DEVELOPMENT OF NEAR INFRARED SPECTROSCOPIC METHODS FOR QUANTITATIVE AND QUALITATIVE ANALYSIS OF MILLETS

Thesis Submitted for the Award of the Degree of

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Physics

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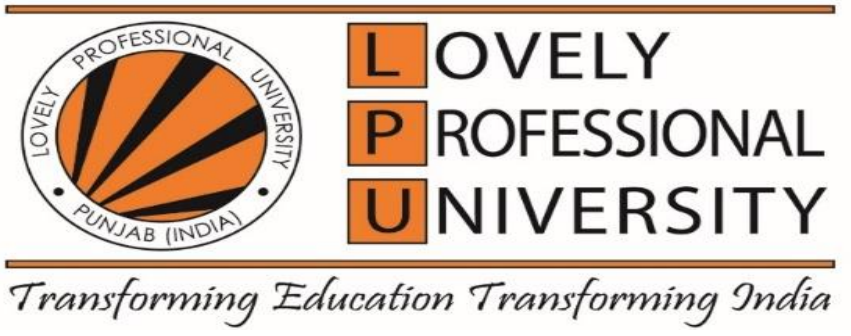

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2023

DECLARATION

I, hereby declared that the presented work in the thesis entitled "DEVELOPMENT OF NEAR INFRARED SPECTROSCOPIC METHODS FOR QUANTITATIVE AND QUALITATIVE ANALYSIS OF MILLETS" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr. Neha Munjal, working as Associate Professor in the Department of Physics of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "DEVELOPMENT OF NEAR INFRARED SPECTROSCOPIC METHODS FOR QUANTITATIVE AND QUALITATIVE ANALYSIS OF MILLETS" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Physics, School of Chemical Engineering and Physical Sciences, is a research work carried out by Pavas, 11813555, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

Millets are commonly referred to as coarse grains and are an excellent source of protein and fiber. Millets are a prominent crop for human health benefits to protect from the risk of diabetes, manage obesity, reduce stress, and help to manage the digestive system. Major millets named as Pearl millet, Sorghum millet, and Finger millet are the most important crop of Asian and African countries. It provides the food with high quality attributes moisture, fat, protein, fiber and carbohydrates. Pearl millet gain the more attention because it can be grown in tropical, sub-tropical and dry areas. It is mostly grown in Africa and India, rich in protein and fiber content and also gluten free in nature. The quality attributes of pearl millet are basically depending upon their proximate compositions i.e. moisture, ash, fat, protein, fiber and carbohydrates that is important to determine accurately. The various standard and chemical methods named as AOAC and AACC standards are available to determine the quality parameters which are hazardous, time consuming and destructive in nature.

For the qualitative and quantitative analysis of food items, near infrared spectroscopy (NIRS) has replaced the laborious, hazardous analytical techniques over the past two decades. NIRS is a rapid, precise, non-destructive, ecologically friendly, and economically advantageous technology that needs little to no sample preparation. Near infrared electromagnetic radiation, which typically ranges from 700-2500 nm, is where spectra are obtained. It is based on overtones and molecular vibration. In the NIR range, molecular vibrations appear in the form of hydrogen bonds between carbon, Sulphur, nitrogen, and oxygen. NIR spectra are complex in nature that depends upon the functional groups absorbed by NIR radiations. The NIR absorbed spectra are correlated with the physical as well as chemical attributes with the various functional groups. In order to connect the sample's physicochemical characteristics with the spectral data, reference and conventional chemical methods are required.

The physical parameters of pearl millet are necessary in designing of equipment for millet grain storage. Physical and chemical properties, as well as Fourier transform infrared

(FTIR) spectroscopic data have been investigated to determine the molecular structure of two varieties, of pearl millet Bajra-1827 and Pioneer 84M88. Both varieties were collected from Haryana, India. At various moisture levels (11.55 to 18.44% db) physical properties of pearl millet grains were measured. Varieties Bajra-1827 and Pioneer 84M88 were assessed for length (L), width (W), thickness (T), geometric mean diameter (D_g), surface area (S), volume (V), sphericity (Ø) and bulk density. The grain from variety Bajra-1827 has observed larger in size as compared to Pioneer 84M88.

Further, pearl millet is a nutritionally rich grain. The physicochemical properties of pearl millet were examined by Association of Officials analytical chemist (AOAC) standard methods. It was observed that the pearl millet has a moisture content was within range of 5.187-14.833%. ash of 1.480-1.880%, crude fat of 3.640-6.560%, fiber of 1.550-4.500%, protein of 5.556-11.946% and carbohydrates of 66.529-79.461% for pearl millet samples. NIR and FTIR spectroscopic technique was employed to analyze the organic material qualitatively and quantitatively, as well as to identify the chemical structure of various inorganic compounds. The spectral data provide the quantitative and qualitative information of organic material pearl millet flour.

Rheology has several uses in the food industry, consumptions, processing, and handling. Rheology is a study that describes the behavior of food materials under the influence of applied stresses and deformations. Rheology information is useful not only for production control and equipment design, but it may also be employed as a significant factor in understanding the behavior of dough during processing and the quality of products made from it. Thus, the viscosity of the various verities was examined using Labman 200 rotational viscometer.

