Industrial Crops and Products 42 (2013) 357–362

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Non destructive estimation of total phenol and crude fiber content in intact seeds of rapeseed–mustard using FTNIR

Manju Bala*, Maharaj Singh

Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur, Rajasthan 321 303, India

ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form 19 May 2012
Accepted 9 June 2012

Keywords:
Crude fiber
Fourier transform near infrared spectroscopy
FTNIR
Rapeseed–mustard
Total phenol

ABSTRACT

In the present study calibration models were developed for non destructive estimation of total phenol and crude fiber content in intact rapeseed mustard seed by Fourier transform near infrared spectroscopy (FTNIR). Rapeseed–mustard (*Brassica* spp.) is an important group of oilseed crops in India. The defatted meal that is left after oil extraction is a high value by-product for animal nutrition and is also a potential source of protein for human nutrition. However the utilization of mustard meal in animal (monogastric livestock such as poultry and pigs) and human nutrition is limited due to high content of antinutritive compounds such as fiber and phenolic. In order to develop genotypes with low or high contents of phenol and fiber fast screening of existing genotypes is required. The traditional methods for estimation of total phenol and crude fiber are destructive and time consuming. A total of 115 rapeseed mustard genotypes were quantitatively analyzed for total phenol and crude fiber content by wet chemical methods, their Fourier transform near infrared spectra was correlated to resulting data by means of partial least square regression and calibration models were developed. The optimal models were achieved with coefficient of determination $(R)^2$ of 0.96 and 0.91 and root mean square error of cross validation (RMSECV) of 0.08 and 0.41 with residual predictive deviation (RPD) values of 4.98 and 3.37 for total phenol and crude fiber, respectively. Test validation resulted in RMSEP of 0.11 for total phenol and RMSEP of 0.28 was observed for crude fiber content.

1. Introduction

Rapeseed–mustard (*Brassica* spp.) is an important group of oilseed crops in India. The oil is an important dietary component, especially in Eastern and North-Western parts of the country (Chauhan et al., 2011). Rapeseed mustard meal that is left after oil extraction is a valuable feedstuff for animal nutrition and a potential protein source for human nutrition. The high nutritional value of seed meal as a result of high energy, protein content, and favorable amino acid composition is restricted due to the presence of antinutritional compounds such as glucosinolates, phenolic acids, phytic acid, and fiber (Alireza Sadeghi and Bhagya, 2009). Phenolics are predominantly located in the embryo and a small amount found in the hull and are often responsible for the dark color, bitter taste, and astringency of *brassica* seed and meal. These compounds were previously considered undesirable because they and/or their oxidized products are known to form complexes with essential amino acids, enzymes, and other substances (Shahidi and Naczk, 1992). These compounds are nowadays emerging as value added products, as they exhibit antioxidant properties (Das et al., 2009; Khattab et al., 2010; Bala et al., 2011) as well as antiviral, anticarcinogenic, anti-inflammatory activities (Vuorela et al., 2005). So genotypes with low or high phenol content could find use.

High content (12–13%) of indigestible fiber limits the use of rapeseed/mustard meal in the diets of monogastric animals (Slominski et al., 1994) as it may influence negatively protein digestibility and bioavailability of minerals such as manganese and zinc. In order to utilize or develop genotypes with low content of phenol and fiber, fast screening of existing genotypes is required. The genotypes which are screened with high phenols could be utilized for industrial antioxidants preparation.

Currently, chemical detergent methods are generally used to estimate total phenolic and crude fiber contents. However, these methods are expensive, time consuming, and require destruction of seed samples. These methods can and are used in breeding programs but sampling and destruction of seeds is a must in these cases. The development of low cost, non-destructive, high throughput screening, and selection techniques to measure total phenolic and crude fiber content would increase breeding efficiency. Newer technological advances have brought a rapid, lower cost analytical technique termed near infrared reflectance (NIR) spectroscopy. The use of NIR spectroscopy has already been reported for the

* Corresponding author. Tel.: +91 5644 260379; fax: +91 5644 260565.
E-mail address: avimithi@gmail.com (M. Bala).

0926-6690/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2012.06.014

non-destructive screening of oil, fatty acids, protein, amino acid, and individual and total glucosinolate content of rapeseed mustard seeds (Font et al., 2004; Hom et al., 2007; Petisco et al., 2010; Chen et al., 2011) in large breeding populations.

The near-infrared (NIR) region is part of the electromagnetic spectrum between the visible and the microwave wavelengths, ranging from 750 to 2500 nm (13,400–4000 cm^{-1}), related to vibration and combination overtones of the fundamental O—H, C—H, and N—H bonds, which are the primary structural components of organic molecules (Williams and Norris, 2002). Near infrared spectroscopy is characterized by low molar absorptivities and scattering, which allows nearly effortless evaluation of pure materials (Cozzolino, 2010). The combination of NIR spectroscopy and chemometrics (multivariate data analysis techniques) could be applied to many foods and agricultural commodities and is widely used in the cereal, oilseed, dairy, horticulture, and other processing industries to predict chemical composition of biological products with high accuracy (Cozzolino, 2009, 2010; Ferrer-Gallego et al., 2011). Moreover, FTNIR spectroscopy is a fast, accurate, and non-destructive technique which requires minimal or no sample preparation, and can be used as a replacement of conventional time-consuming chemical methods. To date, no attempt has been made to determine total phenol and crude fiber content in India, for rapeseed mustard meal based on intact seeds by NIRS. Keeping in view the potential advantages of NIR over chemical methods present study was undertaken to develop calibration models for estimation of total phenol and crude fiber content of Indian rapeseed mustard genotypes and to explore its applicability in identifying variability for these traits.

