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Fourier transform-near infrared reflectance spectroscopy calibration development for screening of oil content of intact safflower seeds

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Abstract

A FT-NIRS calibration was developed for screening of oil content in intact seeds of Safflower. FT-NIR spectra of 204 samples were obtained from 32 scans at a resolution of 1 cm^{-1} throughout the near infrared region (12500-4000 cm^{-1}). Straight line subtraction was found to be the best spectral preprocessing method for the calibration development of oil content in intact Safflower seeds. FT-NIRS calibration developed was cross validated with 26 samples. Reference values showed closed relationship with FT-NIRS value ($y = 0.5354x + 16.093$, $r^2 = 0.853$).

Introduction

Near infrared reflectance (NIR), introduced in 1964, is based on the absorbance of light energy at a given frequency by molecules (or radicals) having a permanent dipole vibrating at the same frequency. Since its first application for determination of moisture in grains and seed, this technique has developed as an analytical method finding application in feed, food, petrochemical, pharmaceutical industries as well as in many other fields also (Panford and de Man, 1990). In oilseeds research laboratories, this technique has been extensively used as a nondestructive technique for rapid estimation of moisture, oil, protein, and fatty acid and anti-nutritional compounds (Kumar *et al*., 2010). In recent years, NIR has been coupled with the mathematical operations known as Fourier transformations (FT) to get FT-NIRS. Fourier transformations is a powerful method to enhance the signal and noise ratio. With FT-NIR spectrometer, data are rapidly collected in time domain and then converted by FT to conventional frequency domain. As the time required for a single scan is reduced, the time required for ensemble averaging is also reduced. FT-NIR spectrometers are an advancement over the conventional instruments having more energy throughput, excellent wave number reproducibility, extensive data manipulative capabilities, accuracy and advance chemometric software to handle calibration development. FT-NIR spectrometer requires smaller quantity of sample in comparison to NIR. With combination of FT and chemo metrics, whole spectrum is recorded instantaneously and simultaneously. FT-NIRS promotes the "green analytical technique" as the use of hazardous solvents and reagents can be avoided using this technique after calibration development. Besides being a nondestructive technique, FT-NIRS also offers advantages such as fast, ease of use in instrumental operation with minimal or no sample processing. Chemo metrics combined with near infrared spectroscopy (NIRS) facilitates estimation of many quality traits on the basis of simultaneous measurement of large amounts of composition related data once the mathematical relationship between the instrumental parameters and quality traits are established (Cozzolino *et al*., 2008). FT-NIRS has been used for the analysis of many agricultural, food products and has become standard technique for estimation of oil content in oilseed crops (Perez- Vich *et al*., 1998; Hom *et al*., 2007). NIR spectroscopy has shown promising results for estimation of oil content in oilseed crops. Safflower is one of the important oilseed crops grown worldwide (Singh, 2006). Safflower seed contains 25-47 % oil rich in nutritionally desirable unsaturated fatty acids and tocopherol (Nagraj, 1993; Velasco *et al*., 2002). A rapid, inexpensive and robust technique is essential for successful crop improvement programme as

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screening of large number of samples is required and also it is difficult to compare seed oil content in breeding programme unless a standard procedure is used. Also, for marketing and processing, a precise estimation of oil content is needed. Keeping this object in view, a FT-NIRS calibration was developed for the estimation of oil content in intact seed of Safflower.

Materials and Methods

Sample and determination of oil content

Two hundred and four Safflower germplasm collections grown in the year 2009 were used for calibration development and validation. Seed samples were oven dried at 38^0C for 24 h and oil content (%) of intact seed samples determined using a pre-calibrated NMR analyzer (Oxford 4000).The sample size was about 20 g and the NMR integration period was approximately 80 second. Instrument was calibrated to display oil content of the seed sample. Standard safflower oil mixed with glass beads was periodically measured to check the instrument settings.The average of the three readings was recorded for each sample.

FT-NIRS analysis

A multipurpose analyzer FT-NIR spectrometer (Matrix I, Brucker Optics, Germany) was used to obtain spectra. About 2.0 g of the intact seed sample was placed into quartz sample cup and placed into sample holder. Sample was then irradiated with near infrared monochromatic light and reflectance was collected with lead sulphide detectors in the frequency range of 4000-12000 cm^{-1} to give a total of 32 data points per sample. Multivariate analysis was performed with OPUS QUANT (Bruker, Germany) for correlating the spectral data to oil content values obtained from NMR analyser. Partial least square (PLS) regression was used for development of model regression. The performance of the final PLS regression model was evaluated in terms of root mean square error of cross validation (RMSECV), standard error of prediction and correlation coefficient (r^2). For cross validation using PLS, leave one sample out method removing the spectrum of one sample was used. Left out sample was predicted with this model and the procedure was repeated with leaving out each sample of the calibration set. Finally, the correlation coefficients between the predicted and standard value were calculated for both the calibration set and test set.