Classification as well as correlation analysis of pearl millet samples based on their near infrared, Fourier transform infrared spectra and proximate compositions using principal component analysis (PCA). Samples were collected from different geographical locations of India. The infrared (IR) spectra of pearl millet samples were collected by using near infrared (NIR) and Fourier transform infrared (FTIR) vibrational spectroscopy and

proximate composition by employing Association of Official Analytical Chemists methods. Furthermore, Principal component analysis (PCA) was performed on the spectral and chemical data to discriminate the pearl millet samples based on the geographical origin. The study was conducted to reveal the efficiency of principal component analysis method in geographical classification of pearl millet samples on the basis of infrared spectral and chemically processed data.

The combination of NIR spectral data and multivariate chemometric approach were employed to develop the statistical calibration model for quality attributes of pearl millet. For the qualitative analysis, the principal component analysis (PCA) was employed to distinguish the pearl millet samples according to their regions. In quantitative analysis, the PLS, iPLS and SiPLS regression methods with various preprocessing were employed to build the calibration model and predict the quality parameters with extra prediction method. The results obtained from NIR technique compared with MIR methods. The PLS regression calibration model using the full NIR wavelength range 700-2500 nm had a coefficient of determination with least value 0.68 and highest 0.93 for moisture, 0.41 and 0.93 for crude fat, 0.29 and 0.91 for crude protein, 0.24 and 0.88 for crude fiber and 0.60 and 0.94 for carbohydrates at SNV and 4DV respectively. Thus, it can be clearly seen that the preprocessing plays the most important role to construct the model for quality parameters. The various types of preprocessing are used to achieve the excellent outcomes and it prevent from scattering effects and unwanted signals and background noise in the spectrum. The iPLS regression and regression coefficients were used to select the effective wavelengths for enhance the accuracy of model. In the iPLS, some intervals of wavelength were taken in the gap of 600, 300, 200 and 100 nm. From these intervals the optimal wavelengths were chosen to build the model. The PLS model were developed using specific wavelengths had higher prediction performance and lower root mean square error of validation than the models developed employing the complete NIR spectrum. The NIR spectrum region yielding the lowest RMSE with high coefficient of determination for moisture content were 1350-1600, 1900-2500 nm, crude fat content was 1900-2500 nm, crude protein was 1300-1600, 1900-2300, 2320-2500 nm, crude fiber was 2000-2100,

2200-2500 and carbohydrates contents were 1500-1700, 2100-2500 nm for pearl millet samples.

The results obtained from mid infrared spectroscopy (MIR region) and compared with near infrared spectroscopy (NIR region). The near infrared spectroscopy, shows the best model performance to predict the proximate composition of pearl millet. The prediction of the parameters of pearl millet using optimum NIR wavelengths may contribute in reducing the expense of the NIR equipment by using fewer monochromators or filters with smaller wavelength ranges to capture spectra.

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Ms. Pavas

(Department of Physics)

Table of Contents

List of Table

List of Figures

List of Appendices

Abbreviations

The abbreviations listed below have been used in this thesis.

AOAC	Association of Official Analytical Chemists
AACC	American Association of Cereal Chemists
NIR	Near Infrared
FTIR	Fourier Transform infrared
PCA	Principal Component Analysis
LDA	Linear Discriminant Analysis
PLS-DA	Partial least squares discriminant analysis
PCR	Principal Component Regression
MLR	Multiple Linear Regression
iPLS	Interval Partial Least Square Regression
SiPLS	Synergy interval Partial Least Regression
R^2_C	Coefficients of determination for Calibration
R^2_V	Coefficients of determination for Validation
RMSEC	Root mean square error of calibration
RMSEV	Root mean square error of validation
R^2_P	Coefficients of determination for Prediction
PCs	Principal components
SG	Savitzky Golay
SNV	Standard normal variate
MSC	Multiplicative Scatter Correction

DT	De-trending
MN	Maximum normalization
S	Smoothing
DV	Derivatives
FT-NIR	Fourier transform Near Infrared
NIPLAS	Non-linear partial least square
ADF	Acid Detergent Fiber
NIRS	Near infrared spectroscopy
SGS	Savitzky Golay smoothing
SVM	Support vector machines
ATR-MIR	Attenuated total reflectance mid-infrared
Vis-NIR	Visible near infrared
SWIR	Short wave infrared

CHAPTER-1

INTRODUCTION OF NIR SPECTROSCOPY & MILLETS

1. Introduction

1.1. Spectroscopy

Spectroscopy is defined as the interaction of matter and electromagnetic radiation. The spectroscopy focusses on emission and absorption spectra. It provides information not only about the arrangement and motion of the outer valence electrons but also gives detailed information about the inner electrons and the angular momentum, magnetic moment, distribution of charge and magnetism of the nucleus.

It was Sir Issac Newton who originated spectroscopy by showing that a prism refracts blue light more than red light, thus forming a band of colours known as 'Spectrum'. There are basically two branches of spectroscopy: -

1. Atomic Spectroscopy
2. Molecular Spectroscopy

Atomic spectroscopy

In the study of atoms, when atom absorbed the energy, the electrons get excited and jump to the higher excited state and due to unstability of electrons in excited state these come back in ground state and emission of energy occurs which is the difference between two energy levels and its result considered as spectrum. This helps in the study of the structure of atoms.

