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Probability-of-growth modelling to optimize the use of hurdle technology to achieve microbiological stability of high moisture processed cheese

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Abstract

This thesis reports a study of the application of hurdle technology to high moisture, low acid ambient shelf-stable hot packed processed cheese analogue (PCA). Hurdle technology makes use of a combination of mild stress factors. A combination of these stress factors can be more effective in inhibiting or inactivating the growth of micro-organism than individual stress factors. The current study focused on the application of hurdle technology to inhibit the growth of the food pathogen, *Clostridium botulinum* (*C. botulinum*). This micro-organism poses a hazard for consumers and is capable of growth in low acid food (pH> 4.5). As there are difficulties in working with *C. botulinum* in laboratory trails, *Clostridium sporogenes* (*C. sporogenes*) was used as an analogue of *C. botulinum. C. sporogenes* is very similar to *C. botulinum* in growth characteristics but is not dangerous.

The effectiveness of selected preservatives on the growth of the target micro-organism was expressed as the probability of growth and was modelled as function of the concentrations of the selected preservatives in nutrient broth. Nutrient broth was initially used as it can be easily and accurately adjusted and controlled in terms of composition, and allows more rapid growth than is observed in PCA. A combination of salt (sodium chloride), sorbic acid (in the form of potassium sorbate), nisin and lysozyme was selected as stress factor. The inhibitory effect of these preservatives was then observed in the high-moisture nutrient broth at pH 7 (the optimum condition for spore of C. *sporogenes* to germinate) at 37ºC for eight weeks. It was found that lysozyme did not have a significant inhibitory effect on *C. sporogenes* in combination with salt, potassium sorbate and nisin. Therefore, the inhibitory effect of salt, sorbic acid and nisin at two different pHs (5.5 and 7) were subsequently evaluated in the nutrient broth at 37ºC for eight weeks.

The probability of growth of *C. sporogenes* was modelled as a function of the concentrations of these selected preservatives at each pH. The results demonstrated that a combination of salt, nisin and potassium sorbate at relatively low concentrations can be used to inhibit growth. The inhibitory effects of the preservatives were pH dependent and their inhibitory effect is higher at pH 5.5. The developed models were validated

using a fresh data set. Finally, the applicability of the developed model was checked in high moisture PCA. The results showed that the developed broth model underestimated the probability of growth in the PCA. Therefore, a specific probability of growth model was developed for the PCA using the PCA instead of nutrient broth as the growth medium. This model accurately predicted the probability of growth of *C. sporogenes* in the PCA for given combinations of preservative concentrations.

The model developed for PCA allows the relative levels of preservatives to be easily quantified without the need for time consuming and expensive experimental work. The model would have limitations in the case of strongly varying formulations, since minor changes in processed cheese formulation or its production, could significantly alter its ability to support toxin production. Therefore, the model is applicable only to PCAs that have formulations similar to that used in this study. The general approach described in this thesis could be applied in the development of other high moisture, low acid foods.

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Table of contents

List of figures

List of tables

List of publications

Peer-reviewed papers

Khanipour, E., McCarthy, O., Flint, S., Golding, M., Palmer, J. and Tamplin, M. Evaluation of the effects of salt, potassium sorbate, nisin and lysozyme on the probability of growth of *Clostridium sporogenes*, *International Journal of Food Science and Technology*, 2014, 49(6), 1506-1512.

Khanipour, E., McCarthy, O., Flint, S., Golding, M., Palmer, J. Ratkowsky, D. A., Ross, T. and Tamplin, M. (2014). Modelling the combined effects of salt, sorbic acid and nisin on the probability of growth of *Clostridium sporogenes* in nutrient broth. Submitted for publication in *International Journal of Microbiology*.

Khanipour, E., McCarthy, O., Flint, S., Golding, M. & Palmer, J. and Tamplin, M. (2014). The growth probability model of *Clostridium sporogenes* as a function of salt, sorbic acid and nisin in processed cheese (In preparation).

Conference presentations

Khanipour, E., McCarthy, O., Flint, S., Golding, M. & Palmer, J. Production of a cocktail of spores of *Clostridium sporogenes* for growth trials in new generation foods. New Zealand Microbiology Society (NZMS) Conference, Convention Center, Palmerston North, 26-28 November 2011 (Poster presentation).

Khanipour, E., McCarthy, O., Flint, S., Golding, M. & Palmer, J. The combined effect of salt, nisin, sorbate and lysozyme on the survival of *Clostridium sporogenes* in broth. New Zealand Microbiology Society (NZMS) Conference, Otago University, Dunedin, 26-28 November 2012 (Poster presentation).

Khanipour, E., McCarthy, O., Flint, S., Golding, M. & Palmer, J. The combined effects of salt, nisin and potassium sorbate at different pHs on the survival of *Clostridium sporogenes* in broth. New Zealand Institute of Food Science and

Technology (NZIFST) Conference, Hawkes Bay Opera House, Hastings 2-4 July 2013 (Poster presentation).

Khanipour, E., McCarthy, O., Flint, S., Golding, M. & Palmer, J. Modelling the growth/no growth of *Clostridium sporogenes* spores in broth as a function of salt, nisin and potassium sorbate concentrations at two pHs. International Conference on Predictive Modeling in Foods (ICPM) Conference, the Institute Pasteur, Paris, 16-20 September 2013 (Poster presentation).

Khanipour, E., Flint, S., McCarthy, O., Golding, M. & Palmer, J. How does the combination of common preservatives affect the survival of *Clostridium sporogenes*. Second Food Symposium. Research Presentation, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, 15 November 2013 (oral presentation).

Khanipour, E., Flint, S., McCarthy, O., Golding, M. & Palmer, J. Probability-of-growth modelling to optimize the use of hurdle technology to achieve microbiological stability of high moisture food product. University of Tasmania's Postgraduate Research meeting, 25 November 2013, Sandy Bay Campus, Hobart, Tasmania (oral presentation).

Khanipour, E., Flint, S., McCarthy, O., Golding, M. & Palmer, J. Evaluation of the effect of salt, potassium sorbate and nisin on the probability of growth of *Clostridium sporogenes*. University of Tasmania's Food Safety Centre Forum, 2-3 December 2013, Sandy Bay Campus, Hobart, Tasmania (oral presentation).

“All models are wrong but some are useful”

George E. P. Box 1979