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Near Infrared Spectroscopy In Prediction Of The Crude Protein Wheat Stored For One Year At 4˚C

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Abstract: Aim of the study was to predict protein content of wheat grain stored for one year at 4˚C temperature using selected Near Infrared Near Infrared (NIR) wavelengths and Chemometrics. The spectra of grains were measured in reflectance mode with the use of lab built NIR filter based pre dispersive spectrometer ranging from wavelength 750nm to 2580 nm. Wavelength set was divided into two sets for all the stored samples. The chemometric methods applied to the reference data and recorded NIR data were analyzed based on principal component analysis (PCA) scores, partial last squared regression (PLSR) model. R^2 values were 0.955, 0.997 for prediction of protein content from PLSR Wavelength Set I and II respectively. Wavelengths with high β correlation coefficients were defined. This study showed that near infrared spectroscopy has potential to distinguish wheat grains stored refrigerator conditions.

Keywords: Near Infrared Spectroscopy, Partial Least Square Regression, wheat

1. Introduction

India is the third largest producer of wheat according to International wheat production statistics with EU and China being 1st and 2nd producer respectively. Wheat is used as a important ingredient in many food items. [FAOSTAT; Kashaninejad et.al., 2009; Mahesh et.al., 2008]. Wheat composition parameters such as kernel weight, protein content, moisture content, carbohydrate content and fat content and rheological properties are measured to check the quality of wheat. Analytical methods being used for the measurement of protein and carbohydrate content of the grains are destructive in nature, time consuming and uses hazardous chemicals. Spectroscopic techniques such as Near Infrared spectroscopy (NIRS) a rapid, non-destructive tool is used as a secondary tool to predict moisture, protein, zeleny distribution and starch in buckwheat flours with multiple correlation coefficients higher than 0.93 [Marie and Oldrich, 2009; JinHwan et.al., 1996, Mukherjee *et al*., 2014]. Wheat flour samples were characterized by developing accurate models using modified PLS and NIR spectroscopy for has been used for prediction of physical, chemical and functional properties of grains [Moroi et.al., 2011; Carol 2003; Carlos 2004]. Near infrared reflectance (NIR) spectroscopy was combined with artificial neural network (ANN) to predict the flour quality using regression models gave good accuracy with coefficient of determination (R^2) greater than 0.832 for all the properties [Avas t.al., 2011, Jnawali *et al.* 2016].Chemical, Physical and nutritive values of wheat were predicted using NIRS for wheat and poultry industry [Owens et.al., 2009]. NIRS was used to predict essential amino acids content for different grains to obtain better quality [Johannes et.al., 2002, Singh *et al.* 2014]. Prediction models using effective wavelengths were proposed for the three constituents. Interval partial least squares

(iPLS), successive projections algorithm (SPA) coupled with multiple linear regression (MLR) regression analysis along with NIRS were used to predict protein content, carbohydrate and crude fat content of millets [Jing Chen et.al., 2013].

Wheat is grown once a year thus it is being stored since ancient times to meet the requirements during the needy time. During storage free radical generation within grains results in molecular damage affecting chemical and quality parameters of wheat depending upon storage condition and time [McDonald, 1999]. Storage dependence on wheat variety was also studied on different wheat varieties during one year at four different storage conditions by measuring total protein content, starch content, hectolitre weight, wet gluten content, and seleny sedimentation using near infrared transmittance spectroscopy and flour titrable acidity using analytical methods [Marie and Dana, 2002].

Wheat grain stored for 180 days was investigated for quality properties using analytical methods [Hakam, 2015]. Changes in biochemical properties of wheat grains stored various temperatures for six months were studied [Zia and Shah, 1999]. It is required to develop an algorithm or a statistical model to predict the quality parameters of wheat grain in a fast, non-destructive and reliable way during storage to maintain the quality of grains.

Objective of this study is develop a fast accurate technique using NIRS and chemometric Principal Least Square Regression (PLSR), analysis prediction of protein content of wheat grains stored at 4 degree for one year.