Molecular Spectroscopy

In the study of molecules, the energy is absorbed by molecules as result in rotational, vibrational and electronic transitions which are depending upon the amount of energy absorbed. Rotational, vibrational and electronic energy levels of molecule are known as molecular energy levels. The transitions of energies can take place between these levels gives the result in the molecular spectrum.[1]

Electromagnetic spectrum represents the all types of radiations in the order of increasing wavelength (decreasing frequency). The spectrum covers the whole range from gamma to radio waves. The IR region is sandwiched between visible and microwaves. When the light radiation is passed through compounds it behaves differently for organic and

inorganic compounds. For organic compound, some of the light is absorbed and for inorganic, the electrons get excited. A molecule absorbs the light according to its molecular vibrations, overtones and harmonics.

The wavelength absorbed with the help of spectrometer. In Figure 1.1 electromagnetic spectrum expressing the energy range from a higher energy, higher-frequency i.e. gamma waves with shorter wavelengths to lower energy, a lower-frequency radio wave with long wavelengths.

- Visible and UV radiations cover the wavelength range from 190-400nm. UV (190-400nm) and visible (400-750nm).
- IR radiations which cover the wavelength from 2.5-15.0µm, which corresponds to wavenumber 4000-650cm^{-1}. Wavelength range from 0.75-2.5 µm constitute the near-infrared region and from 15-25 µm the far-infrared region.

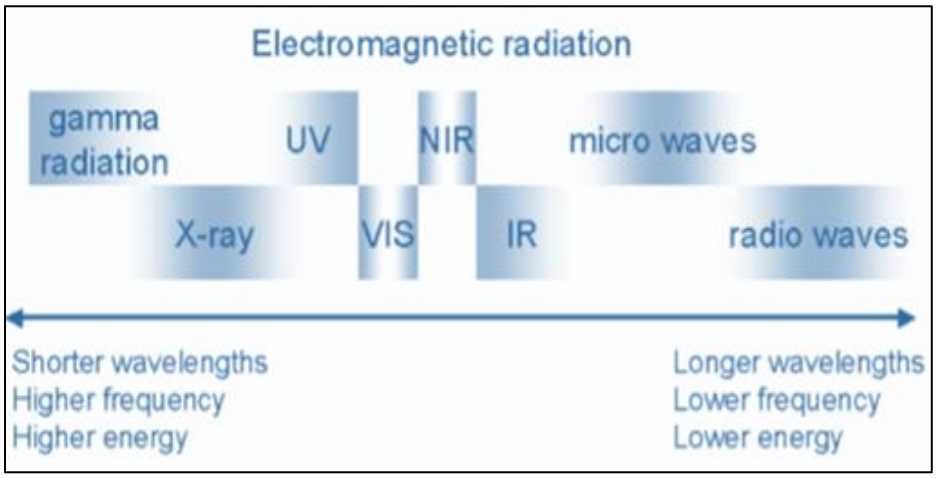

Figure 1.1 Overview of Electromagnetic Spectrum

Source: Torbjörn Lestander, Doctoral Thesis, Swedish University of Agricultural Sciences Umeå 2003

The molecular spectra can be divided into three spectral ranges corresponding to the different types of transitions between molecular energy states.

Pure Rotational Spectra

Only those molecules can produce rotational spectra which have a permanent electric dipole moment (polar molecule). Pure rotational spectra arise due to the change in rotational state of molecule in presence of external electromagnetic radiation, of microwave of far-IR region and also known as microwave spectra having energy 10^{-3} m.

Vibrational Spectra

Vibrational spectra arise due to transition of electrons is from ground state to excited state in vibrational levels of same electronic state and is absorbed in IR-region in electromagnetic spectrum hence it is also known as IR spectra. Vibrational spectra would be observed in those molecules which have change in electric dipole moment on the application of IR wave. The energy associated with IR spectra has the order of 10^{-1}eV.

Electronic Spectra

Electronic spectra arise due to the transition of an electron from one electronic state to another electronic state and visible in UV-region of EM-Spectrum. The typical order of energy associated electronic spectra is 1-10eV. The electronic states of a diatomic molecule can be shown by potential energy graphs as a function of internuclear spacing. On such a graph, electronic changes appear as vertical or nearly vertical lines because they happen so rapidly that the internuclear distance cannot change much during the process. Vibrational transition occurs within different vibrational levels of same electronic state and rotational transition occurs within different rotational levels of same vibrational state as shown in Figure 1.2.

