# Hyperbaric inactivation  a nonthermal approach to inactivate Bacillus subtilis endospores at room temperature

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Introduction:
Hyperbaric inactivation (HI) is a nonthermal, low-pressure-based processing methodology that makes use of moderate hydrostatic pressures (up to 250 MPa) for long dwell times (hours to days) to achieve pasteurization standards in heat and pressure-sensible foods. Recently, this methodology has shown great potential to not only inactivate vegetative microorganisms but also bacterial spores, which are highly resistant either to conventional thermal pasteurization or high-pressure processing, being required temperatures above 100 ºC for several minutes to inactivate them. Considering the importance of bacterial spores for both food safety and shelf-life, it is of upmost importance to evaluate the feasibility of this methodology for endospore inactivation in a range of pH-values important for food safety.

Objectives:
The present work aimed to evaluate the potential of HI to destroy Bacillus subtilis endospores, and the dependence of pH and nutrient-availability for endospore inactivation, and the inherent kinetic parameters. To do so, B. subtilis endospores were inoculated in nutrient-free McIlvaine buffer and Brain-heart infusion broth at three different pH levels (4.50, 6.00 and 7.50) and kept under hyperbaric inactivation (150, 200 and 250 MPa) up to 7 days at uncontrolled room temperature (18-25 ºC).

Results:
The results demonstrated a clear dependence of nutrient-availability and pH upon endospore inactivation under HI conditions, which allowed to fit both linear and non-linear (Weibull, Log-logistic and Biphasic) kinetic models frequently used to describe endospore inactivation patterns. Lower pH values hindered endospore inactivation at 150 MPa, even in the presence of nutrients, which could be surpassed at and above 200 MPa. Additionally, the presence of nutrients accelerated endospore inactivation, which ultimately impacted the inactivation kinetic parameters. Curiously, a pressure increase from 200 to 250 MPa did not accelerate endospore inactivation at both pH 6.00 and 7.50.
Moreover, phase-contrast microscopy images revealed that the endospores were inactivated without reaching the vegetative state, which is an important outcome for food safety.

Conclusions:
There results show that HI can be an interesting approach for the inactivation of bacterial spores at room temperature, without applying any heat, which could be particularly interesting for heat-sensible foods and other matrices.