

## **Inactivation of *Mycobacterium smegmatis* in skimmed and whole milk by means of high hydrostatic pressure and ultra- high pressure homogenization**

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**Short title:** High pressure inactivation of *Mycobacterium smegmatis* in milk

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## Abstract

In this study *Mycobacterium smegmatis* was challenged as a surrogate of pathogenic *Mycobacterium* species in order to evaluate the efficiency of pressure-based technologies in milk. Three strains of this specie (CECT 3017, 3020 and 3032) were inoculated into both skimmed (0.2% fat) and whole milk (3.4 % fat) at an approximate load of 6.5 Log CFU/ml and submitted to high hydrostatic pressure (HHP) treatments at 300, 400 or 500 MPa for 10 minutes at 6 °C and 20 °C. Evolution of the surviving cells of the inoculated strains was evaluated analyzing milk immediately after the treatments and after 5 and 8 days of storage at 6 °C. HHP treatments at 300 MPa were seldom efficient inactivating *M. smegmatis* strains, but lethality increased with the pressure of the treatment in all cases. Generation of sub-lethal injured cells was observed only after 400 MPa treatments since inactivation at 500 MPa showed to be complete. Significant differences were not observed due to either temperature of treatment or fat content of milk, except for strain CECT3032, which showed to be the most sensitive to HHP treatments. Milk inoculated with strain CECT3017 was submitted to ultra-high pressure homogenization (UHPH) treatments at 200, 300 and 400 MPa. Maximum reductions were obtained after the 400 MPa treatments, although less than 3.50 Log CFU/ml of reduction was achieved and not significant differences with respect to 300 MPa treatments were observed. UHPH did not caused a significant number of injured cells. Surviving *M. smegmatis* strains for both kinds of treatments were able to increase counts in milk during the subsequent cold storage at 6 °C. The usefulness of this species as a marker for pressure-based processing seems limited since it shows greater sensitivity than some pathogenic species including other *Mycobacteria* reported in previous studies.