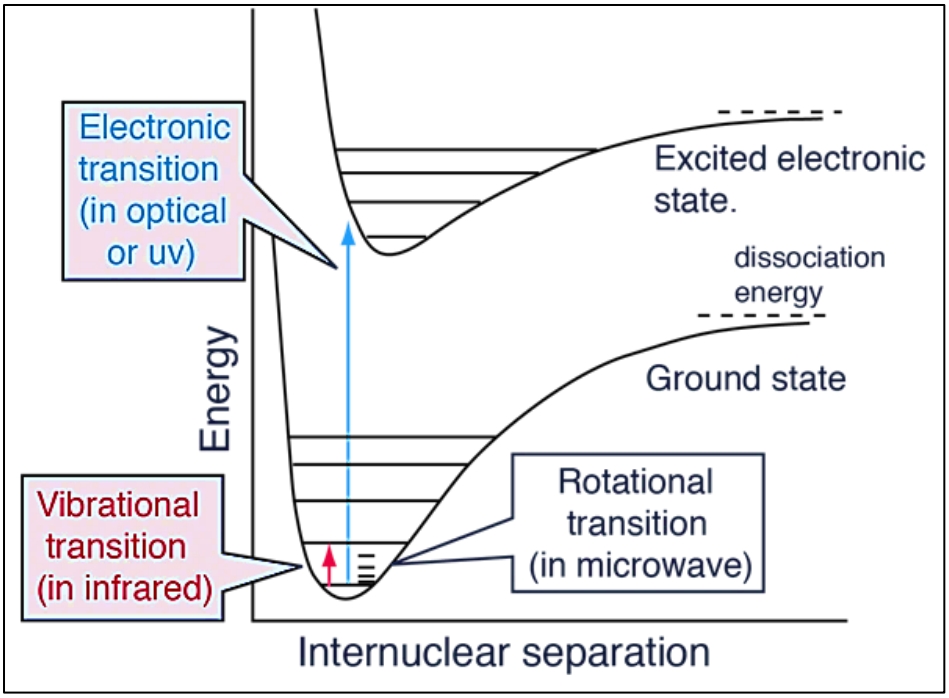

Figure 1.2 Molecular spectra with rotational, vibrational and electronic transition

Source- http://hyperphysics.phy-astr.gsu.edu/hbase/molecule/molec.html

Vibrational-Rotational Spectra

Vibrational-rotational molecular spectra are recorded in the near-infrared region of the electromagnetic spectrum and emerge from transitions between vibrational energy levels coupled with the same electronic state of the molecule. This can be seen in the molecules with permanent dipole moments, such as heteronuclear diatomic molecules. When the nuclei of such molecule vibrate relative to each other, the nuclear distance changes. Hence, the dipole moment changes periodically with the frequency of vibration of the molecule. Thus, the presence of an oscillating dipole moment is a must for interaction with the oscillating electric field of radiation[1].

The molecule acting as a Harmonic Oscillator

The near-infrared spectra are attributed to the vibrations of the nuclei in the diatomic molecule along the internuclear axis. It is treated as a harmonic oscillator. The potential then parabolic and of the form given by

$$V(r) = \frac{1}{2}k(r - r_e)^2 = \frac{1}{2}kx^2 \quad (1.1)$$

Where k present for the force constant, r_e is the equilibrium internuclear distance and x shows the displacement of the oscillator from the equilibrium position. And the allowed energies for the harmonic oscillator is given as

$$E = \left(v + \tfrac{1}{2}\right) h\omega_{osc.} \quad (1.2)$$

Where v represents the vibrational quantum number that can take integral values; v = 0, 1, 2, 3, 4 and so on. The selection rule for harmonic oscillator within vibrational changes are $\Delta v = \pm 1$.

Molecule acting as Anharmonic Oscillator

The molecules do not obey exactly the law of simple harmonic oscillator. If the bond length between the atoms is increased, the molecule breaks at a certain point - molecule dissociates into atom. Although, for small compressions and extensions the bond may be taken as elastic. In contrast to the harmonic oscillator, energy levels are no longer equidistant, and selection rule $\Delta v = \pm 1$ gets expanded to transitions among more than one energy levels. In addition, an asymmetric Morse function is used to present the potential energy curve as shown in Figure 1.3.

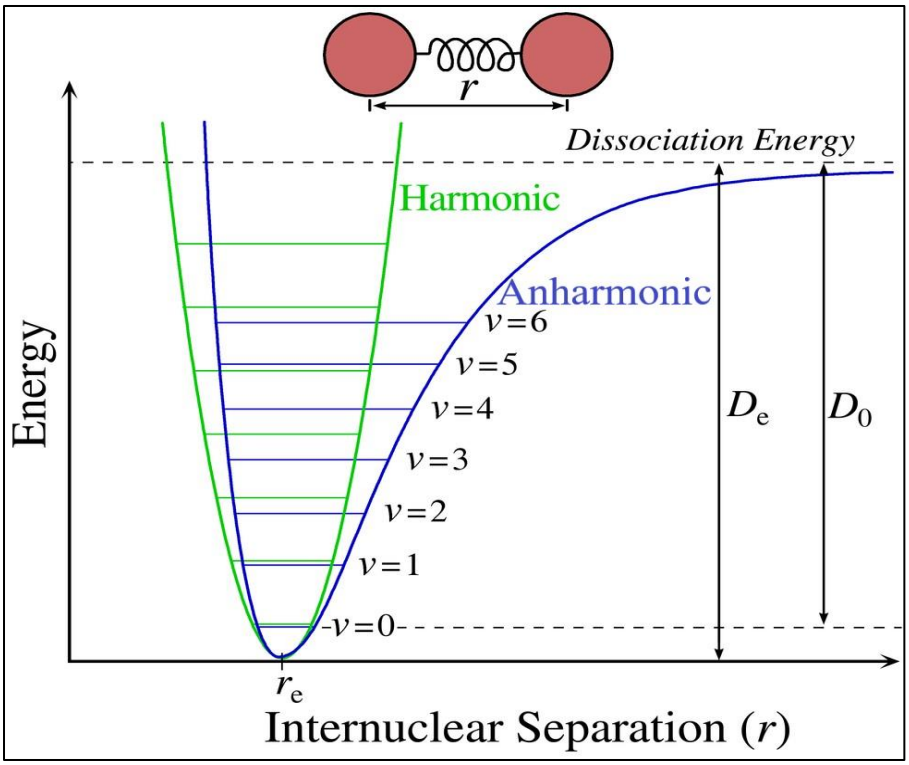

Figure 1.3 The Morse curve: energy of diatomic molecule under harmonic and anharmonic oscillator

Source: (CC-By-SA-3.0; Created by Mark Somoza March 26 2006)

As, the harmonic oscillator would give a single band at wave number, which is the classical frequency of vibration of the molecule. The actual infra-red spectrum is, however, found to consist of an intense band at ω, plus a number of weak bands at wave numbers slightly lesser and lesser than 2ω, 3ω.... The observation of overtones indicates that the selection rule $\Delta v=\pm1, \pm2, \pm3\ldots$ (v can be any number). When $\Delta v=\pm1$, the fundamental band occurs and transition from 0 to 1 level and $\Delta v=\pm2, \pm3$...and so on shows the first and second overtone transition from 0 to 2 and 0 to 3 shown in Figure 1.4 that express anharmonicity. This means that the dipole moment of the molecule is not strictly linear with respect to the internuclear displacement.

