DEVELOPMENT OF MULTIVARIATE REGRESSION MODEL FOR QUANTIFICATION OF PROXIMATE CONTENT IN VIGNA RADIATA USING FOURIER TRANSFORM –NIR SPECTROSCOPY

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Abstract: The Fourier Transform Near Infrared (FT-NIR) absorbance spectra (12800-3600 cm⁻¹) of 222 green gram samples was used to build calibration models for the determination of the content of protein, fat and carbohydrate. The samples that comprised the dataset had an average composition of 22.18% of protein, 1.30% fat, and 50.72% carbohydrate. Multivariate regression was used to develop the quantitative models for protein, fat and carbohydrate compounds. The root mean square error of cross validation (RMSECV) was 0.191 (R² = 91.52) for protein, 0.0271 (R² = 88.54) for fat and 0.765 (R² = 93.62) for carbohydrate. A fast, simple and accurate method to quantify the proximate content of green gram was developed by using FT-NIR spectroscopy. The results showed that FT-NIR spectroscopy could support chemical analysis methods.

Key words: FT-NIR Spectroscopy, green gram, protein, first derivative, calibration

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INTRODUCTION

Green gram (Vigna radiata) is one of the important pulse crops in India. It is a protein rich staple food [1]. It contains about 24 percent protein, which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. The biological value improves greatly, when wheat or rice is combined with green gram because of the complementary relationship of the essential amino acids. The biochemical composition changes the structure of the grains. It influences the engineering properties of grains reported by [2-4]. Most common methods reported for the determination of protein content are kjeldahl and lowery method. But these methods are tedious, time-consuming, destructive, not economically viable and as they require highly skilled operators.

Fourier transform near-infrared (FT-NIR) spectroscopy is an analytical technique that has gained popularity in recent years for analyzing a wide variety of samples used in used in nutritional, pharmaceutical, petrochemical, textile and agricultural industries. The major strength of FT-NIR spectroscopy is a rapid technique, nondestructive, accurate and that can be employed as a replacement for time-consuming chemical methods. It is a non-invasive method for the characterization of fat, nitrogen and moisture content in cocoa powder [5] and protein and moisture in fishmeal [6]. Indistinctly, many authors have used different ranges of wavelengths, which extend from the visible spectrum to the NIR in order to estimate multiple properties and they have established different wavelengths ranges. But until now there is not consent between researchers which is the best wavelengths range to study each grain parameters due to different grain nature, type, characteristic and influence of grain grow environments [7, 5] conclude that the second derivative of NIR is the recommended procedure to quantify fat, nitrogen, and moisture content in cocoa powders by infrared spectroscopy and also showed that no single wavelengths were strongly correlated with the protein, fat and carbohydrate content of cocoa powder, which indicates the difficulty of using selected wavelengths or bans to accurately predict the protein, fat and carbohydrate content of the selected product.

On the other hand, India still lags behind the countries with a well-developed of grain sector, especially in processing technology, quality and taste improvement, variety improvement and storage techniques. The grain chemical composition also changes during ozone fumigation [20, 21]. The aim of the present study was to evaluate the potential of NIR-Spectroscopy, as a rapid and non-destructive method to predict the protein, fat and carbohydrate content of the green gram. On the other hand, NIR models were developed based on partial least square (PLS) technique.

MATERIAL AND METHODS

Raw material. Green gram was procured from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore of Tamil Nadu, India and used for the study. The green gram was cleaned manually to remove all foreign materials.

Sample preparation. The initial moisture content of the samples was determined by hot air oven drying at 103 ±2°C for 5 hours [8]. The initial moisture content of green
gram was found to be 11.18% (db). In order to maintain the moisture content of green gram for this study, the samples were kept in a refrigerator at 4±2°C. The required quantity of green gram sample was withdrawn and equilibrated at room temperature (29±3°C) before conducting different tests [9].

**Protein, Fat and Carbohydrate determination.** Protein content was estimated using Kjeldahl method. The fat content of the green gram was estimated by the method described by [10]. Soxhlet extraction with petroleum ether was used for determination of the fat content. The percentage of carbohydrate content in the green gram sample was determined by the method reported by [11].

