

Title: Effect of Sublethal Shocks on *Staphylococcus Aureus* Envelope Properties: Relationship with the Development of Cross-Resistance to Pulsed Electric Fields

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Abstract: Pulsed Electric Fields (PEF) is one of the most promising alternatives to heat treatments for food preservation but much research effort is still required to fully elucidate the factors affecting microbial inactivation by this technology. The aim of this work was to study the effect of sublethal shocks of different nature on the properties of *Staphylococcus aureus* envelopes and its relationship with the development of cross-resistance to PEF. *S. aureus* cells were exposed to sublethal shocks (acid, alkaline, oxidative, osmotic, heat and cold) and then their membrane fluidity (fluorescence anisotropy), surface charge (cytochrome-C binding) and surface hydrophobicity (2-phase partition) were determined. Results obtained indicate that only heat shock resulted in a significant change ($p < 0.05$) of *S. aureus* membrane fluidity (r value increased up to 0.05 units). Heat shock also resulted in an increase (10.6 %) in surface hydrophobicity. Finally, heat and alkaline shocks resulted in an increase in the surface negative charge (up to a 23 %) of *S. aureus* cells. On the other hand, heat and alkaline shocks were the only ones triggering an increase in PEF resistance (3- and 6-fold increase in the time for the first decimal reduction). Neither the changes in surface charge nor the heat and alkaline shock-dependent development of PEF resistance required *de novo* protein synthesis, thus suggesting that surface charge would play a major role in *S. aureus* PEF resistance.

Keywords: Stress resistance; non-thermal technologies; foodborne pathogens; food preservation