Near infrared spectroscopy coupled with radial basis function neural network for at-line monitoring of *Lactococcus lactis* subsp. fermentation

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**Abstract** In our previous work, partial least squares (PLSs) were employed to develop the near infrared spectroscopy (NIRs) models for at-line (fast off-line) monitoring key parameters of *Lactococcus lactis* subsp. fermentation. In this study, radial basis function neural network (RBFNN) as a non-linear modeling method was investigated to develop NIRs models instead of PLS. A method named moving window radial basis function neural network (MWRBFNN) was applied to select the characteristic wavelength variables by using the degree approximation (Da) as criterion. Next, the RBFNN models with selected wavelength variables were optimized by selecting a suitable constant spread. Finally, the effective spectra pretreatment methods were selected by comparing the robustness of the optimum RBFNN models developed with pretreated spectra. The results demonstrated that the robustness of the optimal RBFNN models were better than the PLS models for at-line monitoring of glucose and pH of *L. lactis* subsp. fermentation.

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1. Introduction

*Lactococcus lactis* subsp. as a kind of probiotics has been used in food fermentation and used as a producer of Nisin which is one kind of biopreservative (Lv et al., 2004). Nisin composed of 34 amino acids is a lantibiotic with a very strong bactericidal effect, which is one of the safe, efficacious and non-toxic natural food grade biopreservatives with international permission (Cheigh and Pyun, 2005; Delves-Broughton et al., 1996; Soriano et al., 2004; Loir et al., 2005). *L. lactis* subsp. is usually used as an expression vector with its many advantages which are listed as follows: firstly, *L. lactis* subsp. secretes fewer
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proteins, which makes its extraction, separation and purification process much simpler; secondly, the degradation of the expression products could be greatly reduced as the low activity of extracellular enzyme secreted by *L. lactis* subsp. However, it was reported that *L. lactis* subsp. fermentations required harsh nutrition of broth and culturing conditions (Vuyst, 1995; Ashraf et al., 2013), therefore, the fermentation parameters should be strictly controlled to elevate the yield and the quality of fermentation. The nutritious component concentrations especially glucose and the pH of the broth must be strictly controlled during fermentation due to their significant effect on bacterial growth and the yield of the products. It should be an ideal idea that glucose and pH could be at-line measured, and then take appropriate feedback adjustments according to the measuring results, such as adding glucose, acid or base and so on during *L. lactis* subsp. fermentation processes (Butt et al., 2015). Though the traditional electrochemical probe for monitoring pH is very popular, it must be adjusted using pH standard solutions before application and the high temperature would shorten the life of the probe (Surhio et al., 2014). The electrochemical probe for monitoring glucose is much expensive and its operation is cumbersome, which restrains its application. It was reported that near infrared spectroscopy (NIRs) could be used for simultaneous at-line monitoring of some fermentation parameters such as glucose, biomass and pH etc. during fermentation using fiber optic cables (Fernández-Novalesa et al., 2008). As optic cables could withstand sterilization temperatures and the other fermentation conditions, NIRs could simultaneously strictly monitor and control the fermentation parameters under the extreme conditions. However, the data of NIRs are numerous and the information of the spectra would be usually interfered by the bubbles which are produced by agitation, the changing shape of the microbes and the variational viscosity of the broth, and so on. It is a challenge to parse the NIRs (Teixeira et al., 2009; Cervera et al., 2009; Rinnan et al., 2009). Chemometric methods were usually recommended to parse NIRs. Nowadays, principal component analysis (PCA) and partial least squares (PLS) as linear chemometric methods are usually used for NIRs modeling. Both of them have many advantages for linear multivariate analysis such as effectively reducing data dimensions, fast calculation and simple modeling processes with few modeling parameters. However, it was not so satisfying to use PCA and PLS for complex system modeling such as fermentation broth, food-processing monitoring, pharmaceutical process monitoring, and so on. Non-linear modeling methods should be considered under these situations (Madakyaru et al., 2012; Batool et al., 2015).

Artificial neural network (ANN) method is one of the most popular non-linear modeling methods. The fundamental principle of ANN simulates the work of the brain. Radial basis function neural network (RBFNN) is a kind of a three-layer feed-forward neural network with many advantages such as simple operation, fast calculation, good generalization, great robustness, and so on. RBFNN as the nonlinear modeling method has been widely used in many fields, such as the pattern recognition and function approximation (Bashera and Hajmeerb, 2000; Liu et al., 2010; Du et al., 2007). In this paper, RBFNN was used for modeling the correlation between the NIRs and the two parameters (glucose and pH) instead of the PLS method. The results of PLS models were reported in the literature (Guo et al., 2012).

