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Rapid estimation of single cell oil content of solid-state fermented mass using near-infrared spectroscopy

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ABSTRACT

Calibration model using near-infrared reflectance spectroscopy (NIRS) for estimation of SCO content in solid-state fermented mass was established. The NIRS calibration model was derived by partial least-squares (PLS) regression and prediction of SCO contents of independent solid-state fermented mass samples fermented by different oleaginous fungi showed the model to be rapid and accurate, giving R^2-value higher than 0.9552 and root mean standard error of prediction (RMSEP) value lower than 0.5772%. The established NIRS calibration model could be used to estimate the SCO contents of the solid-state fermented masses and will provide much convenience to the research of SCO production in solid-state fermentation.

1. Introduction

Microbial oils, called single cell oils (SCO), have potential commercial applications as nutraceuticals, pharmaceuticals, feed ingredients for aquaculture and feedstock for producing biodiesel (Lewis et al., 2000; Miao and Wu, 2006). The high production cost of SCO is a major barrier to its commercialization (Ratledge, 2004). Solid-state fermentation (SSF), that is, cultivation of microorganisms on solid substrates in the absence or near absence of free water (Pérez-Correa and Agosin, 1999), has experienced particular interest due to many advantages of this bioprocess in comparison to submerged fermentation (SmF), e.g., smaller bioreactor volume, reduced downstream processing cost, higher productivity, simpler technique, reduced energy requirement, low wastewater output (Burke and Cairney, 1997; Kim et al., 1985). Some researches (Conti et al., 2001; Gema, 2002; Peng and Chen, 2007; Peng and Chen 2008; Stredansky et al., 2000) were engaged to produce SCO in solid-state fermentation from cereals, orange peel or straw in order to reduce the production cost and increase the production scale to satisfy the huge market requirement of SCO. In such researches it is necessary to estimate the SCO contents of the fermented masses to determine the fermentation results. The classical method for estimating the SCO content was complicated, time-consuming and reagent-consuming as described by Folch et al. (1957), hence not suited to estimate large quantities of samples. Another method of rapid estimation of SCO content was established by Kimura et al. (2004) using nile red fluorescence, but it was unfitted to the samples of solid-state fermented mass. Therefore a rapid and accurate method is needed to be established for estimation of the SCO content in solid-state fermented mass.

Recently, the development of near-infrared reflectance spectroscopy (NIRS) techniques and chemometrics have resulted in rapid detection for chemical components and been widely applied in the fields of food chemistry (Cen and He, 2007), pharmaceutical technologies (Roggo et al., 2007) and determination of chemical composition of straw (Jin and Chen, 2007; Liu and Chen, 2007). NIRS is a well-established technique for detection of chemical components as it has many advantages when compared with classical chemical and physical methods (Brenna and Berardo, 2004; Pontes et al., 2006). One of the advantages of NIRS is that it can record the multi-frequency and co-frequency information of organic molecules, which involves the response of molecular bonds C–H, N–H, C–O and O–H. Then the "fingerprint" of the sample will be built via a feature spectrum. This shows the possibility of using spectra to determine complex attributes of organic structures, which are related to gene, inner tissues and sensory characteristics. Due to large hidden information in spectral data, at present, particular attention has been paid to the data mining of numerous spectral data with chemometrics, which extends and improves the potential application of NIRS. Thus, it is effectively combined with multivariate analysis such as stepwise multiple linear regression (SMLR), principal component regression (PCR), and partial least-squares (PLS) analysis which provide the possibility to extract features from spectra and investigate spectral properties of samples (Lu et al., 2000).

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In this research, the rapid method for determination of SCO content of solid-state fermented mass using near-infrared reflectance spectroscopy (NIRS) is reported. This method is based on the construction of multivariate calibration models combining spectrometric data and traditional chemical analysis results obtained with conventional method. NIRS was used to develop NIR calibration model for the SCO content and to predict the SCO contents of new fermented mass samples. The method established in this research will be used to determine the SCO content of solid-state fermented mass rapidly and accurately.

2. Methods

2.1. Instrumentation and software

A Nicolet Nexus FT-NIR spectrometer (Thermo Nicolet Corporation, USA) was used to obtain NIRS spectra. The system TQ Analyst 6.2 (Thermo Nicolet Corporation, USA) was used for calculation and analysis of the spectra.

