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Quantifying the *Bacillus weihenstephanensis* and *Bacillus licheniformis* spore recovery considering the sporulation, the heat-treatment and the recovery conditions

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OBJECTIVE(S)

Spore-forming bacteria development in food is a major cause of food spoilage and food poisoning, leading to economical losses. Empirical models have been developed to predict spores in variable environments after heat-treatment (Mafart *et al.* 2010). The aim of this study is to understand and quantify *Bacillus weihenstephanensis* and *Bacillus licheniformis* spore recovery taking into account the properties induced by sporulation environments, the heat-treatments intensity and the recovery conditions. A predictive model, based on physiological parameters, will be designed to describe and quantify the spore recovery.

METHODS(S)

Bacillus licheniformis strain AD978 was isolated from raw dairy ingredients and was kindly provided by ADRIA Développement (Quimper, France) and *Bacillus weihenstephanensis* KBAB4 strain was kindly provided by the Institut National de la Recherche Agronomique (INRA, Avignon, France).. The sporulation was performed in sporulation mineral buffer (pH7.00) at two different temperatures: 12°C and 30°C for *Bacillus weihenstephanensis* and 45°C and 20°C for *Bacillus licheniformis*. Spores were treated at 95°C, 100°C and 105°C following the capillary method, in buffered peptoned water (pH 7.00). Then, a method of dilution-inclusion in the recovery media has been used for survivor counts (Baril *et al.*, 2011). The spores were incubated in Brain Heart Agar plates at pH ranging from 4.50 to 8.00 and incubation temperature ranging from 4°C to 40°C for *Bacillus weihenstephanensis* and 15°C to 60°C for *Bacillus licheniformis*.

RESULTS

Inactivation kinetics have been obtained for each heat-treatment conditions and recovery temperature and pH. An optimal recovery has been observed at around 30°C for *Bacillus weihenstephanensis* and 45°C for *Bacillus licheniformis*. Moreover, stronger are the conditions of heat-treatment, stronger is the impact of the recovery medium. The spore heat-sensitivity (z_T value) was constant regardless to the recovery pH and temperature. Only the apparent heat-resistance (δ value: time for the first decimal reduction) was affected by the temperature and pH. Bigelow-like models, using z'_T values, have been previously used to fit the data but these models led to over-estimations and predict possible recovery at 15°C, while *Bacillus licheniformis* AD978 is not able to grow at this temperature. The new model is based on the growth physiological parameters (minimal, optimal and maximal recovery temperature and pH), which are often available in scientific literature and have a real physiological meaning. It also avoids over-estimation of the predicted heat-resistance out the range of temperature and pH allowing the growth.

CONCLUSIONS AND IMPACT OF THE STUDY

The new model would be useful to predict spore recovery after a heat treatment. The parameters used in this model can be obtained by elicitation of expert opinion, allowing a possible extension to other bacterial species of concern for food safety and quality.

REFERENCES

Baril, E. *et al.* 2011. Modeling heat resistance of *Bacillus weihenstephanensis* and *Bacillus licheniformis* spores as function of sporulation temperature and pH. *Food microbiology*. 30 29–36.

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