Monitoring of polypeptide content in the solid-state fermentation process of rapeseed meal using NIRS and chemometrics

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Abstract
This study combined Fourier transform-near infrared (NIR) spectroscopy and chemometrics, to monitor changes in peptide content during the solid-state fermentation of rapeseed meal. A NIR calibration model was established by performing spectral scanning on 81 samples and using interval partial least squares. The results showed that coefficient of determination ($R^2$) and root mean square error of cross-validation could achieve 0.9441 and 0.654 g/L for polypeptide content. Furthermore, the predicted and experimental values of the two parameters in an external validation set showed similar changes throughout the fermentation.

Practical applications
The results show that near-infrared spectroscopy is a promising method to monitor the chemical parameters of rapeseed meal during solid fermentation.

1 | INTRODUCTION

Global rapeseed meal production is reported to be a little over 25 Mton/year, and second to soybean meal in worldwide meal production (Sutter, Thevenieau, Bourdillon, & De Coninck, 2017). Rapeseed meal is a by-product obtained after the extraction of oil from rapeseeds, and is primarily composed of proteins, lignocellulosic fibers and minerals (Jin et al., 2016). Rapeseed meal is an excellent animal feed protein resource with very good basic amino acid balance. However, the digestibility of rapeseed meal for animals is poor owing to the presence of anti-nutritional substances such as glucosinolates, phytic acid, tannins, and so forth. (Shi et al., 2015). The antinutrients significantly impede the exploitation of rapeseed meal as feed or a potential source of protein for human nourishment; and as a consequence, previous research has focused on removing these substances (Żuchowski, Pecio, Jaszek, & Stochmal, 2013). Presently, rapeseed meal is recognized as potential source of bioactive peptide. Most studies have involved the use of various proteolytic enzymes to release ACE-inhibitory and antioxidant rapeseed peptides (Mäkinen, Streng, Larsen, Laine, & Pihlanto, 2016). Short peptides may be easily absorbed with strong antioxidant activity (Dai, Zhang, He, Xiong, & Ma, 2017); another approach is to convert the protein in rapeseed meal into a more digestible polypeptide by microbial fermentation.

Currently, methods such as liquid chromatography-UV (LC-UV) (Chen, Garrido Arias, Adams, & Van Schepdael, 2014), biuret technique (Hortin & Meilinger, 2005), modified bicinchoninic acid assay (Kapoor et al., 2009), and UV–VIS spectrophotometry has been used to determine the peptide concentration in biological samples (Sapan & Lundbald, 2015), and so forth. These methods, however, are considered traditional. Traditional methods have a limitation of meeting large-scale industrial production. As a consequence, exploring the use of recent and more accurate methods in determining the peptide concentration in biological samples is worthwhile. Near infrared (NIR) technology can respond to this need. It is an optical method which is fast, reliable, and nondestructive. Near-infrared spectroscopy (NIRS) has received wide application in the food, pharmaceutical, agriculture, and fermentation industry due to its advantages of being rapid, noninvasive, and cost-effective (Jiang, Liu, Xiao, et al., 2012b; Tong, Du,
Zheng, Wu, & Wang, 2015). Hongqiang and Hongzhang (2008) have shown that NIRs can be used for the prediction of moisture content and biomass. Jiang, Liu, Mei, et al. (2012a) have successfully applied Fourier transform-NIR (FT-NIR) and other spectroscopy methods to the determination of pH and moisture content in solid state fermented products. To the best of our knowledge, no study has been published on the use of NIRS and chemometrics in the determination of peptide in fermented rapeseed meal. Chemometrics helps to better interpret chemical information and relate quality parameters or physical attributes to data obtained from analytical instrument (Mishra, Srivastava, & Panda, 2018).

The main aim of this work was, therefore, to detect the polypeptides in solid-state fermentation of rapeseed meal using NIR spectroscopy and chemometrics.

2 | MATERIALS AND METHODS

2.1 | Preparation of rapeseed meal and bacteria strains

Rapeseed meal was purchased from China Zheng Da Oil Company, China. The main components of the rapeseed meal were: protein content (40%), moisture content (9%), and glucosinolate (46 μmol/g). Rapeseed meal was sieved (with a 40 mesh sieve) and stored at 4 °C for further processing.

