 48 h and 7 days) and freezing (−20 °C for 7 days, 1 month and 3 months) on the concentration of cylindrospermopsin (CYN) and microcystins (MCs) (MC-LR, MC-RR and MC-YR) in lettuce and spinach leaves. Vegetable samples were spiked with a stock solution of each toxin containing 0.75 µg toxin/mL (equivalent to 0.75 µg/g fresh weight) and subjected to the corresponding conservation process. Samples were extracted and quantified with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). In the case of CYN, only in spinach samples the refrigeration process caused a significant time-dependent decrease in its concentration (48 h and 7 days). However, this preservation process was not shown to be effective in reducing the concentration of this toxin in lettuce. Moreover, the freezing process in spinach didn't show differences in the CYN content between the control and the experimental groups. In general, results obtained in MC concentrations showed that the refrigeration process in both lettuce and spinach caused a significant decrease in toxin concentrations, and this decrease was higher in lettuce. Moreover, freezing caused a significant reduction in MC concentrations in spinach after 3 months. These results showed the differences in the toxin content depending on the type of process selected (refrigeration versus freezing) and vegetable species. Furthermore, more studies are needed to study the influence of different storage processes on cyanotoxin concentrations in vegetables humans consume to assess the risk of human exposure to these cyanotoxins in a more realistic way.

Keywords: cylindrospermopsin; microcystins; spinach; lettuce; refrigeration; freezing



Citation: Casas-Rodríguez, A.; Diez-Quijada, L.; Prieto, A.I.; Jos, A.; Cameán, A.M. Effects of Refrigeration and Freezing in Cylindrospermopsin and Microcystin Concentrations on Leaves of Lettuce (*Lactuca sativa*) and Spinach (*Spinacia oleracea*). *Biol. Life Sci. Forum* **2022**, *14*, 18. <https://doi.org/10.3390/blsf2022014018>

Academic Editor: Vitor Gonçalves

Published: 19 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Author Contributions: Investigation, A.C.-R., L.D.-Q., A.I.P., A.J. and A.M.C.; Methodology, software, formal analysis and data curation, A.C.-R., L.D.-Q. and A.I.P.; Original draft preparation, A.C.-R.; Review and editing, L.D.-Q., A.I.P., A.J. and A.M.C.; Conceptualization, resources, supervision, project administration and funding acquisition: A.J. and A.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: The Spanish Ministerio de Ciencia e Innovación (PID2019-104890RB-I00 MICIN/AEI/10.13039/501100011033) for the financial support. A.C.-R. thanks MICINN for the predoctoral grant (PRE2020-094412). L.D.-Q. thanks Junta de Andalucía for the grant (POSTDOC_21_00130).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon request from the authors.

Conflicts of Interest: The authors declare no conflict of interest.