Results and Discussion

Infrared spectroscopy, a vibrational spectroscopic method coupled with chemometric techniques has emerged as an analytical technique to quantify quality traits in oilseeds. Although, nuclear magnetic resonance (NMR), also a nondestructive technology for estimation of oil content in oilseeds has reduced the analysis time significantly but not considered enough for meeting the market requirements (Panford and de Man, 1990). Earlier, Panford *et al*. (1988) reported estimation of oil content in ground Safflower achne using monochromator based NIR spectrometer. Elfadl *et al*. (2010) also reported NIRS calibration model for estimation of oil content in ground samples of Safflower. We report a FT-NIR calibration for screening of oil content in intact seeds of Safflower. Samples were divided into 4:1 ratio for calibration and validation group respectively and oil content (%) in samples estimated by a pre-calibrated NMR with the oil content values obtained from Soxhlet extraction. The oil content in safflower seed ranged from 28.29 - 38.79%. FT-NIR spectra of 204 samples were obtained from 32 scans at a resolution of 1 cm-1 throughout the near infrared region (12500-4000 cm^{-1}). These spectra were subtracted against background air spectrum (Figure 1A).

NIR spectrum of sample depends on the chemical composition and on the physical characteristics of the sample which are usually observed as the background and noise of the spectrum (Chen *et al*., 2007). Therefore, preprocessing options are used to eliminate offset or different linear baselines. Processing of the recorded average spectra data of Safflower seeds were carried out using the following preprocessing options: (1) no spectral data processing (2) constant offset elimination (3) straight line subtraction (4) vector normalization (5) min-max normalization (6) multiple scattering correction (7) first derivative (8) second derivative (9) first derivative + straight line subtraction (10) first derivative + vector normalization and (11) first derivative + multiple scattering. Preprocessing options were used to optimize the validation parameters in terms of rank, RMSECV and residual prediction deviation (RPD) values (Table 1). As compared to no spectral processing, the spectra of safflower seeds were significantly improved by preprocessing options. Constant offset elimination enhances the intrinsic matrix absorption of the material, thereby, enabling the extraction of significant information from the matrix of original spectra.

Fats and oils are constituted of fatty acids esterified with glycerol, a tri hydroxyl alcohol, with

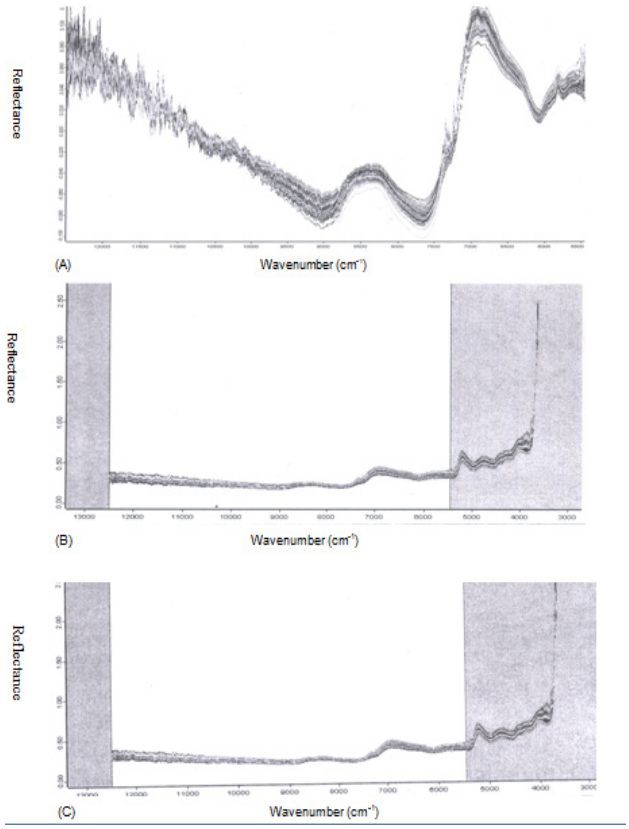

Figure 1. (A) Typical FT-NIR reflectance (log1/R) spectra obtained from Safflower seed samples (B) effect of straight line subtraction spectral preprocessing on original spectra of Safflower seed (C) effect of constant offset elimination spectral preprocessing on original spectra of Safflower seed