The allowed vibrational energy levels of the anharmonic oscillator are given by

$$E=\left(v+\frac{1}{2}\right)h\omega_e-\left(v+\frac{1}{2}\right)^2 h\omega_e x_e \quad (1.3)$$

Whereas, ω_e is oscillation frequency in form of wavenumbers, x_e expressed as anharmonic constant.

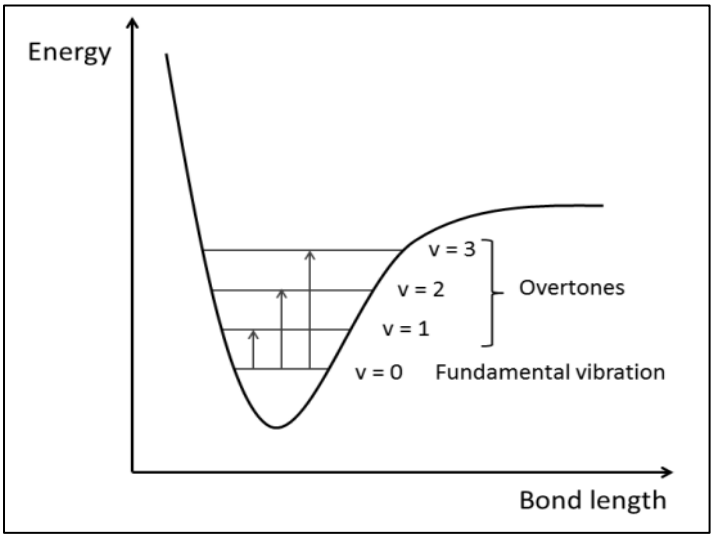

Figure 1.4 Molecule behave like anharmonic oscillator

Source- Abatement of volatile organic compounds by combined use of non-thermal plasma and heterogeneous catalysis (Thesis)

1.2 Near-Infrared Spectroscopy (NIR)

Historically, William Herschel discovered the Near Infra-Red (NIR) radiations in 1800. In the 1930s, NIR region below 2500 nm was given low priority in the chemical industry. In the 1960s the situation has changed about NIR spectroscopy and by the use of this technology can determine protein and moisture content in a sample of grains. In the 1980s NIR has been used in many applications in different fields because of its non-destructive, rapid and green technology.

Principle of Near-Infrared Spectroscopy (NIR)

Near-Infrared spectroscopy (NIRS) based upon vibrational energy and its range 780-2500 nm. In case of organic compound, the molecular vibrations are produced in the NIR region.

The basic principle of NIRS is grounded on overtone and combination vibrations of the molecules and intensities will become decreases from fundamental to next overtone. Fundamental and overtone vibrations evaluated by anharmonicity of the diatomic molecule. NIR spectrum absorbs which consist of overtones and combination bands of vibrations of C-H, O-H, and N-H functional groups. The first overtone exists in the NIR region because the X-H functional group absorbs at wavenumbers must be greater than 2000 cm^{-1}.

Bands are becoming weaker in intensity towards shorter wavelengths. The lower NIR intensities imply that solid samples do not need dilution and the impacts of non-linearity are less probable owing to powerful absorption. The energy (per photon) of infrared light is very less as compared to visible, ultraviolet and x-rays. Infrared light is therefore relatively safe since its photons cannot break down molecules.

NIR method commonly used today not only in the chemical, pharmaceutical, and food sectors but also in the forestry and agricultural sectors.

The absorption of infrared radiations causes a molecule to be excited from a lower to a higher vibrational state and each state is associated with a number of closely spaced rotational levels. All bonds in the molecule are not suitable of absorbing infrared energy. The bonds that are followed by a change in the molecule's dipole moment are referred to as IR active transitions. These are responsible for the absorption of energy in IR region. Whereas, vibrational transitions that do not result in a change in the dipole moment of the molecule are not directly absorbed and are referred to as IR inactive.

Instrumentation

Near-IR (NIR) spectroscopy instrumentation is nearly comparable to those for the UV and mid-IR ranges. It consists of a various element including source, dispersive component, and a detector. The dispersive components consist of gratings or prism.