2. Material and Methods

2.1 Sample collection and Storage

Wheat grains were collected from local markets of Punjab, India and then cleaned and packed in zip lock bags to keep the humidity of the samples constant. Grains were placed in refrigerator with temperature maintained at 4^0C. Sample analysis were started after the initial storage of grains for 4 months. Samples were grinded using Glen Mini Grinder GL 4045G for collection of NIR spectra and reference analysis.

2.2 NIR analysis

Lab made NIR spectrometer in pre dispersive reflectance mode was used as a rapid and non destructive technique to collect the spectra in the range of 700-2580 nm with discrete intervals. When NIR radiation is incident on the sample, sample undergoes molecular overtone vibration and combinational vibration depending upon the bonds present in the sample. The spectra obtained are complex due to overtone and combinational vibration bands. To understand the complex nature of the NIR spectra and to find their relation with the physicochemical properties there is need of a chemometric analysis.

NIR Source used for the analysis was Philips Tungsten Halogen Lamp 50W/12V. Dispersive element used was filters (company). The reflected light from the grounded samples was collected by PbS Detector (Thorlabs) and data was noted down by visualizing on a two channel Oscilloscope (Key sight). Each spectrum was collected in duplicate and the mean was taken. Absorbance was calculated using Lambert's Beer Law. NIR spectra for grains were collected every alternate month for one complete year after initial storage of 4 months.

2.3 Reference Analysis

Chemical Analysis were done to measure the physicochemical properties, mainly ash content, moisture content, crude fat content, crude protein content and carbohydrate content of wheat grain according to the Association of Official Analytical Chemists (AOAC) methods. Experiments were performed for all the stored grains in triplicate to reduce the experimental and manual errors. Chemical analysis was done every alternate month for all the stored grains.

2.4 Statistical Analysis

Property	Range	Standard deviation	Mean
Protein	8.516574-13.05736	1.285227	11.19467
Carbohydrate	75.02351-82.88749	2.443908	78.49878

Chemometric analysis of the Reference data and NIR spectral data was done using statistical software Unscrambler X 10.3. Sample Data were divided in to two sets namely calibration and prediction set in a ratio of 4:1. The spectral data as well as reference data were preprocessed before the analysis. The spectral data was smoothened using Savitzky Golay Algorithm and then maximum Normalization and linear baseline correction was applied to the data. Principal Component Analysis (PCA) was performed on the complete data set using Non Linear Partial Least Square (NIPLAS) Algorithm to develop relation between the samples (score plot) and spectral variable (loading plot). Analysis of the data were done using Principal component analysis, Partial Least Square Regression (PLSR) using NIPLAS algorithm. Correlation (R^2) and Root Mean Square Error (RMSE) for PLSR was calculated. The minimum number of wavelengths or important spectral variables required to predict the protein content of the wheat samples were determined using regression coefficient. Wheat quality under study in varied depending due to different varieties.

3. Results and Discussions

3.1 Reference analysis

Statistical results for the protein and carbohydrate content for wheat samples stored for one year under different storage conditions are shown in Table 1.

Table 1: Statistical parameters for wheat for protein and carbohydrate

3.2 NIR Spectrum

Near Infrared spectra of samples was observed from 780-2500nm. The samples absorption peaks were observed in the ranges 780-860nm, 960-1340nm, 1600-1900nm, 1960-2094nm, 2100-2180nm refers to the C-H, O-H, N-H stretch first, second overtone and combination band vibrations respectively due to protein, carbohydrate, fiber, crude fat present in the samples [Jerry and Lois, 2012]. The peak absorbance of the spectrum may be due to protein and carbohydrate content so statistical analysis was used to develop a regression model to predict the protein and carbohydrate composition individually.