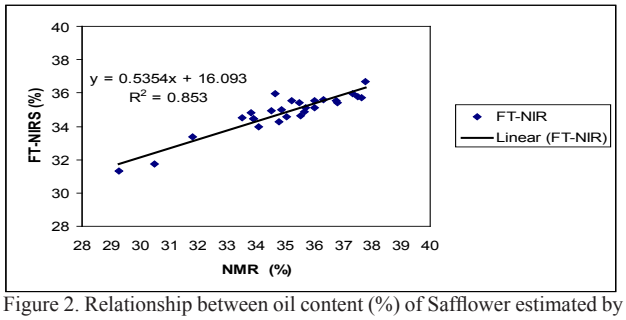

Figure 2. Relationship between oil content (%) of Safflower estimated by NMR and FT-NIRS methods

different carbon number, position of double bond and fatty acids attached to glycerol. Because of this, different oilseeds have different wavelengths assigned to oil band due to interactions between peaks, particle size, chain length and degree of saturation and unsaturation (O'brien, 2004). The wavelength for oil in whole safflower seed obtained in the present study were in the range of 800-1836 nm (12493.3-5446.6 cm^{-1}). Earlier, also in ground safflower achne optimal wavelength for oil ranged from 1000-2175 nm (Panford *et al*., 1988; Elfadl *et al*., 2010)

Spectral data and the chemical data are written in the form of matrices, in which each row represents a sample spectrum. Rank (the number of PLS vectors)

Table 1. Optimization of spectral preprocessing method for estimation of oil content (%) in intact seeds of Safflower using FT-NIRS

Preprocessing method	Rank	RMSECV	RPD	R^2	Spectral range (cm^{-1})
Multiple scattering	5	0.41	0.312	0.89	12493.3 - 4246.7
First derivative	5	0.71	1.75	0.68	7502.1 - 5446.3
Second derivative	7	0.63	1.98	0.74	6102 - 4246.7
Straight line subtraction	6	0.28	4.49	0.95	12493.3-5446.3
Constant offset elimination	6	0.28	4.49	0.91	12493.3-5446.3
Vector normalization	5	0.41	3.02	0.89	5450.1-4597.7
Min-max normalization	5	0.39	3.13	0.89	12493.3-4597.7
First derivative + straight line substraction	5	0.65	1.91	0.72	7502.15446.3
First derivative + vector normalization	5	0.71	1.75	0.67	7502.14597.7
First derivative + multiple scattering	5	0.7	1.77	0.68	7502.1-5446.3
No spectral processing	3	2	1.24	0.12	12493.3-5446.3

is crucial for the quality of the calibration model. Rank below 10 is desirable (Yang *et al*., 2008). All the spectral preprocessing method had rank below 10 (Table 1). RMSECV is a quantitative measure for the preciseness with which the samples are predicted during validation. RMSECV values obtained using different preprocessing methods were in the range of 0.277-0.709. Lowest value of RMMSECV (0.277) was obtained using straight line subtraction preprocessing method and it was maximum in case of first derivative. Residual Prediction Deviation (RPD) is qualitative measurement of the assessment of validation results. Larger the RPD, the better is the calibration. RPD values greater than 2.5 is desirable as lower value can result from the narrow range of reference value (Cozzolino *et al*., 2008). RPD values ranged from 0.312 - 4.49. Straight line subtraction and Constant offset elimination preprocessing methods had the highest RPD value (4.49). All the preprocessing methods except first derivative had high coefficient of determination (R^2) ranging from 0.675-0.950, exhibiting high correlation between the oil content data and spectral data.

Straight line subtraction (Figure 1B) was found to be the best processing method for the calibration development of oil content in intact Safflower seeds. Accordingly, the calibration parameters were developed by using the information from the best spectral region. Using stepwise elimination of outliers from the calibration graph, the efficiency of the method was gradually improved. 1828 selected data points generated from 47 standard samples were finally used to develop the calibration. Calibration was validated by comparing the oil content values estimated by NMR and FT-NIRS calibration methods

in 26 samples. Values showed closed relationship (y = 0.5354x +16.093, r^2 =0.853) (Figure 2). Although calibration equation based on ground samples are reported to be more accurate than those based on the intact seed sample possibly due to homogeneity of ground sample and surface and size diversity of intact seeds. However, long period of grinding leads to the destruction of oil bodies as well as an increase in temperature of the sample forming conglomerates, which cannot be eliminated by following mixing. Further, intact seed after measurement of oil concentration can be utilized for the breeding purposes.

Conclusion

The present investigation revealed that FT-NIRS could be successfully utilized for rapid mass screening for oil content in intact seeds of Safflower. Thus, enhancing the effectiveness of quality breeding programme aiming at developing Safflower varieties with high oil content.

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