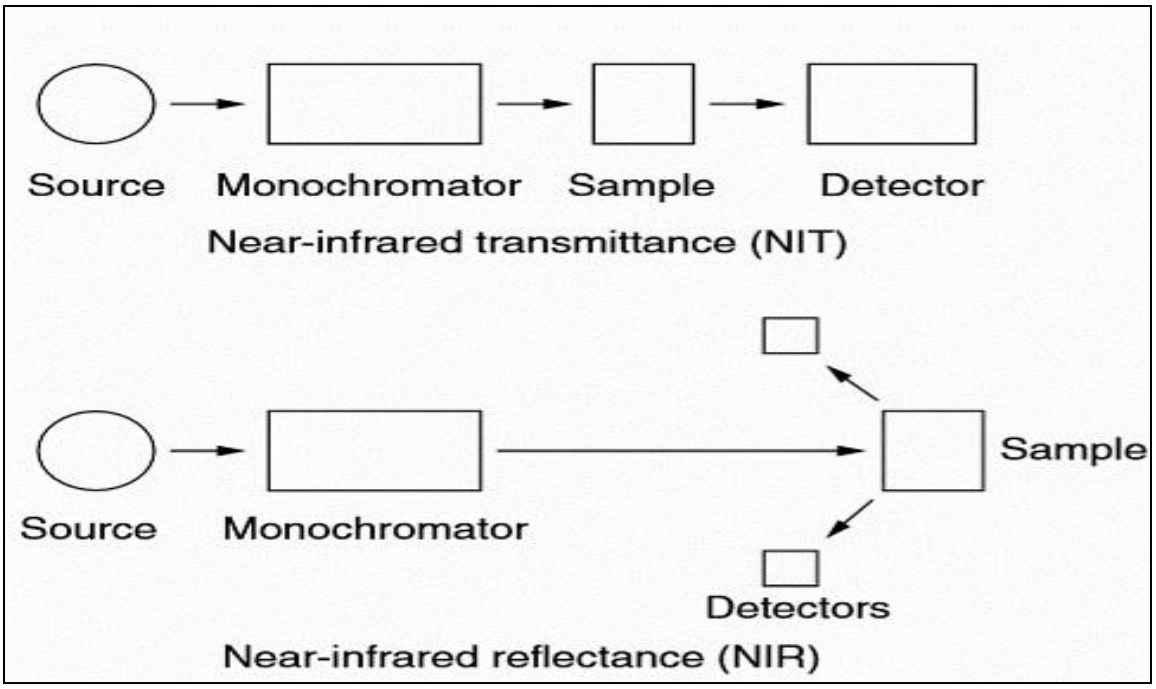

Figure 1.5 Basic design of Near Infrared Spectrometer

Source: Donald and A.Burns (2007).

Figure 1.5 shows that basic diagram of Near Infrared Spectroscopy which consist of transmittance and reflectance. These are two basic instruments which are commonly used in NIRS. The difference between both designs can appear when assembling the spectroscopic measurements.

In the reflectance measurements, the light penetrates only $1\text{-}4\times10^{-3}$m from the surface of the sample. When the energy of small penetration passes into the sample it will bring about greater variation then transmittance technique (when measuring non-homogeneous sample).

The path length of the sample in the transmittance measurement is investigated into spectrum measurements. Due to transmittance technique measurement can be done on large particles. There is more front surface scattering in case of the higher frequency energy i.e.,800 to 1400 nm in context to low frequency. For transmittance measurements, the size of the particle can be small but it should not be too small, that signals do not get detected by the detectors. Figure 1.6 shows the Sketch map of NIR Spectrometer.

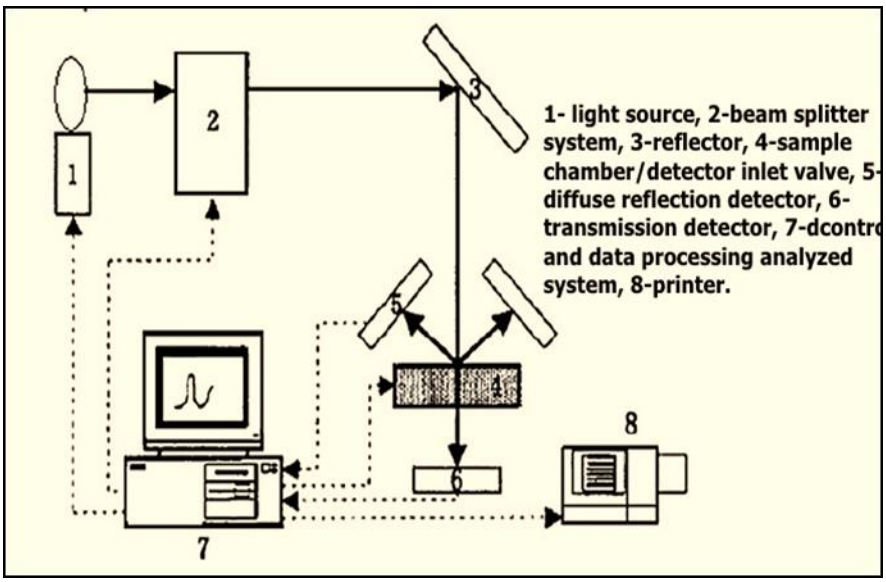

Figure 1.6 Sketch map of NIR Spectrometer

Source: Donald and A.Burns (2007).

To compensate, the effective research equipment would be capable of both transmittance and reflectance.

For analytical measurements, the common sources are incandescent or quartz halogen light bulbs as they emit in the near infrared region.

Light-emitting diodes (LEDs) in the required range have a larger lifetime and spectral stability and is also used as a source. Detectors are used to check the absorbance of the sample at a particular wavelength. Silicon-based CCDs are suitable for detection in the range of 700-1100 nm. InGaAs and PbS devices are used for the detection in the higher range in NIR. In few NIRS instruments, both InGaAs and silicon-based detectors are used in the single instrument so that detection in the range of UV, Visible and NIR can be detected simultaneously. The range of Near Infrared instrument depends upon the source and the detector used for the instrumentation process.

