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学 位 の 種 類	博士（食料革新学）
学 位 記 番 号	博 甲第 9718 号
学位授与年月	令和 2 年 9 月 25 日
学位授与の要件	学位規則 第４条第１項該当（昭和２８年４月１日文部省令第９号）
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学位論文題目	The Development of Rapid Growth Potential Analysis Method of Foodborne Pathogens by Real-Time PCR （リアルタイム PCR による食中毒菌の迅速増殖特性解析法の開発）

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Abstract of thesis

Foodborne illness is widely distributed globally in both developed and developing countries at an unprecedented rate while global food demand is projected to increase sharply. To secure the microbial safety of food, the practice of an intelligent hurdle' combination is widely applied in food manufacturing practice. In relation to microbial hurdle technology, the boundary limit of growth or no-growth of bacteria in such stress conditions can be predicted by the approach of predictive microbiology that can be used for specific types of microorganisms, physio-chemical properties of food, and the used hurdles. However, most of predictive models are utilizing the application of conventional culture methods that have been recognized for having several limitations. A highly sensitive and specific real-time PCR quantification method that provides the ability to yield high throughput quantification results in a short period of time should be considered as an alternative data collection tool for bacterial growth in food materials to construct predictive models.

In this thesis, the author compared the real-time PCR quantification results to the conventional method by agar plate (both selective and non-selective medium) that considered as a gold standard for bacterial enumeration, as well as to the most probable number (MPN) method. The sensitivity and specificity of this assay were examined in pasteurized product and in food materials with naturally occurring microbial background flora. The accuracy of

the developed model constructed by real-time PCR data was validated by means of observation obtained from the existing models in ComBase and MRV databases. The performance of four primary models in predicting the growth parameters of foodborne pathogens from various tested conditions was also evaluated. The inhibitory effect of pH, water activity (a_w), and temperature on the growth of target pathogens was investigated and the growth prediction under combined conditions was tested.

The author targeted on the growth monitoring study of *Staphylococcus aureus* and *Listeria monocytogenes* under the adjusted a_w level by various salt and sugars contents, as well as the pH adjustment by HCl and NaOH. The initial inoculum level of artificially inoculated samples was set as 10^4 to 10^5 CFU/ml or CFU/g. The incubation temperatures in all experiments were set at 4°C to 35°C and fluctuating temperature scenario performed for *Salmonella enteritidis* in chicken juice was set at 5°C and 30°C, while for *L. monocytogenes* study in pasteurized milk was set at 2°C, 8°C, 12°C, 15°C, and 30°C. Samples for agar method were adequately diluted in phosphate buffered-saline and spread on selective and/or non-selective medium using spiral plater, then incubated at 35°C or 37°C for 24-48 h. The MPN samples were appropriately diluted and transferred to 10 mL Fraser broth prior to the incubation at 35°C for 24-48 h. The samples for real-time PCR quantification were immediately stored at -20˚C until all samples were collected for DNA extraction. The target gene for *S.* Enteritidis quantification was the *inv*A gene fragment, the *hly*A gene fragment for *L. monocytogenes*, and the *nuc* gene fragment for *S. aureus*.

Previously, the author has succeeded the initiation of a rapid quantification by real-time PCR method for *S. enteritidis* in chicken juice samples as reported in Chapter I, where all growth data from this experiment showed goodness of fit with the prediction. The results of this study were also reflecting the existing prediction data in MRV and ComBase. The goodness of fit of the developed model to MRV was demonstrated by a proportion of relative error (pRE) of 1.00, accuracy factor (A*f*) of 1.10, bias factor (B*f*) of 0.96, root mean square error (RMSE) of 0.09, and R^2 of 0.97. The goodness of fit to the Combase predictor was demonstrated by pRE of 0.67, A*f* of 1.24, B*f* of 0.81, RMSE of 0.34 and R^2 of 0.61. *S.* Enteritidis growth prediction model in chicken juice samples with naturally occurring background microflora provide useful knowledge for the further development of growth prediction studies from many other kinds of foodborne pathogens in various food materials. This study also showing that the real-time PCR has a powerful advantage to measure the number of target genes in a large number of samples.