Characteristic wavelength variables selection is a key step in the development of RBFNN model (Chu et al., 2004). Moving window radial basis function neural network (MWRBFNN) is a wavelength interval selection method for multi-component spectra analysis. Its fundamental principle is similar to that of moving windows partial least square (MWPLS) (Du et al., 2004; Khaskheli et al., 2015). Briefly, MWRBFNN builds a series of RBFNN models in a fixed size window that moves over the overall spectral region and then locates useful spectral regions in terms of the best capability of RBFNN models reaching a desired error level. MWRBFNN provides a viable approach to eliminate the extra variability generated by non-composition-related factors such as the perturbations in experimental conditions and physical properties of samples. A salient advantage of MWRBFNN is that the calibration model is very stable against the interference from non-composition related factors. Moreover, the selection of wavelength variables in terms of the best capability of the models enables the reduction of the size of a calibration sample set (Kasemsumran et al., 2004; Naureen et al., 2014).

In this paper, the established models have been optimized by selecting the suitable spectra pretreatment methods and the optimum parameters of MWRBFNN models such as *W*: the size of the moving window; *Wc*: the number of selected wavelength variables; *nc*: the number of selected moving windows; the number of hidden nodes and the spread constant. The capability parameters of the RBFNN models would be compared to those of PLS models for choosing the suitable modeling methods.

2. Materials and methods

2.1. Microorganism, medium components, fermentation and spectra measurement can be seen in literature (Guo et al., 2012)

2.1.1. Analytical methods

60% of the total samples were randomly selected as the calibration samples, and the remaining samples were selected as the prediction samples (external validation samples which were not used for calibration), the statistic glucose concentrations and pH values of the samples are shown in the Table 1. RBFNN was employed to develop the models for quantitative analysis of the glucose and the pH of fermentation broth with NIRs using Matlab R2010a (MathWorks, Inc., USA). The degree of approximation (*Da*) was used as the criterion for optimizing the developed models. The definition of *Da* is given by Eq. (1):

\[
Da = \frac{n_c \times \text{RMSEC}}{n} + \frac{n_p \times \text{RMSEP}}{n} + \frac{\text{RMSEC} - \text{RMSEP}}{n} \tag{1}
\]

where *n* is the total number of samples, *n*<sub>c</sub> is the number of calibration samples, *n*<sub>p</sub> is the number of prediction samples.

| Components     | Samples numbers | Average | Ranges        |
|----------------|-----------------|---------|---------------|
| Glucose (g/l)  | 145             | 9.768   | 2.210–18.258  |
| pH             | 120             | 6.082   | 4.670–7.690   |

Table 1 The statistical values of the glucose concentration and pH.
and $c$ is the constant which can be adjusted case by the chart. In the presented study, the $c$ for monitoring glucose and pH were 5 and 0.5 respectively. RMSEC is the root mean square error of calibration set; RMSEP is the root mean square error of prediction set.

The characteristic wavelength variables were selected by MWRBFNN. The MWRBFNN procedure was compiled by Matlab 2010a (Mathwork, USA). The basic steps of MWRBFNN procedure is as follows: A RBFNN model is developed with the wavelength variables in the moving window which starts at the first wavelength of the spectra and has been optimized by selecting the suitable number of hidden nodes using $Da$ as criterion. The maximum $Da$ value is recorded as the value of the first wavelength in the $Da$ spectra. The moving window moves down a wavelength. And than its’ wavelength variables are used for developing the RBFNN model which is also optimized as the first RBFNN model and the maximum $Da$ is recorded as the value of the second wavelength in the $Da$ spectra. It was done till the moving window moves across overall spectra regions and the $Da$ spectra are recorded for identifying the characteristic wavelength regions (Safi et al., 2015).

The characteristic wavelength variables were selected by combining several moving windows with the maximum $Da$ values. The RBFNN model is developed by the selected characteristic wavelength variables and optimized by selecting the suitable number of moving windows with the maximum $Da$ values ($n_a$), the number of hidden nodes and spread constant. The spread constant is fixed at 2.0 in this optimizing procedure. Finally, the RBFNN model with selected wavelength variables is optimized by selecting the suitable spread constant using $Da$ as criterion.