1.3 NIR Spectra collection and wavelength selection

The time-consuming, hazardous analytical methods have been replaced by near infrared (NIR) spectroscopy to fulfil the requirement for product quality improvement in the food and agriculture industries. Over the last ten years, chemometric and other vibrational spectroscopic methods, including mid-infrared, NIR, and Raman methods, have become effective tools for process and quality control. The basis of near infrared spectroscopy (NIRS) is the absorption of electromagnetic radiation, which typically ranges from 700-2500 nm. It is based on overtones, combinations and molecular vibrations.

In the NIR region the broad bands are formed and overlapped. The molar absorptivity is smaller in NIR region and it can be easily penetrate as compared to MIR region. NIR radiations behave like a wave with simple harmonic motion characteristics, including frequency and wavelength. The vibrations in chemical bonds of molecules are produced by the NIR spectroscopy and vibrations behave like the simple harmonic oscillator. According to the molecule's centre of mass, each atomic and molecular vibration is considered an individual vibration. In the NIR region, molecular vibrations appear in the form of hydrogen bonds in between carbon, sulphur, nitrogen, and oxygen as shown in Table 1.1. The spectrum highly depends upon the fundamental groups that can be absorbed by NIR light and these groups are interconnected to the fundamental chemical and physical constituents of a substance.

Table 1.1 Wavelength and functional groups in NIR region

Wavelength (nm)	**Functional Groups**
800	C-H 3rd overtone, O-H 2nd overtone
1100	O-H combinations, N-H 2nd overtone
1300	C-H 2nd overtone
1420	C-H combinations
1600	N-H, O-H 1st overtone
1800	C-H 1st overtone
2200	O-H, N-H combinations
2500	C-H combinations

1.4 Multivariate data Analysis and Chemometric

The data obtained from NIR spectra is multivariate in nature, because there are huge number of data points available for each sample. The complexity in spectra comes from the overlapping of the peaks. The spectra give the physical as well as chemical information present in organic samples. The chemometric approach are required to reduce the complexity and to determine the valuable chemical information i.e. fat, moisture and protein present in organic sample. The NIR spectra treated by some mathematical procedures to remove the unwanted information like effect of particle size and effect of noise in spectra without losing the important and relevant information. The development of model is based upon the dependent (Y-matrix) and independent variables (X-matrix) represented in Figure 1.7. The model development states the correlation in between X (references values) and Y (absorbance of spectral data) matrix to predict the property quality attribute of sample in future.

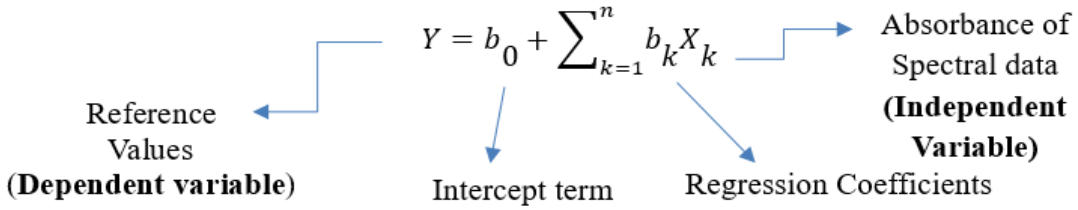

Figure 1.7 Explanation of regression equation with dependent & independent variable

Spectral Pre-processing

To minimize noise or undesirable information from spectra and enhance prominent chemical information, spectral data must be pre-processed or pre-treated. The noise in spectra comes from the sample preparation or spectra collection by instrument during scanning. The weak absorbance bands in NIR spectra are more affected as compare to strong absorbance bands. The spectral collecting condition plays an important role in existence of unwanted information and that should be under controlled. To overcome the

noise and undesired information to spectra should be scanned multiple times. Some of efficient methods to decrease scatter-induced baseline offsets are standard normal variate (SNV) and multiplicative scatter correction (MSC). To adjust for baseline shifts and intensity variances carried on by changes in route length, normalisation methods can be used. The resolution of overlapping peaks is improved by derivatives because they eliminate continuous offsets.

1.5 Quantitative multivariate data Analysis

The multivariate data analysis and chemometric approach are most prominent in the application part of NIR spectroscopy. The overlapping in peaks and broad bands in NIR spectra are resolved by the chemometrics. Once the calibration model has been developed, this approach can be used as quantitative measurements. The absorption bands occurring at the higher wavelengths resolved more easily as compared to absorbance band occurred in lower wavelength in NIR spectra. In the NIR spectrum all the variables (wavelength) are required but it is necessary to treat all the variables with multivariate regression techniques as shown in flow chart in Figure 1.8.