**FT-NIR spectroscopy.** The FT-NIR spectra were collected on multipurpose analyzer (MPA) (Bruker Optics, Ettlingen, Germany) equipped with an integrated Michelson interferometer combined with OPUS software (v. 7.2 Bruker Optics, Ettlingen, Germany). It was used for spectral acquisition and instrumental control. For the current study spectra’s were recorded in diffuse reflectance mode on the green gram sample with sphere macro sample integrating sphere measurement channel over the range 12800–3600 cm⁻¹ at room temperature. For each calibration standard, the spectrum was attained by averaging three spectral scans. Each spectrum was the average spectrum of 64 scans.

**Chemometrics.** Multivariate analysis was used for quantitative and qualitative analysis. In the present study the in built software (OPUS/Quant 7.2) with the instrument was used for multivariate calibration which exclusively uses the PLS algorithm for the calibration and is designed for the quantitative analysis of spectra consisting of bands showing considerable overlap. It correlates more spectral information using larger spectral range with reference value of calibration set. This leads to a higher degree of precision with reduced chance error [12]. Partial Least Square algorithm (PLS), which was proven to be effective in many quantitative applications [13], was used in the present study. The OPUS 7.2 software was used for PLS analysis. The samples (222) were divided in the two sets, viz., calibration sample and validation sample set randomly, each set containing 111 samples. The quality of the calibration models for prediction of protein, fat and carbohydrate content was checked by cross validation of the models. It has been reported that the number of samples to develop the calibration model should cover the desired quantification range for the specific analyses, with a minimum of 20–50 samples depending on the complexity of the problem [14].

**Data analysis.** The spectral data were analyzed using PLS regression with various preprocessing techniques. In this study three spectral preprocessing methods were applied comparatively; it includes first derivative, vector normalization and first derivative plus vector normalization. Vector normalization normalizes a spectrum by first calculating the average intensity value and subsequent subtraction of this value from the spectrum. This method is used to account for different samples thickness [15].

**Model accuracy.** The performance of final PLS model was evaluated in terms of coefficient of determination ($R^2$) and root mean square error of cross validation (RMSECV). The accuracy of the validation models is obtained according to the highest values of $R^2$ and RPD and lowest RMSECV.

$$SSE = \sum [Residual]^2$$

(1) where:

*Residual* [-] - experimental value-Predicted value
The coefficient of determination \( R^2 \) gives the proportion of variability of the property that is described by the model.

\[
R^2 = \left( 1 - \frac{SSE}{\sum (y_i - y_m)^2} \right) \times 100
\]

where:
- \( y_i \) - observation of experimental value,
- \( y_m \) - mean of the reference results for all samples.

\[
RMSECV = \sqrt{\frac{\sum (\tilde{y}_i - y_i)^2}{n}}
\]

where:
- \( n \) - number of samples in the validation set,
- \( \tilde{y}_i, y_i \) - measured and predicted value of the \( i \)th observation in the test set.

The number of PLS factors included in the model is chosen according to the lowest \( RMSECV \).

**RESULTS AND DISCUSSION**

**Distribution of data sets.** The 222 samples of green gram that comprised the dataset under study had an average composition of 22.18% of protein, 1.30% of fat and 50.72% of carbohydrate. The protein content in the dataset ranged from 20.5 to 23.4%, the fat from 1.16 to 1.48% and carbohydrate from 45.1 to 55.5%. The samples can be split into three groups according to their protein content. The group with high protein content included 98 samples and had an average protein content of 22.8%, ranging from 22.4 to 23.4%; the intermediate protein content group included 104 samples and had an average protein content of 21.8%, ranging from 21.2 to 22.3% and the group with lower protein content included 20 samples and had an average protein content of 21%, ranging from 20.5 to 21.1%. The uneven distribution of the data leads to better prediction by the model and can be used for wide range of samples [16].

**Spectra analyzes.** Fig. 1 shows the FT-NIR spectra of green gram samples which have major peaks at absorbance units (wave numbers) of 4721.1, 5168.5, 5662.2, 6780.8, 8346.8 and 10028.6 cm\(^{-1}\). The peak and depression in the spectra showed the strong and weak absorbance characteristics of green gram within the region of study. Almost spectra of all samples are parallel (Fig. 1), which means the response of the detector for the sample is linear within the range of study and thus may give better results [17].