The size of the moving window is a key parameter of MWRBFNN for selecting wavelength variables. Therefore, the MWRBFNN procedure had been carried out when the size of the moving window was 5, 7, 9 and 11 for glucose and 21, 31, 41 and 51 for pH values which were set due to the preliminary experiments. The RBFNN models developed by raw spectra and each pretreated spectra were optimized by MWRBFNN and selected the most efficacious spectra pretreatment methods by using $Da$ as criterion.

NIRs were pretreated by Fast Fourier Transform (FFT), Savitzky–Golay Smoothing, first derivative, second derivative and Standard Normal Variate (SNV) methods with different sizes of pretreatment window using Origin 8.5.1 SR2 b315 (OriginLab Corporation, USA). The developed models were optimized by selecting efficacious spectra pretreatment methods with the suitable sizes of pretreatment windows, the characteristic wavelength variables, the number of hidden nodes and the spread constants by MWRBFNN.

3. Results and discussion

3.1. Samples

Sampling scheme greatly affects the NIRs model generalization as the NIRs would be influenced by different batches of fermentation with different conditions, fermentors or different fermentation phases (Cervera et al., 2009). 15 batches of $L$. lactis subsp. fermentation were carried out under different conditions which were designed by Box–Behnken design in 3 different fermentors. Broth samples were drawn out at an interval of 1 h and the glucose concentration and their pH values were determined by reference methods immediately. The glucose and pH profiles of each batch of $L$. lactis subsp. fermentation are shown in Fig. 1. As can be seen, glucose concentrations and pH values continually decreased since 2 h, and then, became stable after 7 h, which was indicating that 0–2 h was in incubative phase, 2–7 h was in growth phase and 7–10 h was in stable phase of $L$. lactis subsp. fermentation. The calibration samples were collected from each phase of different batches of $L$. lactis subsp. fermentation, which was good for the generalization of the calibration models.

It was proposed that infrared spectroscopic techniques, mainly NIR (780–2526 nm) and MIR (2500–40,000 nm), had in recent years become important tools in the context of bioprocess monitoring to rapidly assess the state of the culture. Both NIR and MIR spectroscopy allow a fingerprint of the culture bulk within a few minutes (Teixeira et al., 2009). However, there are several interfering factors such as aeration intensity, high biomass concentration, viscosity, filamentous production strain, temperature, microorganism morphology etc, that negatively influence the quality of chemometric models built for

![Figure 1](image.png)

Figure 1 The glucose and pH profiles of each batch of Lactococcus lactis subsp. fermentation ((A) glucose, (B) pH).
at-line or on-line monitoring of the cell culture or fermentation processes. In this paper, the NIRs of the samples are shown in Fig. 2. As can be seen, the NIRs of the samples showed strong absorption peaks in range of 1400–1600 nm which was the absorption of water O–H octave-band (Pasquini, 2003). The characteristic peaks of the target components have been concealed. Chemometric techniques such as PCA, PLS, ANN, etc. allow researchers to explore the relationship between NIRs and analyte concentration changes and remove the noise induced by the above mentioned interfering factors. PLS as a linear modeling method was used for monitoring the nisin titer, biomass, pH and glucose of \( L. \text{ lactis} \) subsp. fermentation broth had been reported in the literature (Guo et al., 2012). The selection of calibration method is one of the most important factors affecting the measurement accuracy with NIRs (Mireeia et al., 2014; Kiyani et al., 2014). The frequent non-linearity of the calibration models used in NIRs is the main source of large errors in analyte determinations with this technique. Non-linearity in this type of system arises from factors such as the multiplicative effect of differences in particle size among samples or an intrinsically non-linear absorbance–concentration relationship resulting from interactions between components, hydrogen bonding, etc (Blanco et al., 2000). In this paper, RBFNN as a non-linear modeling method was used for developing NIRs models for monitoring glucose and pH of \( L. \text{ lactis} \) subsp. fermentation broth expecting a better result than PLS models.

3.3. Selection of efficacious wavelength variables, the number of hidden nodes, spread constants and the spectra pretreatment methods

Selection of characteristic wavelength variables, the suitable number of hidden nodes and spread constant and the size of moving window of MWRBFNN and the suitable spectra pre-treatment methods greatly influence the measurement accuracy of NIRs. The influences of the selected characteristic wavelength variables, the size of moving window, the numbers of hidden nodes, the spread constants and spectra pretreatment methods on RBFNN \( Da \) values were studied respectively and the results are shown in Table 2.