Bacillus subtilis (for fermentation) was purchased from China Center of Industrial Culture Collection (CICC). A ring of bacteria was transferred into a basic medium, the medium was placed in a biochemical incubator (stationary culture 24 hr) to strain activation. The activated bacteria inoculated sterilized-medium was incubated for 24 hr at 30 °C and 180 r/min to prepare a fermented seed liquid.

2.2 | Fermentation and chemical analysis

Fermentation was carried out in 3-L laboratory fermentation tanks. The medium consisted of 400 g of sterilized rapeseed meal and 400 mL of sterile water, and 10% (8 mL) fermented seed liquid was added into each medium. Each strain, for fermentation, was used at 36 °C. Nine fermentations were conducted, and each lasted for 8 days with 9 data sets.

After each fermentation, a 20 g sample was picked and stored at 4 °C. The medium needed to be stirred every time when the sample was picked to ensure the necessary ventilation. Five gram (5 g) of each of the stored sample was then mixed with 100 mL of distilled water for the determination of amount of polypeptides.

The mixture was stirred for half an hour and centrifuged at 5,000 r / min for 30 min. Two milliliters (2 mL) of the supernatant was mixed with 2 mL of 10% TCA and allowed to stand for 10 min. The 4 mL mixture was centrifuged at 12,000 r / min for 15 min. After centrifugation, 2 mL of supernatant was mixed with 8 mL of biuret reagent and allowed to stand for 10 min, after which the absorbance was measured at 540 nm using UV-visible spectrophotometer (Persee, CHN). The polypeptide content of the sample was obtained by comparing the absorbance curve of Gly-Gly-Tyr-Arg.

2.3 | Spectrum collection

Near-infrared spectroscopy is a result of the complex pattern of scattering and absorption of many structures and biochemical components due to the stretching and vibrational effects of C-H, N-H, and O-H groups (Wang & Peng, 2017). Diffuse reflectance spectra of samples were collected using a FT-NIR spectrometer (Nicolet, USA), which covers from 4,000 cm⁻¹ to 10,000 cm⁻¹ and is equipped with a fiber optic probe. The polypeptide in the wavelength range has better absorption. An air background spectrum was collected as a reference prior to carrying out spectrum measurements. Spectrum was collected by directly inserting the fiber probe into the sample at room temperature (25 °C). All spectral measurements were performed in triplicate.

2.4 | Interval partial least squares

Interval partial least squares (IPLS) gives a general idea of the relevant information in different spectral intervals; it focuses on more relevant spectral regions and eliminates the interference of other regions. At that gist, each segment of the spectral interval PLS modeling to determine the model deviation of each section, selecting the area with the lowest prediction error during verification (Villar et al., 2017).

2.5 | Calibration of NIR spectroscopy models

Near-infrared spectroscopy models related to compound concentrations are usually established prior to using the NIRS. In this study, the tentative samples were first determined by chemical analysis of chemical value, and then the NIR spectroscopy calibration models for monitoring the polypeptide content in the fermentation process of rapeseed meal were established. The performance of the IPLS regression calibration model is usually assessed by the determination of the coefficient (R²), the cross-validation root mean square error (RMSECV), and the root mean square error of prediction (RMSEP).

3 | RESULTS AND DISCUSSION

3.1 | Chemical analysis

The fermentation of the rapeseed meal showed that microbial fermentation is beneficial for degrading rapeseed protein into polypeptide and consequently improves the amino acid composition (Xie et al., 2015). In this study, the initial polypeptide content of fermentation medium (rapeseed meal) was approximately 30 mg/g. A total of 81 samples collected from 9 fermentation tries were divided into a calibration set (72 samples from the first 8 fermentation tries) and a validation set (9 samples from the last fermentation tries) for the establishment of multivariate calibration models. Each set of data was obtained at the same fermentation conditions. Figure 1 shows the standard curve of peptides measured by UV–visible spectrophotometer. In addition, the rapeseed peptide contents during the entire fermentation process of rapeseed meal ranged from 30 to 180 mg/g. This result is similar to what has been reported by He et al. (2012), that after mixed solid-state fermentation, rapeseed peptide increased...
by 8.1%. Thus, a wide range of polypeptide content facilitates model calibration and monitoring of rapeseed meal fermentation.

