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ORIGINAL ARTICLE

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.

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 processes 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 took 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 (PLSs) 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 (Basheera 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; W_n: the number of selected wavelength variables; n_w: 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):

$$Da = \frac{c}{\frac{n_c \times RMSEC}{n} + \frac{n_p \times RMSEP}{n} + |RMSEC - RMSEP|} \quad (1)$$

where n is the total number of samples, n_c is the number of calibration samples, n_p is the number of prediction samples

Table 1 The statistical values of the glucose concentration and pH.

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