3.4. Selection the characteristic wavelength variables

MWRBFNN was employed to select the characteristic wavelength variables with the fixed sizes of the moving window and the results are shown in Table 2. The \( Da \) spectra are shown in Fig. 3. As can be seen, the characteristic wavelength variables for monitoring glucose distribute in 800–920 nm, 990–1030 nm and 1320–1850 nm regions. The characteristic wavelengths for monitoring pH distribute in 1460–1560 nm and 1800–1850 nm, respectively. Via optimization by MWRBFNN, the number of the selected wavelengths for monitoring glucose and pH were 62 and 71 respectively.

3.5. Selection the number of hidden nodes

The effect of the number of hidden nodes on \( Da \) was investigated when the spread constant was fixed at 2.0 and the other parameters of RBFNN model were fixed at constant values which are shown in Table 2 and the results are shown in Fig. 4, as can be seen, \( RMSEC \) decreased along with the increase of the number of hidden nodes. However, \( RMSEP \) decreased firstly and rose later along with the increase of the

| Components             | \( W^1 \) | Pretreatment methods        | Windows | \( W^2_n \) | \( n^3_w \) | \( RMSEC \) | \( RMSEP \) | \( Da \) | \( NH^4 \) | Spread |
|------------------------|---------|-----------------------------|---------|-------------|------------|------------|------------|--------|--------|--------|
| Glucose concentration  | 7       | Original spectra            | 62      | 22          | 1.2987     | 1.3076     | 3.8133     | 19      | 0.53000|
|                        | 5       | Savitzky–Golay smoothing    | 31      | 17          | 1.9816     | 1.9834     | 2.3200     | 9       | 0.53000|
|                        | 7       | FFT                         | 21      | 33          | 2.0296     | 2.0073     | 2.4475     | 5       | 0.92000|
|                        | 5       | First order derivative      | 11      | 28          | 1.8983     | 1.9528     | 2.5323     | 9       | 0.98000|
|                        | 11      | Second order derivative     | 51      | 64          | 1.8358     | 2.0805     | 2.2956     | 11      | 0.00037|
|                        | 11      | Standard normal variate     | 26      | 4           | 2.0014     | 2.0204     | 2.4656     | 9       | 0.61000|
| pH                     | 20      | Original spectra            | 44      | 4           | 0.3774     | 0.3754     | 1.3204     | 17      | 0.21000|
|                        | 30      | Savitzky-Golay Smoothing    | 21      | 34          | 0.3593     | 0.3589     | 1.3906     | 18      | 0.21000|
|                        | 20      | FFT                         | 41      | 33          | 0.2942     | 0.2967     | 1.6799     | 17      | 0.33000|
|                        | 30      | First order derivative      | 51      | 104         | 0.3316     | 0.3361     | 1.4972     | 18      | 0.00056|
|                        | 40      | Second order derivative     | 51      | 127         | 0.3532     | 0.3625     | 1.3652     | 20      | 0.00029|
|                        | 30      | Standard normal variate     | 71      | 12          | 0.2439     | 0.2417     | 2.0390     | 28      | 0.13000|

1 \( W \): the size of the moving window; Windows: the size of pretreatment window.
2 \( W^2_n \): the number of selected wavelength variables.
3 \( n^3_w \): the number of selected windows.
4 \( NH \): the number of hidden nodes.
number of hidden nodes. Consequently, the trends of the Da curves for monitoring glucose and pH rose firstly and then decreased later, namely the prediction of model would become worse when increasing too many hidden nodes. This phenomenon was named over-fitting. The optimum numbers of the hidden nodes for monitoring glucose and pH with the highest Da values and the lowest RMSEP were 19 and 28 respectively.

3.6. Selection of the spread constants

The effect of the spread constants on RMSEC, RMSEP and Da value was investigated and the results are shown in Fig. 5. As can be seen, the RMSEC, RMSEP and Da value become stable when the spread constant of RBFNN models for monitoring glucose and pH were larger than 0.44 and 0.13 respectively. The optimum spread constants of RBFNN models for monitoring glucose and pH with the highest Da values were 0.53000 and 0.13000, respectively.
RBFNN has a better predictive capability. It was suggested was lower than that of the PLS model, meaning that model. The RMSEP monitoring the glucose were much better than those of the PLS models. In this research, the result supports the view that RBFNN model for monitoring pH with NIRs pretreated by SNV had the highest \( \text{RMSEC} \) and the lowest \( \text{RMSEP} \). It was concluded that the best methods for PLS modeling were SNV and FFT for determination of glucose and pH in the literature (Guo et al., 2012). It was suggested to select suitable spectra pretreatment method case to the modeling methods. In this research, the result supports the view that RBFNN has better fault tolerance on comparing to the PLS modeling method, the requirement of spectra pretreatment methods was less harsh. SNV pretreatment method which can remove the multiplicative interference of scatter and particle size work well in PLS model for monitoring glucose and RBFNN model for monitoring pH (Chu et al., 2004; Bampi et al., 2013).

