

WHAT CULTURE-BASED ANALYSES DON'T TELL AND FLOW CYTOMETRY DOES ABOUT VIABLE BUT NON-CULTURABLE MICROORGANISMS

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Microbiology pays a main attention to characterize and limit bacterial contaminations in food, and the viable but non-culturable (VBNC) state of microorganisms cause a great threat to food safety and public health as the use of culture-based only microbiological methods might direct to a risky under-appreciation of a food contamination. New fast and accurate analytical methods for VBNC bacteria are needed to assess the effectiveness of treatments to ensure healthy food preservation. We have investigated flow cytometry (FCM) as a fast and consistent method to evaluate the induction of *E. coli* ATCC 25922 strain into a VBNC state after mild heat treatment of fruit juices in combination with oregano essential oil (OEO). At first, *E. coli* was grown in Brain Heart Infusion (BHI) medium at 37 °C for 24 h and treated at 55 °C, 60 °C and 65 °C for 30 min with and without OEO. Subsequently, microbial challenge test was performed by artificially contaminating commercial and organic fruit juices with *E. coli* strain at 5 Log CFU/mL. The combination of mild heat treatment (65 °C) and OEO (100 ppm) for 5 min was applied and samples were stored at 4 °C and 37 °C up to 48h. Bacterial survival ratio was estimated using culture-based methods and flow cytometry. Cell sorting of the different sub-populations in terms of viability obtained after treatments was also performed and their growth on culture media was assessed using a single-cell approach. Results obtained in vitro revealed that the samples incubated at 37 °C for 24h after the treatments at 55 °C for 30 min with OEO (50 and 100 ppm), showed a long-term inactivation of bacterial growth, although a percentage of damaged cells is able to recover growth activity at optimum temperature for the strain. Instead, combined treatments at 65°C for 5 min led only to a temporary reducing effect of the alive population; indeed, it was found to be ineffective to stop long-term contaminations. Results obtained in vivo revealed no bacterial growth of the challenge microorganisms after the combined treatment both in culture-based and in FCM assay, while curing with OEO only was not sufficient to reduce the microbial load below the limits by law for bacterial contamination. Our FCM results show adding OEO to fruit juice rise the efficiency of mild heat treatment, and low thermal treatment alone can be considered as a VBNC inducing factor.

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