3.2 | Choosing the best pretreatment strategy

To eliminate the unavoidable noise in the scanning process of the instrument, as well as reducing the impact brought by various external factors and simplifying the operation process of the data processing, a series of preprocessing should be performed on the spectral data obtained by the instrument detection (Blanco, Coello, Iturriaga, Maspoch, & Pagés, 2000). Standard normal variate correction (SNV) and multiplicative scatter correction (MSC) could eliminate the influence of scattering due to nonuniformity of particles on the spectrum. Savitzky–Golay (S-G) was an effective method to eliminate baseline noise and improve signal-to-noise ratio. Furthermore, derivative correction was used to eliminate the baseline shift and drift (scattering) in the spectrum, interference from other backgrounds, resolve overlapping peaks, and improve resolution and sensitivity (Figure 2). Table 1 summarizes the results obtained by applying different pretreatments strategies.

3.3 | Calibration models by IPLS regression

Multivariate calibration models based on NIR spectroscopy have several important parameters, including wavelength range, spectral preprocessing methods, latent variable (LV), RMSEP, and so forth (Liang et al., 2013). In this study, IPLS regression was used to establish models to quantify polypeptide. Various preprocessing methods including SNV, MSC, S-G, first derivative, and second derivative were used. The spectral range was divided into 15 segments and combined arbitrary 4 segments to establish the model. Several models were established based on different pretreatment methods. From all the models, the best results are listed in Table 1, where the minimal RMSECV and RMSEP and the maximal $R^2$ were shown. The best

![Standard curve of peptides measured by UV-visible spectrophotometer](image)

![FT-NIR spectroscopy images after different pretreatment methods](image)
predictive IPLS models for polypeptide content showed RMSECV = 0.5299 g/L and $R^2 = 0.943$ with 18LVs. Near-infrared spectroscopy has been shown as a fast and nondestructive method to determine the various parameters in the fermentation industry.

The correlation plots between chemical reference values and FT-NIR prediction values of the polypeptide content of rapeseed meal samples in the calibration set are shown in Figure 3. As can be seen from Table 1 and Figure 3, the optimal calibration model provides the values of the polypeptides in good agreement with those obtained from the chemical analysis. Consequently, it can be concluded that FT-NIR spectroscopy can be used to measure, with high accuracy, the polypeptide content of rapeseed meal (under fermentation conditions) by FT-NIR spectroscopy.

### 3.4 Application of models in the rapeseed solid-fermentation process

To assess the FT-NIR calibration model established in solid state fermentation of rapeseed meal, the last trial of data was used as an external validation set. Figure 4 shows the correlation between changes in polypeptide content in rapeseed meal solid state fermentation and the predictions obtained from the FT-NIR spectral calibration model. The predicted and measured values of the 2 parameters showed similar variations over the entire fermentation period. The RMSEP and $R^2$ between the predicted and measured values of the polypeptide content were 0.272 g/L and 0.9897. All these results suggested that the calibration models have good predictive capacity for polypeptide content (1.2–8 g/L).

### 4 CONCLUSIONS

The results of this study show that the use of NIR spectroscopy and the chemometric method, IPLS regression offers an efficient and rapid approach for the concurrent determination of polypeptide in rapeseed meal under fermentation conditions. The predicted values obtained by the near-infrared spectroscopy model are consistent with those obtained by the UV–VIS spectrophotometry. The overall results indicated that using fiber-optic FT-NIR spectroscopy to monitor the changes of polypeptide content during solid state fermentation process of rapeseed meal has a good outcome. Thus, this study provides a viable approach for the detection of other chemical constituents.

Applying a well-established mathematical model to industrial...
production can help to achieve on-line monitoring of chemical constituents and provide real-time signals related to process and final product quality.

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