Non-destructive determination of the total bacteria in flounder fillet by portable near infrared spectrometer

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ABSTRACT

There is an emerging trend of non-destructive and onsite analysis of microbial contaminations for better food safety. A new strategy for determination of total bacterial in fish products (flounder fillets) was established using a portable near infrared spectrometer. Results revealed that the pretreatment of near infrared spectrum by the wavelet transform could significantly improve the accuracy and precision of the analysis. In comparison to usually exploited partial least squares regression (PLS), a combination of genetic algorithm (GA) and back-propagation artificial neural network (BP-ANN) exhibited much better efficiency, and the correlation coefficient ($R$) and root mean square error (RMSE) of the prediction model were calculated as 0.985 and 0.095, respectively, and validated as 0.966 and 0.083, respectively. These results allowed us to suggest a promising potential of the established technique for non-destructive and onsite monitoring of total bacteria in fishery products.

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1. Introduction

Microbial contamination is considered one of the most important hazards for fishery products. Traditional techniques for determination of total bacteria in food are usually based on agar plate culturing and counting. Though these methods were well established and widely used with proven accuracy, repeatability and reproducibility, they are time-consuming, laborious and destructive in nature, which makes them unsuitable for onsite monitoring of large number of samples.

The development of simple, economical and accurate non-destructive analytical techniques for microbial contamination now receives increasing attention all over the world. For example, Peng et al. (2011) reported hyperspectral scattering for determination of total bacteria in beef, and Alexandrakis, Downey, and Scannell (2012) used Fourier transform mid-infrared spectroscopy for non-destructive analysis of the spoilage of intact chicken muscles. Near infrared spectroscopy (NIR) has also been reported for detection of bacteria in various food stuffs including vegetables, chickens and fishes (Alexandrakis, Downey, & Scannell, 2011; Egidio et al., 2009; Tito, Rodemann, & Powell, 2012). In comparison with traditional methods, such a NIR analysis was demonstrated much faster and simpler, however, there are still some tasks for its real application for food stuffs: i) most of previous studies were performed with large and expensive desktop devices, which indicated relatively poor flexibility and adaptability in resource-limited environments; ii) previous researches on aquatic products were very limited, and the sensitivity and precision were exhibited significantly lower than other techniques. For example Tito et al. (2012) built a NIR model for total bacteria in Atlantic salmon with partial least squares regression (PLS), but the value of correlation coefficient ($R$) and root mean square error (RMSE) was validated as only 0.64 and 0.32, respectively.

In this paper, a portable NIR spectrometer was exploited for determination of the total bacteria in fishery products. Moreover, a combination of genetic algorithm (GA) and back-propagation artificial neural network (BP-ANN) was developed to increase the accuracy and precision of the analysis. Based on the results, the efficiency of the developed technique was validated with flounder samples, and its potential for onsite monitoring of sea foods was also evaluated and discussed.

2. Material and methods

2.1. Reagents and materials

Plate Count Agar (PCA) was purchased from Beijing Land Bridge Technology co., LTD (Beijing, China). Flounders (Paralicthys...
olivaceus) were purchased alive from Jusco supermarket (Qingdao, China), and stored in seawater until further use.

2.2. Sample preparation

For each experiment about 3–4 flounders were killed and the muscles were cut into 35 fillets (with only white flesh) of about 2 cm × 2 cm × 1 cm (length × width × thickness), each with an approximate weight of 5 g. Then the fillets were packed into sterilized plastics separately, and stored at 4 °C for eight days. Every 12–24 h 3 fillets were sampled randomly for determination of the total bacteria. Therefore in each experiment 27–33 fillets were sampled and analyzed covering the whole process of preservation. The experiment was repeated four times, and a total of 120 fillet samples were analyzed for construction and validation of NIR models.