3.7. Selection of spectra pretreatment methods

As shown in Table 2, the capability parameters including \( \text{RMSEC} \), \( \text{RMSEP} \) and \( Da \) of RBFNN model with raw NIRs for monitoring glucose were better than those of the models with pretreated NIRs. This result indicated that the spectra pretreatment methods investigated in this paper were not so suitable for calibrating the effective information of glucose in the NIRs of \( L.\ lactis \) subsp. fermentation broth. The RBFNN model for monitoring pH with NIRs pretreated by SNV had the highest \( Da \) values and the lowest \( \text{RMSEC} \) and \( \text{RMSEP} \). It was concluded that the best methods for PLS modeling were SNV and FFT for determination of glucose and pH in the literature (Guo et al., 2012). It was suggested to select suitable spectra pretreatment method case to the modeling methods. In this research, the result supports the view that RBFNN has better fault tolerance on comparing to the PLS modeling method, the requirement of spectra pretreatment methods was less harsh. SNV pretreatment method which can remove the multiplicative interference of scatter and particle size work well in PLS model for monitoring glucose and RBFNN model for monitoring pH (Chu et al., 2004; Bampi et al., 2013).

3.8. The comparison of the optimum RBFNN models and PLS models

The developed RBFNN models for monitoring glucose and pH have been optimized by selecting characteristic wavelength variables, the number of hidden nodes, and the spread constants and efficacious pretreatment methods. The efficacious spectra pretreatment methods for glucose and pH were without the pretreatment method and SNV method, the numbers of the characteristic wavelengths were 62 and 71, the suitable numbers of hidden nodes were 19 and 28, and the optimum spread constants were 0.53000 and 0.13000, respectively. The optimum RBFNN models with the optimized parameters were used for determining the glucose concentration and the pH values of all the samples and the results are shown in Fig. 6. The correlation coefficient of the calibration set (\( Rc \)) and the prediction set (\( Rp \)) of the optimum RBFNN model for monitoring glucose and pH were over 0.9000. The comparison of capability parameters between the optimum PLS models and RBFNN models are shown in Table 3. As can be seen, the \( Rc \) and \( \text{RMSEP} \) of RBFNN model for monitoring the glucose were much better than those of the PLS model. The \( \text{RMSEP} \) of RBFNN model for monitoring pH was lower than that of the PLS model, meaning that RBFNN has a better predictive capability. It was suggested that the non-linear modeling method was better than linear ones for NIRs and this conclusion was also supported by several literatures as follows: Bampi et al. (2013) applied NIRs with PLS and ANN to predict the average droplet size and water content in biodiesel emulsions respectively. The results indicated that the predictive capability of ANN models was better than those of PLS models. Nie et al. researched on using PLS, back propagation-artificial neural networks (BP-ANN), multiple linear regressions (MLR) and least square-support vector machine (LS-SVM) to develop models based on NIRs data for quantitative analysis of chrysin and galangin in Chinese propolis and concluded that BP-ANN had the best robustness. Fulop and Hancsok (2009) compared to the prediction efficiency of the models for determining the oleic acid concentration of vegetable oils developed by PCA-MLR, PLS, PCA-ANN and GA-ANN methods and concluded that the GA-ANN model was the best.

4. Conclusions

In this paper, NIRs combined with RBFNN were used to real-time monitor glucose and pH during \( L.\ lactis \) subsp. fermentation. The calibration and external validation samples were collected from 15 batches of the \( L.\ lactis \) subsp. fermentation in three different 5 l fermenters. The fermentation conditions including agitation rate, temperature, inoculate, air flow rate, seed age, work volume of 15 batch fermentations were designed by central composite design method, the samples were collected at every fermentation phase. Therefore, the model developed by these representative samples would have good generalization. Comparing to PLS models, RBFNN model shows better robustness in this research. This paper successfully combined NIR spectroscopy technology with MWRBFNN method to monitor the glucose concentration and pH during \( L.\ lactis \) subsp. fermentation, this paper could offer reference on key parameters for further NIR spectroscopy technology to real-time monitor bacterial fermentations.

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