3.3 Chemometric Analysis

PCA loading plot results showed that the wavelength range can be divided into two spectral group: 700-1620nm (Group1) and 1660-2177 nm (Group 2) (Fig. 1). Group I has the wavelengths in fourth quadrant and Group II in first quadrant of PCA plot. The wavelengths at the boundaries of two groups correspond to the C-H vibrations which show that the C-H vibration of the samples stored at different temperatures may be undergoing a change in vibrational frequency that results in the variation of wavelengths.

From the NIR data along with the reference data, predictive models was developed using PLSR for protein.

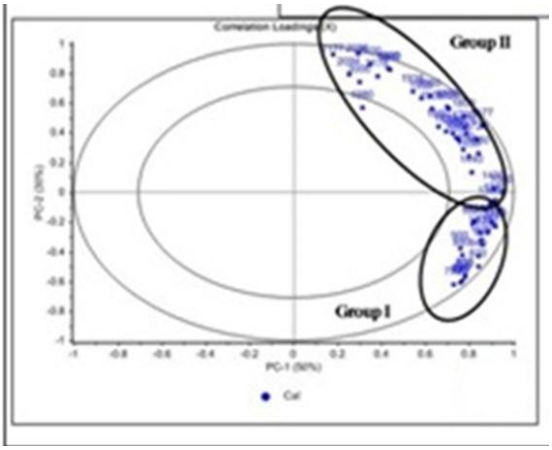

Figure 1 Principal Component Analysis for the samples

3.4 Partial Least Square regression Analysis

Partial Least Square Regression was applied using the NIPLAS algorithm and full cross validation technique was used for validation. As mentioned above Savitzky Golay Smoothening, maximum Normalization and Linear Baseline correction were used for preprocessing before regression analysis. It was observed that first four factors were enough to predict the protein and carbohydrate content of the stored grains and it accounted for 99% of variations in the data set. Values of R^2 and RMSE for calibration (R^2_c and RMSEC) and validation (R^2_v and RMSEV) are shown in Table 2. The results show that the models are able to predict the protein content of samples stored.

Table 2. PLS regression model predicted vs. reference value result for protein prediction

Wavelength set	**R^2_C**	**RMSEC**	**R^2_V**	**RMSEV**
Prediction of Protein using PLS Regression Model				
Group I	0.955	0.09	0.915	0.117
Group II	0.997	0.023	0.975	0.071

The weighted regression correlation coefficients (β-Coefficient) were used as a parameter to know the important wavelengths responsible for the prediction of dependent variables in a Partial Least Square Regression Analysis. Higher the magnitude of the coefficient more important is the variable. Negative values of the variables show that they have negative correlation with the independent variable. On the basis of the β-coefficents most favorable wavelengths for prediction of protein and carbohydrate contents for the samples were identified and are listed in Table 3. Wavelengths 1450nm, 1490nm, 1540nm, 1780nm, 1820nm, 1930nm, 2100nm, 2170nm, 2180nm having high β-coefficient for prediction of carbohydrate content correspond to O-H stretch 1st overtone, C-H stretch 1st overtone, O-H stretch and C-O stretch 2nd overtone, HOH deformation combination, O-H stretch and C-O bend combination, Asymmetric C-O-O stretch third overtone bands and wavelengths 1143nm, 1400nm, 1440nm, 1500nm, 1820nm, 1900nm, 1930nm, 1960nm, 1980nm, 2100-2180nm important for protein content prediction correspond to C-H 2nd overtone, O-H 1st overtone, N-H 1st overtone, Asymmetric N-H stretch/amide H^{b} combination, Asymmetric C=O strech 4th overtone, C=O stretch/ amide III^{b} combination bands [Jerry and Lois, 2012].

Table 3: Wavelengths for different groups with high value of B- coefficients from the PLSR model for protein content prediction for the 4^{0}C stored samples.

Protein	
4^0C	
Set I	**Set II**
820	1400
860	1900
870	1933
880	1938
940	1940
1064	1960
1100	2100
1140	2177
1300	2178
1340	

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