Application of High-Intensity Pulsed Electric Fields for the stabilization of tiger nut milk

SUMMARY

Tiger nut milk is a traditional plant-based beverage from the Valencian Community that holds a Protected Designation of Origin in the L'Horta Nord area of the province of Valencia. Recent scientific studies support the functional properties of natural tiger nut milk, a fact that has increased consumer interest in this plant-based drink. The refrigerated shelf life of this product is very short; furthermore, its starch content hinders its preservation through thermal treatments. Therefore, the application of non-thermal technologies is being investigated to ensure its microbiological safety and quality, as alternative solutions to conventional thermal processes.

This doctoral thesis evaluates the effectiveness of High-Intensity Pulsed Electric Field (HIPEF) technology as a non-thermal method for the microbiological stabilization of tiger nut milk. The study analyzes the inactivation by HIPEF of the intrinsic aerobic mesophilic microbiota of this food, the spoilage microorganism *Enterobacter aerogenes*, and the pathogen *Bacillus cereus*. Additionally, the antimicrobial effect on *B. cereus* of adding *Stevia rebaudiana* Bertoni (stevia) infusion and the synergy of its combination with HIPEF treatments for pathogen control in tiger nut milk are studied. These infusions have sweetening properties compatible with the organoleptic characteristics of the beverage.

In the first phase, the technical parameters of the HIPEF treatment combined with moderate temperatures were defined to reduce the aerobic mesophilic microbiota in tiger nut milk. Survival kinetics were obtained at different electric field intensities, and the shelf life of the processed tiger nut milk was studied under refrigerated conditions at abuse temperature. The survival curves showed significant reductions in microbial load, which allowed an extension of the refrigerated shelf life. In the second phase, in a model substrate for tiger nut milk, the inactivation of *E. aerogenes* by HIPEF was investigated, evaluating the effect of the cellular physiological state on the effectiveness of the treatments. It was shown that the growth phase has a determining effect on the microorganism's resistance, with cells in the stationary phase

exhibiting the greatest resistance. For this physiological state, survival kinetics allowed the selection of effective treatments that reduced the population of this spoilage organism.

In the third phase, the research focused on the inactivation of vegetative cells of *B. cereus*, both in a model substrate and in sterilized tiger nut milk. In the model substrate, the inactivation kinetics of three *B. cereus* strains by HIPEF were studied. These kinetics characterized the resistance of the strains, identifying the most resistant strain, with which the inactivation study in tiger nut milk was conducted. The food matrix had a protective effect on the survival of *B. cereus*, which significantly reduced the effectiveness of the treatments. Finally, the effect of adding stevia infusions to the tiger nut milk formulation and their combination with HIPEF treatments against the most resistant *B. cereus* strain was investigated. Stevia exerted a bactericidal effect and generated sublethal damage that allowed control of pathogen growth during storage at abuse temperatures. The combination of technologies enhanced the bactericidal effect and sublethal damage caused by stevia, identifying a combination that ensured the microbiological safety of the product under temperature abuse conditions.

The results obtained provide strong evidence on the potential of HIPEF, especially when combined with mild thermal treatments or natural antimicrobials, as a possible technological alternative to preserve refrigerated tiger nut milk.