

ON THE ASSESSMENT OF AN AGAR OVERLAY METHOD FOR THE RECOVERY OF HEAT-INJURED *LISTERIA INNOCUA* IN PARSLEY

Fátima A. Miller¹, Bárbara F. Ramos¹, Teresa R. S. Brandão¹, Paula Teixeira¹, Cristina L. M. Silva¹

¹Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Porto, Portugal

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The development of methods to detect pathogenic bacteria, such as *Listeria monocytogenes*, in thermally processed foods is crucial for assessing products' safety.

Current methods for *Listeria* detection require enrichment techniques. When the objective is to quantify bacterial cells, researchers exclude the enrichment steps, choosing merely a selective media for microbial enumeration. However, the selective agents present in these media inhibit repair of injured cells, that might recover and regain their pathogenicity.

The objective of this work was to assess a methodology for the detection of heat-injured *L. innocua*. A non-selective medium (Tryptic Soy Agar + 0.6 % yeast extract; TSAYE) was overlaid on a selective medium (Palcam agar + selective supplement). Studies were carried out in parsley (*Petroselinum crispum*), using *L. innocua* as an indicator of the pathogenic specie. Parsley was artificially contaminated with *L. innocua*, packed in vacuum-sealed plastic bags and submitted to heat treatments at 52.5, 60.0 and 65.0 °C, using a thermostatic water bath. Samples were removed at different times, diluted and directly inoculated onto Palcam medium and on agar overlaid plates for posterior analysis of *Listeria* inactivation behaviour. Higher microbial counts were observed when the overlay method was used (at least one log difference was observed during all inactivation processes). The top layer of TSAYE allowed heat-injured cells to recover. After diffusion of selective agents from Palcam agar to TSAYE, typical *Listeria* colonies were formed with inhibition of the parsley's natural microflora.

This methodology ensures reliable heat-injured cells enumeration, attaining accurate quantification of bacteria.