In Chapter 2, the author has successfully developed the growth monitoring of *L. monocytogenes* in pasteurized milk samples under constant and fluctuating temperature conditions by real-time PCR method. A high correlation was obtained between bacterial growth rate and incubation temperature, where the R^2 of the slope was calculated as 0.993 and 0.996 for real-time PCR and conventional culture method, respectively. Moreover, the obtained maximum specific growth rate (μ_{max}) data plots were correlated with 188 *L. monocytogenes* μ_{max} data points from the existing model in the ComBase database, with an R^2 of 0.961 for real-time PCR and of 0.931 for the conventional culture method. The prediction results fell within ± 20% of the relative error zone, showing that real-time PCR quantification could be used for fast, sensitive, and specific bacterial growth monitoring with high throughput results. Real-time PCR should be considered a promising option and a powerful tool for the construction of a bacterial growth prediction model for safety risk analysis in the dairy industry.

The author also has successfully evaluated the growth behavior of *S. aureus* under various a_w conditions adjusted by sugars and salt with satisfactory results, as reported in Chapter 3. The inhibitory capacity of each compound was investigated, where galactose performed higher inhibitory capability compared to other compounds. This indicates that although the materials set under the same a_w level, the inhibitory effect is specific to the type of additive applied in the product. Therefore, the food industry should consider the effect of various components for the construction of bacterial growth predictive modeling and not rely solely on the final adjusted a_w value. In this study, the performance of four kinds of primary models was also evaluated. Baranyi and Roberts' model was showing better goodness of fit since it has the lowest mean squared error (MSE) with better A*f* and B*f* values, while the three-phase Buchanan model was the least fit model compared to Baranyi and Roberts', Huang and modified Gompertz models.

In Chapter 4 and 5, the author showed that the real-time PCR method was successfully construct the growth prediction model of *L. monocytogenes* in ground pork samples as a function of temperature, a_w, and pH. However, the selective agar medium was underestimating *L. monocytogenes* cell count from the ground pork sample. On the other hand, the data from MPN method was showing similarity to the real-time PCR quantification results. The obtained growth parameters from *L. monocytogenes* in ground pork samples were further used to predict the growth rate of *L. monocytogenes* by the secondary Cardinal model. Great agreement between the actual growth rate under combined effects quantified by real-time PCR and the prediction by secondary Cardinal model was obtained, where the pRE was obtained as 1, A*f* as 1.0951, B*f* as 0.9283, RMSE as 0.0029 and R^2 as 0.9928. The predicted μ_{max} values from Cardinal model were showing good agreement to 263 ComBase data with A*f* and B*f* were calculated as 1.063 and 1.213, respectively. The results of this study indicate that a novel real-time PCR quantification technique combined with mathematical prediction model is highly potential to determine food formulation factors in food hurdle approach.

Abstract of assessment result

【Review】

Foodborne pathogens remain a major public health concern worldwide. Among various kinds of the source for foodborne disease from pathogenic bacteria, non-typhoidal *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* are considered as the major cause for foodborne disease. To estimate the potential risk of foodborne disease, the development of rapid growth prediction tool is necessary. However, until now, the experiments were performed in the limited environmental conditions (e.g. sterilized food materials or laboratory medium) by using conventional culture methods. The conventional culture methods, including the MPN technique, has been recognized to have several limitations such as high cost, time-consuming, labor-intensive, and cannot be used for routine test with large number of samples. So far, there is no report about the construction of high accuracy and specificity model for chicken juice, pasteurized milk, and ground pork products. The applicant has developed a rapid and specific quantification method for the application of prediction model by using real-time PCR. The method developed was successfully used to generate numerous growth data, further used to establish the predictive

models. This method has a potential to be widely used in food industry to estimate the risk of foodborne pathogens. This thesis provides scientific basis and innovation for the advancement of food science and technology.

【Result】

The final examination committee conducted a meeting as a final examination on June 19, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during the question-and-answer session. All the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Food Innovation.