2.3. Microbiological analysis

The fillet sample was at first analyzed by the portable NIR spectrometer (wavelength 600–1100 nm, FQA-NIR GUN, FANTEC Research Institute of Japan, Shizuoka, Japan) under aseptic conditions. The NIR spectrometer was at first calibrated with sterilized glass slides and standard provided with the instrument, and the result showed that the slides had no significant effect on the basic spectrum. As demonstrated in Fig. 1, the fillet was placed on sterilized polythene (food grade, 200 mm × 200 mm, length × width), and then two glass slides (usually used for microscope, each of 25.4 mm × 76.2 mm × 0.8 mm, width × length × thick, sterilized by immersing in 75% ethanol for at least 30 min) were overlapped together on the surface of the fillet to make the obtained spectrum fall in the working range of the NIR spectrometer. The probe of the spectrometer was closely put on the slides, and for each side of the fillet sample, 6 points uniformly distributed on the surface were chosen and scanned. The average of the total 12 scans from each sample was used for determination of the corresponding total bacteria by mapping of the spectra matrix. The calculated data were then trained by PLS or ANN models to fit with the results of plate count, and normalized into the range of [−1, 1].

2.4. NIR data analysis

2.4.1. Pretreatment of NIR data

The NIR spectrum was pretreated by the db5 wavelet transform (Daubechies, 1992). The absorbance values of each sample (average of 12 points) contained 256 data points from 600 nm to 1100 nm, and for 120 samples a matrix of 256 × 120 was constructed. For wavelet transform, the matrix was decomposed into three layers of wavelet coefficient, in which the detail signal and the approximation signal were re-constructed. The second layer of detail coefficient was chosen as input data according to previous studies (Hu et al., 2007), and the number of data points of each spectrum was decreased from 256 to 70.

2.4.2. Analysis by the partial least squares regression (PLS)

The PLS regression model was directly created by Function Plsregress. The results of bacteria analysis (both NIR and aerobic plate count) from 90 samples were used for the construction of the model, and other 30 samples were used for validation of it. The correlation coefficient (R) and the root mean square error (RMSE) were used to evaluate the efficiency of the model.

![Fig. 1. The scheme of NIR determination.](image)

![Fig. 2. The typical NIR absorbance spectra of a flounder sample at the 1st day (A) and the 7th day (B). The results represented the data of 10 parallel determinations.](image)
2.4.3. Analysis by the artificial neural network (ANN)

The construction of BP-ANN was according to the reference (Hassoun, 1995). In brief, the system consisted of one input layer, one or more hidden layers, and one output layer. There were weight matrix “W” and threshold value “b” at each layer. The imported spectra moved through the input layer, hidden layer, and output layer sequentially, and then RMSE were calculated by comparing the result of NIR with that of aerobic plate count. If the RMSE was not acceptable, it would go back in the opposite direction to the input layer, and the “W” and “b” would be modified automatically to reduce the RMSE. The system was repeatedly trained with 90 samples, until the RMSE was less than the pre-set value.

A combined strategy of GA and BP-ANN was used for data analysis according to previous studies (Marengo, Bobba, Robotti, & Lenti, 2004). The data from NIR (pretreated by wavelet transform) and aerobic plate count were used as input data and output data, respectively. 90 samples were used to construct the model, and other 30 samples were used to validate the model. The $R$ and RMSE were used to evaluate the efficiency of the model.

3. Results and discussion

3.1. The NIR characteristics of flounder samples

Among the 120 flounder samples, the aerobic plate count was determined in the range of 3.7 log CFU/g – 9.8 log CFU/g with significant increase from the 1st day to the end of the preservation, which represented a complete process of fishery products from freshness to spoilage. Similar as that of the aerobic plate count, there was also a gradual increase in the NIR absorbance value with the prolonged storage time (Fig. 2, representing the 10 parallel determinations of a flounder sample at the 1st day and the 7th day, respectively), indicating a possible correlation between the NIR spectrum and the total bacteria in the flounder samples.

