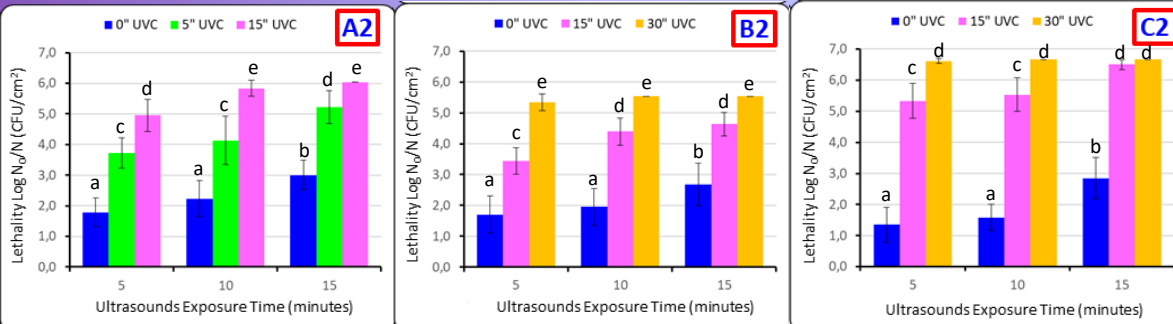


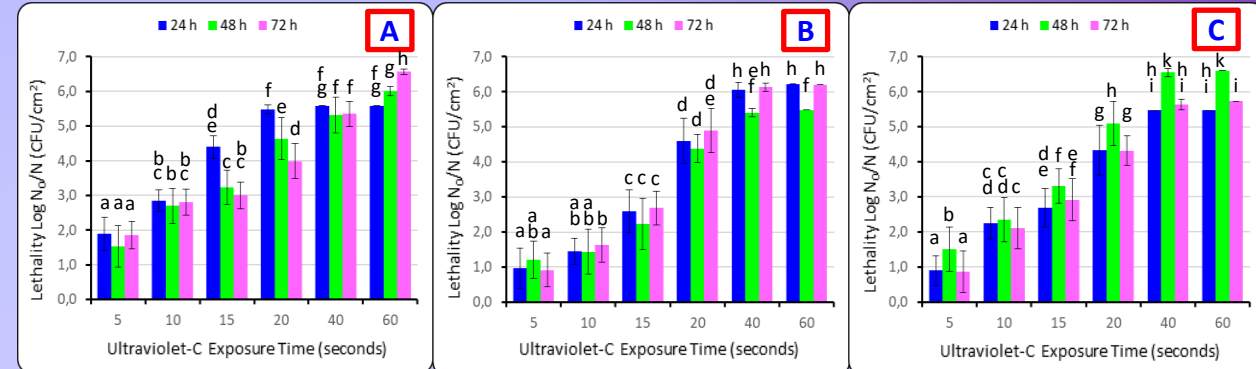
This study is based on the hypothesis that UVC treatment in combination with US shows synergistic and/or additive phenomena. It was proposed to determine the bactericidal and disintegrating effect of UVC treatments combined with US against biofilms of *Listeria monocytogenes* and *Bacillus subtilis* (spore and vegetative forms) formed at different times.

## Specific objectives

- To establish the differences in lethality between different microorganisms and their states (*i.e.* sporulated and vegetative).
- To determine if the UVC technology reaches microbial lethality for different stages of maturity in biofilms and microorganisms, establishing from what dose (*i.e.* exposure time) they could be considered as a disinfectant effect (*i.e.* reductions of  $\geq 4$  Log).
- To know if the US technology effectively disintegrates biofilms and to determine if the disintegration caused by the US facilitates the bactericidal effect of the UVC technology.
- To observe if the state of maturity of a biofilm influences the elimination of bacterial cells after the application of the proposed treatments.



**Figure 2.** Lethality of cells forming biofilms of (A2) *L. monocytogenes*, (B2) spores of *B. subtilis* and (C2) vegetative cells of *B. subtilis* produced at 48 h (A2, B2 and C2), after the combined application of US and UVC at different treatment doses. The same letter on the bars, within each independent graph, indicates that "There are no significant differences" ( $p > 0.05$ ).



**Figure 1.** Lethality of cells forming biofilms of (A) *L. monocytogenes*, (B) spores of *B. subtilis* and (C) vegetative cells of *B. subtilis* obtained after applying different doses of UVC. The same letter on the bars, within each independent graph, indicates that "There are no significant differences" ( $p > 0.05$ ).

## Conclusions

- L. monocytogenes* was the most sensitive microorganism to UVC-US treatments, followed by the vegetative forms of *B. subtilis* and the sporulated forms were the most resistant.
- The UVC treatments, with and without the US, were effective with the intensity and times tested, to obtain reductions of  $\geq 4$  Log in the microorganisms studied for the three maturation states.
- The application of US proved to be effective, increasing lethality in combination with UVC and showing synergism values.
- The three stages of maturation of the biofilms, in general, showed that there was greater resistance to treatments at a higher maturity of the structure, although this was not always the case.
- Finally, it could be considered that the application of UVC to surfaces is a potential disinfectant technology, especially when US is applied as a disintegrating process if there is biofilm formation.