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# Development and validation of an extensive stochastic model for the simultaneous growth of Listeria monocytogenes and lactic acid bacteria – A case study with naturally contaminated cold smoked Greenland halibut

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### OBJECTIVE

The objective of the present study was to develop and validate a combined stochastic model for growth of *Listeria monocytogenes* and lactic acid bacteria (LAB).

## METHODS

An existing deterministic model for the simultaneous growth of *L. monocytogenes* and LAB (Mejlholm and Dalgaard, 2007, 2009) was expanded into a stochastic model using the Analytica software. The stochastic model includes the effect of 12 environmental parameters as well as their interactive effects, together with the effect of microbial interactions between *L. monocytogenes* and LAB. To evaluate the performance of the stochastic model, a total of 24 storage trials with naturally contaminated cold-smoked Greenland halibut (CSGH) was carried out at 5, 8 and 12 °C. Samples were supplied by a Danish seafood processer from a withheld batch of CSGH being positive for *L. monocytogenes*. The variability in product characteristics was determined by analysis of five samples. In addition, 56 samples of CSGH were collected from the same company during a period of 12 months to obtain a more thorough description of the variability in product characteristics and in the occurrence of *L. monocytogenes* and LAB. In contrast to traditionally produced CSGH, all the examined samples were added acetic and lactic acids in order to improve the safety of the product.

#### RESULTS

Product characteristics of CSGH used for the storage trials were: Water phase salt  $(3.31 \pm 0.41\%)$ , pH  $(6.13 \pm 0.15)$ , phenol (10.1  $\pm 0.8$  ppm), water phase acetic acid (3011  $\pm 472$  ppm) and water phase lactic acid (7657  $\pm$  1162 ppm). The initial concentration of L. monocytogenes in CSGH was 0.13  $\pm$ 0.28 log (cfu/g), with all samples being positive. No growth of L. monocytogenes was observed at  $5^{\circ}$ C whereas an increase in the concentration was seen for some of the storage trials at 8 and 12°C, with the maximum population density (MPD) of the pathogen reaching approx. 1.7 log (cfu/g) at both temperatures. By including a relative lag time (RLT) of 4.5 for L. monocytogenes (Ross, 1999), MPDs of 1.3, 1.6 and 3.2 log (cfu/g) were predicted by the stochastic model at 5, 8 and 12°C, respectively. Without a RLT of 4.5, the corresponding predictions were 2.8, 3.6 and 4.2 log (cfu/g). Product characteristics of the 56 samples of CSGH were: Water phase salt ( $3.10 \pm 0.53\%$ ), pH ( $6.12 \pm 0.16$ ), phenol (10.4  $\pm$  4.2 ppm), water phase acetic acid (3586  $\pm$  1061 ppm) and water phase lactic acid (9701 ± 1954 ppm). Assuming a shelf life of 28 days at 5 °C, L. monocytogenes was predicted to grow to no more than 1.5 log (cfu/g) in CSGH. Without a RLT of 4.5, the corresponding prediction was 3.4 log (cfu/g). Predicting growth of L. monocytogenes in CSGH without addition of acetic and lactic acids resulted in a MPD of 3.3 log (cfu/g) and more than 25% of the samples were estimated to exceed the regulatory limit of 2.0 log (cfu/g).

# CONCLUSIONS AND IMPACT OF THE STUDY

It was clearly demonstrated that to accurately predict the MPD of *L. monocytogenes* in naturally contaminated CSGH both the lag time and the effect of microbial interaction needs to included. Without these effects the MPD of *L. monocytogenes* was predicted to be up to 100.000 times higher than observed and high percentages of samples were estimated to exceed 2.0 log (cfu/g). Furthermore, it was shown that CSGH with added acetic and lactic acids complied with the EU-regulation (EC 2073/2005) on *L. monocytogenes*, even when the variability in product characteristics was taken into account. Without the addition of acetic and lactic acids, CSGH constitutes a high-risk product with the potential of causing listeriosis.

# REFERENCES

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