2. Materials and methods

2.1. Sample collection

Seed samples of rapeseed mustard 115 genotypes used in present study were obtained from the germplasm section of Directorate of Rapeseed Mustard, Bharatpur, Rajasthan, India.

2.2. Chemical analysis

The seeds were grounded and extracted with hexane in a Soxhlet extractor for 9 h. Oil content was measured gravimetrically. The defatted meals were air dried and ground into a fine powder. Total phenol content was determined by the method of Singleton et al. (1999). Total phenol content was expressed as % as well as mg g^{-1} gallic acid equivalent on seed meal basis. For the calibration of NIR percent values were used. Crude fiber content was estimated by the method of Ahuja and Bajaj (1999) and values were expressed as percent cellulose equivalent.

2.3. FTNIR spectroscopy

For the FTNIR measurement, the seed samples were poured into glass vials and set into sample holder for the spectral acquisition. NIR spectra were collected by using a FTNIR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with an integrative sphere, over the range 12,800–3600 cm^{-1} (780–2780 nm) at 1 nm interval and were stored. The spectrum of each sample was the average of 32 scans. OPUS spectroscopy software (v. 6.0 Bruker Optics, Ettlingen, Germany) was used for spectral acquisition and instrumental control.

2.4. Data pre-processing

Data pre-treatment using mathematical transformation (e.g., derivatives, multiple scatter correction, smoothing) of the NIR spectra were applied to enhance spectral features and/or remove or reduce unwanted sources of variation. The spectral datasets were correlated with total phenolic and crude fiber content by using partial least squares (PLS) regression algorithm. To evaluate the calibration performance of the developed models cross validation was used and also a test set validation was performed. Various statistics such as the coefficient of correlation (R^2) in cross-validation and the root mean standard error in cross-validation (RMSECV) as well as residual predictive deviation (RPD) were computed. The RMSECV is the prediction error of a calibration model and it is defined as the standard deviation of differences between spectral data and reference values in the cross-validation sample set. This value gives the average uncertainty that can be expected for predictions of future samples. The optimum calibrations were selected based on minimum value of RMSECV. In test set validation, root mean square error of prediction (RMSEP) was computed. The residual predictive deviation (RPD), defined as the ratio between the standard deviation of the population's reference values and the standard error of performance (RMSECV/RMSEP) was used to verify the accuracy of the calibration models developed. The higher the value of the RPD the greater the probability of the model to predict the chemical composition in samples set accurately. An RPD value range between 2.4 and 3.00 is considered poor and the models could be applied only for very rough screening, while an RPD value greater than three (range 3.1–4.9) and greater than five (range 5–6.4) could be considered fair and recommended for screening purposes and good for quality control, respectively (Williams and Norris, 2002).

3. Results and discussion

3.1. Chemical analysis

The range of values obtained for rapeseed mustard germplasm using wet chemical methods for total phenol content and crude fiber content are presented in Table 1. The total phenol content in seed varied from 0.78% (7.8 mg g^{-1} GAE) to 2.39% (23.9 mg g^{-1} GAE). The mean phenol content was 1.69% (16.9 mg g^{-1} GAE). The minimum value was reported in 'PT-30' genotype of *Brassica rapa* var toria, while maximum value was observed in 'CC-220' genotype of *Brassica juncea* (L.). Total phenol contents (as gallic acid equivalent) have been reported in the range of 9.16–16.13 mg g^{-1} in canola meal (Khattab et al., 2010) and 14.11–21 mg g^{-1} (Alireza Sadeghi and Bhagya, 2009; Bala et al., 2011) in *B. juncea* meal. So in the present study values were similar to and in range as reported by other workers. The results of crude fiber content were expressed as percentage on seed meal basis. The crude fiber content of the samples ranged from 5.12 to 12.06% on the basis of seed meal showing variability for this trait, and the mean crude fiber content was 8.71%. The minimum value was reported in 'Sheetal' variety of *Brassica napus* while maximum value was observed in 'Panchali' genotype of *B. rapa* var toria. The values of these parameters were in approximation with the data of other workers who have reported crude fiber content in Indian genotypes in the range of 6.3–18.9% (Chauhan and Kumar, 2011).

3.2. FTNIR spectra

To perform the NIR calibration model for prediction of total phenol and crude fiber content of rapeseed mustard seeds spectra were collected in whole available spectral domain 12,800–3600 cm^{-1} (780–2780 nm). Fig. 1 represents the spectra of samples taken in the whole NIR range. It is clear from the figure that spectral patterns of all the samples were found to be similar across the whole wavelength range along the *X*-axis, however along the *Y*-axis changes among different samples were obvious. Prediction equations for