Major peaks at wave numbers of 4721.1 and 5168.5 cm\(^{-1}\) may be due to the stretching vibrations of \( NH \) and \( 2\times C=O \) (esters) bonds of protein. Peaks at 5662.2 and 6780.8 cm\(^{-1}\) may be due to first overtone of -CH and ArNH\(_2\) bonds of amine (NH\(_2\)) groups. Peaks at 8346.8 and 10028.6 cm\(^{-1}\) may be due to second overtone of symmetric stretching of -CH\(_3\) bonds of methyl groups and second overtone of ArNH\(_2\) bonds of amine (NH\(_2\)) groups. The vibration of the NH, -CH, ArNH\(_2\) and -CH\(_3\) molecules are caused by ingredients such as protein, fat and carbohydrate.

The NIR region contains several bands that often overlap, making it difficult to extract spectral parameters of the individual bands [13]. Multivariate analysis with
partial least square technique has provided a way of overcoming these problems through empirical models. Despite the lack of distinct peaks, it has been shown the PLS can extract relevant information for quantitative determinations [18].

Whole range of wave-number was split a fixed interval to know the group of the most effective wave-number for prediction of protein, fat and carbohydrate. After several pre-processing were choice the best model using each multivariate analysis method (PLS), the results to each parameter analyzed are summarized in Tab. 1. In the application of PLS algorithm, it is generally known that the spectral pre-processing methods and the number of PLS factors are critical parameters [13]. The main advantages of PLS is that the resulting spectral vectors are directly related to the constituents of interest; also when analyzing systems that have constituent concentrations that are widely varied and number of samples are not very large PLS offers satisfactory results [19]. The performance of the final PLS factor was evaluated in terms of correlation coefficient of determination \( R^2 \), root mean square error of cross-validation (RMSECV) and the root mean square error of prediction (RMSEP). The optimum number of factors is determined by the highest \( R^2 \) and lowest value for RMSECV and RMSEP.

Fig. 2 (a) and (b) shows the \( R^2 \) and RMSECV values plotted as a function of PLS factors for determining protein, fat and carbohydrate content with first derivative and vector normalization method as the pre-processing technique. Seen from figure, \( R^2 \) value increased up to certain limit reached a maximum value and thereafter maintained the value for quantitative model for carbohydrate content but there is no significant change in protein and fat content model. Similarly quantitative model for carbohydrate content the RMSECV value decreases sharply with initial factors and maintain the value as PLS factor increases.

![FT-NIR spectra of green gram](image)

*Figure 1. FT-NIR spectra of green gram*

The linear regression plot of the validation data sets for the best model showing measured versus predicted protein, fat and carbohydrate content is presented in Fig. 3 (a), (b) and (c) respectively. The equation of the straight line for this cross validation plots of the calibration data sets is represented as \( y = 0.9134x + 1.9237 \) \( (R^2 = 91.08) \) for protein, \( y = 0.8945x + 0.1373 \) \( (R^2 = 88.11) \) for fat and \( y = 0.9366x + 3.2316 \) \( (R^2 = 91.08) \) for carbohydrate content.
93.18) for carbohydrate showing good performance by this model in predicting protein, fat and carbohydrate content of the green gram samples. The best model was selected based on high value of correlation coefficient and low RMSECV values (Tab. 1). The offset and the slope for the equation of the regression line for this set were 1.916 and 0.914, 0.137 and 0.895, 3.234 and 0.937 for protein, fat and carbohydrate respectively. The results of this study clearly demonstrated the efficiency of FT-NIR for this application.

![Graph (a)](image1)

![Graph (b)](image2)

Figure 2. Effects of number of PLS factors on $R^2$ (a) and RMSECV (b) for the validation model

Table 1. $R^2$, RMSECV and RMSEP values corresponding to PLS factor for determining protein, fat and carbohydrate content with different spectral pre-processing methods