2.2. Samples

The samples of fermented mass were, respectively fermented by six species of oleaginous fungi in solid-state fermentation from steam-exploded wheat straw under different fermentation conditions (Peng and Chen, 2008). Five of the oleaginous fungi, such as *Microsphaeropsis* sp. *Phomopsis* sp., *Cephalosporium* sp., *Sclerocystis* sp. and *Nigrospora* sp., were endophytic fungi isolated from oleaginous plants (Peng and Chen 2007). Another oleaginous fungi was *Mortierella ramanniana* conserved in our laboratory. After solid-state fermentation the fermented masses were harvested and dried off at 80 °C then milled to particles which could all passed 40-mesh. The milled samples were collected and used for both chemical analysis of the SCO content and NIRS spectra scanning. As shown in Table 1, samples of fermented masses by *Microsphaeropsis* sp. and *M. ramanniana* were employed to develop the calibration model, and samples of fermented masses by all of the six oleaginous fungi were used for validation.

2.3. Spectrum collection, pre-processing and analysis of spectral data

The NIRS spectrum of each sample of fermented mass (about 1.0 g) loaded in a quartz cuvette (internal diameter = 20 mm, depth = 70 mm) was obtained by scanning with a Nicolet Nexus FT-NIR Systems spectrometer. The settings of the parameters in the experiment were as follows: the spectral range was 4000–10,000 cm^{-1}, number of scans was 64, and the resolution was 8 cm^{-1}. The detector has an integrating sphere to collect scattered radiation. Each sample spectrum was divided by the background spectrum so as to remove the effects caused by the instrument and atmospheric conditions hence ensuring that the peaks in the final spectrum were due solely to the sample. Every sample was scanned twice, and the two spectra were averaged. The averaged spectrum was pre-processed by a Karl Norris Derivative Filter to filter out noise, improving the signal-to-noise ratio of the data. Duplicate scans for the detected diffuse reflectance (R) of each sample were measured and averaged, and then transformed into apparent absorbencies ($\log 1/R$). Quantitative analysis was based on the absorption peak of samples (Liu and Chen, 2007).

Table 1
Fermented mass samples used in the research

Fungi	Samples	
	Development of the calibration model	Validation
Microsphaeropsis sp.	33	11
Mortierella ramanniana	35	12
Sclerocystis sp.	0	12
Phomopsis sp.	0	9
Cephalosporium sp.	0	10
Nigrospora sp.	0	10

2.4. Reference method for analysis of SCO content

SCO content of fermented mass sample was analyzed by Folch method (Folch et al., 1957) which was based on the weights of extracted SCO and dry fermented mass.

2.5. Multivariate calibrations

In order to relate the spectral data to the reference data, multivariate analysis was performed with a commercial spectral analysis program (TQ Analyst 6.2). Partial least-squares (PLS), stepwise multiple linear regression (SMLR), principal component regression (PCR) were used to optimize the model and the error between modeled and reference values, the root mean standard error of cross-validation (RMSECV), was reported. Validation of the model was performed by being used to predict the SCO contents of new fermented mass samples, giving the multiple coefficient of determination (R^2), the root mean standard error of prediction (RMSEP) (Kays and Barton, 2002; Liu and Chen, 2007).

$$\mathrm{RMSECV} = \sqrt{\frac{\sum(\hat{C}i - \hat{C}i)^2}{n-1}} \quad (1)$$

$$\mathrm{RMSEP} = \sqrt{\frac{\sum(\hat{C}i - \hat{C}i)^2}{m-1}} \quad (2)$$

where Ci is the reference measured values; $\hat{C}i$ is the NIRS modeled values; n is the number of samples in the model; m is the number of samples in the validation.

3. Results and discussion

3.1. NIRS model for SCO content of fermented mass

A total of 68 samples of fermented masses by *Microsphaeropsis* sp. and *M. ramanniana* were selected to develop the NIRS model of SCO content. SCO contents of these samples, analyzed by conventional Folch method (Folch et al., 1957), ranged from 3.2% to11.4%, the average value was 7.24%, and the S.D. was 0.42%.

The 68 fermented mass samples were scanned with a Nicolet Nexus FT-NIR Systems spectrometer. The scanned NIR spectrum of samples was smoothed and differentiated by the Karl Norris

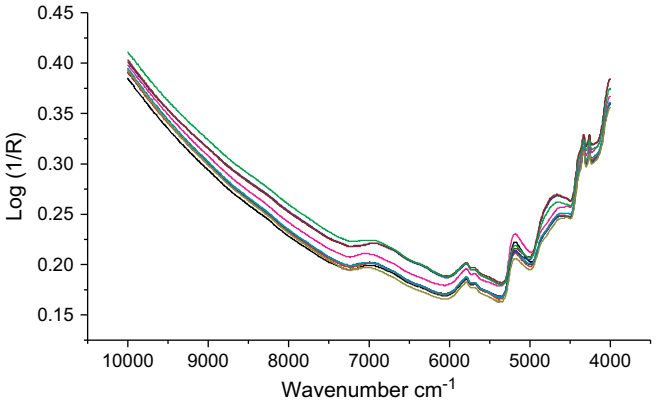

Fig. 1. NIR spectra of some solid-state fermented masses.