3.2. NIR analysis of the total bacteria by PLS model

A PLS model for the total bacteria was at first developed without pretreatment of the NIR spectrum. Based on the calibration curve, the value of $R$ and RMSE of the model was calculated as 0.937 and 0.18, respectively, and validated as 0.916 and 0.40, respectively (Fig. 3). To a large extent the relatively poor efficiency of the model may be attributed to the noise from the background. Therefore, to improve the accuracy and precision of the analysis, the wavelet transform was exploited for the pretreatment of the NIR spectrum. With such a pretreatment, the low-frequency background could be effectively removed while most of useful signals could be preserved (Fig. 4). After this modification a significant enhancement in the PLS model was observed (Fig. 5): the value of $R$ and RMSE of the calibration curve was calculated as 0.948 and 0.17, respectively. For validation of the model, the value of $R$ and RMSE was calculated as 0.952 and 0.33, respectively. These results allowed us to suggest the wavelet transform a useful tool for improvement of the NIR analysis.
3.3. NIR analysis of the total bacteria by ANN model

Overall the efficiency of the established PLS model was still not perfect enough as expected. Therefore, a combined strategy of GA and BP-ANN was further developed to improve the analysis. GA was derived from the theory of Darwinism and genetics, and has been exploited as a novel tool for global optimization (Huo & Xie, 2005; Conn, Gould, & Toint, 1991). BP-ANN is now considered much more effective than PLS for the solution of complex problems, especially when a non-linear relationship is involved (Marengo et al., 2004).

The training of GA system usually needs 50–200 generations (Goldberg, 1989), and here it was set as 100 generations. In fact, after 20 generations of training, the average fitness of the GA system coincided well with the theoretical optimum (Fig. 6). When the value of goal expectation was set as 0.01, the mean square error of the whole GA-BP-ANN system was reduced to 0.0091 after 244 times of training, which indicated a feasibility of the established model for further analysis (Fig. 7).

Using 90 flounder fillets as samples, the value of $R$ and RMSE of the GA-BP-ANN model for the total bacteria was determined as 0.985 and 0.095, respectively (Fig. 8). The model was further evaluated by a cross-validation procedure with the other 30 samples, and the value of $R$ and RMSE was calculated to be 0.966 and 0.083, respectively. These results indicated much better accuracy and precision of the GA-BP-ANN model for refrigerated fish samples than those of usually exploited PLS models; in fact such a efficiency for total bacteria was demonstrated excellent even in comparison to that of NIR analysis for other food stuffs such as chicken (Feng & Sun, 2013) and beef (Peng et al., 2011). Though the difference in chemical composition (such as fat), color and processing conditions (such as heating, freezing and smoking) may affect the accuracy and precision of the NIR analysis, the excellent flexibility and adaptability of the GA-BP-ANN system indicated a promising potential of the developed technique also for other fishery products after necessary modification of the models.

Fig. 5. The efficiency of the PLS model after wavelet transform of absorbance spectrum for analysis of the total bacteria in flounder samples during storage at 4 °C. (A) The calibration curve of the model. The value of the x-axis is a normalized value related to the log 10 of total bacteria, and the value of y-axis is a normalized value related to NIR absorption; (B) the validation curve of the model.

Fig. 6. The fitness of the Genetic Algorithm (GA) system.

Fig. 7. The training result of the BP-ANN.

Fig. 8. Theabo
4. Conclusions

A new method for the total bacterial counts in refrigerated flounder fillets was established and validated with a portable NIR spectrometer. We also reported that, a pretreatment of the NIR spectrum by the wavelet transform was able to improve the accuracy and precision of the analysis significantly. Moreover, a combined strategy of GA and BP-ANN was exhibited much better efficiency in comparison to the usually exploited PLS model. These results allowed us to suggest the established technique as a new and promising tool for non-destructive and onsite monitoring of the total bacteria in fishery products.

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References