<table>
<thead>
<tr>
<th>Protein</th>
<th>Pre-processing technique</th>
<th>PLS factors</th>
<th>$R^2$ (Validation)</th>
<th>RMSECV</th>
<th>$R^2$ (Calibration)</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-processing</td>
<td>7</td>
<td>91.24</td>
<td>0.350</td>
<td>92.34</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>Pre-processing technique</td>
<td>PLS factors</td>
<td>$R^2$ (Validation)</td>
<td>RMSECV</td>
<td>$R^2$ (Calibration)</td>
<td>RMSEP</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>--------------------</td>
<td>--------</td>
<td>--------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>No pre-processing</td>
<td>7</td>
<td>86.88</td>
<td>0.029</td>
<td>88.23</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Vector normalization</td>
<td>8</td>
<td>87.85</td>
<td>0.0279</td>
<td>89.95</td>
<td>0.0259</td>
<td></td>
</tr>
<tr>
<td>First derivative</td>
<td>9</td>
<td>88.54</td>
<td>0.0271</td>
<td>91.06</td>
<td>0.0245</td>
<td></td>
</tr>
<tr>
<td>First derivative plus Vector normalization</td>
<td>8</td>
<td>88.09</td>
<td>0.0276</td>
<td>90.54</td>
<td>0.0251</td>
<td></td>
</tr>
</tbody>
</table>

### Carbohydrate

<table>
<thead>
<tr>
<th>Pre-processing technique</th>
<th>PLS factors</th>
<th>$R^2$ (Validation)</th>
<th>RMSECV</th>
<th>$R^2$ (Calibration)</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-processing</td>
<td>6</td>
<td>93.29</td>
<td>0.785</td>
<td>94.27</td>
<td>0.737</td>
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<tr>
<td>Vector normalization</td>
<td>7</td>
<td>93.62</td>
<td>0.765</td>
<td>94.18</td>
<td>0.745</td>
</tr>
<tr>
<td>First derivative</td>
<td>6</td>
<td>93.37</td>
<td>0.780</td>
<td>94.09</td>
<td>0.749</td>
</tr>
<tr>
<td>First derivative plus Vector normalization</td>
<td>5</td>
<td>93.47</td>
<td>0.774</td>
<td>93.96</td>
<td>0.755</td>
</tr>
</tbody>
</table>

(a) Predicted value vs. True value

(b) Predicted value vs. True value
CONCLUSIONS

A rapid and simple FT-NIR procedure to estimate protein, fat and carbohydrate content in green gram was developed using a calibration model. The model was developed using the spectral region 3600 – 12800 cm⁻¹. Lower values of RMSECV and relatively higher values of \( R^2 \) showed that NIR spectroscopy has potential to predict the quality of green gram nondestructively with almost same accuracy as that of laboratory method. The results presented in this work show that FT-NIR can be used as quick, simple, specific and easy automatic tool to predict the content of protein, fat and carbohydrate in green gram samples. It might be an application for green gram quality monitoring in the grain processing industry and various green gram research stations using FT-NIR spectroscopy.

BIBLIOGRAPHY


RAZVOJ MULTIVARIJANTNOG MODELA REGRESIJE ZA
APROKSIMATIVNU PROCENU SADRŽAJA VIGNA RADIATA
KORIŠĆENJEM FURIFEJOVE TRANSFORMACIJE – NIR SPEKTROSKOPIJE

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Sažetak: Furijeova transformacija blizu infracrvenog (FT-NIR) absorbovanog
spektra (12800-3600 cm⁻¹) kod 222 uzorka zelenog pasulja korišćena je da se izrade
kalibracioni modeli za određivanje sadržaja proteina, masti i ugljenih hidrata. Uzorci
koji su činili skup podataka imali su srednji sadržaj od 22.18% proteina, 1.30% masti i
50.72% ugljenih hidrata. Multivarijantnom regresijom su razvijeni kvantitativni modeli
proteina, masti i ugljenih hidrata. Srednje kvadratno odstupanje unakrsne procene
(RMSECV) bilo je 0.191 ($R^2 = 91.52$) za proteine, 0.0271 ($R^2 = 88.54$) za masti i 0.765
($R^2 = 93.62$) za ugljenih hidrata. Tako je razvijen brz, jednostavan i precizan metod za
kvantitativnu procenu sastava zelenog pasulja upotrebom FT-NIR spektroskopiju. Rezultati
su pokazali da FT-NIR spektroskopija može da podrži metode hemijske
analize.

Ključne reči: FT-NIR spektroskopija, zeleno zrno, protein, pasulj, prvi derivat, kalibracija

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