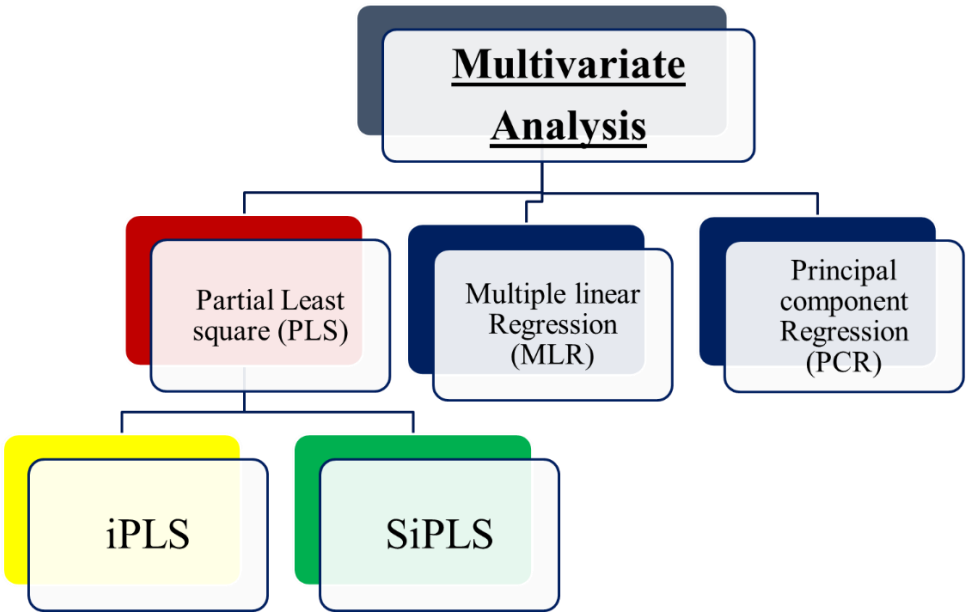

Figure 1.8 Flow chart of multivariate analysis methods

These regression approaches are based on the concept that only a limited number of spectral data components may be utilized in regression equations. These components are determined by applying the appropriate mathematical terms. Thus, it is the way to get the most relevant chemical information from the spectral data to develop the calibration regression model. The calibration regression model development states that the NIR spectral data is secondary data and the accuracy depends upon the reference data of sample.[2]

Reference data

The reference method must be carefully chosen as the precision. The accuracy of the NIR approach is determined by reference method. The reference analysis sample size should be equivalent to the spectral sample size for content uniformity analysis. In addition, the interval between the reference analysis and the NIR readings may be critical.

Multivariate calibration modelling

After collection of spectral data and reference chemical data, the next step is to development of calibration model. The calibration model may be constructed using raw or pre-processed spectral data. In the calibration model, the mathematical equation is built in between reference values that determined by standard methods and NIR spectral raw or pre-processed data. There are various aspects for developing a calibration model. Firstly, the sample should be organic in nature for direct measurement e.g. moisture or protein will get absorb in the NIR region because of presence of O-H and N-H bonding. Secondly, the indirect measurement can be done where the sample does not absorb in the NIR region e.g. salt content. These types of samples can be measured through co-variation.

The aim of development of model is to determine the straight line fit in the reference and NIR spectral data. In the multivariate analysis, the only selective wavelengths are utilizing in MLR, PCR and PLS (iPLS, SiPLS) regression. MLR technique are used only when a limited number of variables (wavelengths) are available. There are number of multivariate algorithms for model development but the principal components regression (PCR) and partial least squares regression (PLSR) are most widely employed for quantitative NIR

calibration modelling. The more precise results are obtained from the PLS regression calibration model. In the PLS regression the primary factors are more important for model it gives the more accuracy with less prone to errors. PLS is frequently easier to apply and optimize for large data sets than PCR. The interval partial least square (iPLS) and synergy iPLS regression techniques are applied to reduce the complexity of model and to enhance the prediction performance of model. The regression methods are employed to achieve the most exact and precise calibration model. The accuracy of calibration model is determined by the parameters named as coefficient of determination (R^2_C) and the root mean squared error of calibration (RMSEC). Once model is developed with various regression techniques then it is validated or predicted with unknown samples.[3–7]

1.6 Qualitative multivariate data Analysis

To discriminate between different classes of commodity or to detect on adulterated food, the qualitative analysis through NIR spectroscopy is the best suitable method. These types of problems can be resolved by comparison of authentic materials by unknown samples. When NIR spectra are collected for various samples, the typical spectrum may vary for each class. The variation in spectra depends upon the variation in biological properties of sample or variation in class of samples. These variations are found due to the different varieties, geographical origin/region and season. The application of NIR approach give the confirmation of authenticity of food and differentiation between the classes of food[8,9]. The quantitative analysis and recognized method compare the NIR spectra and search for differences and similarities of spectra. The classification models are developed to correct the classification as much possible. The initial step is to consider about how many classes there should be and always examine what criteria must be met before being allocated to a certain class. In the application of qualitative analysis of NIR spectroscopy, there are two approaches are present i.e. unsupervised and supervised.

Unsupervised approach

Unsupervised approach is mostly used at where the classification structure is not determined. The principal component analysis (PCA) is employed to determine the possible relationship in between the samples.

Supervised approach

The supervised approach are generally used to develop the classification model e.g. linear discriminant analysis (LDA), Partial least square-LDA (PLS-LDA) and artificial neural network (ANN).[10]

1.7 Fields of Applications

There are numerous commercial applications of NIR spectroscopy such as for examining the quality of food products, in pharmaceutical sciences, and it is widely used in astronomy.[11,12]

Agriculture

Near-infrared spectroscopy is widely used in agriculture for determination of the quality of fruits, vegetables, beverages, oils, dairy products, eggs, and many various agricultural products. Identifying the composition of agricultural products is commonly used because this technique is very precise, reliable, fast, non-destructive and requires less time and knowledge to perform.[13,14]

Astronomical Spectroscopy

For analysing atmosphere of a stars, the NIR is commonly used. The vibrational and rotational spectra of molecules like cyanide, carbon monoxide, titanium oxide etc. can be observed, which gives the valuable information about the spectral of stars.

In addition, astronomical spectrographs were designed to identify exoplanets by using the parent star's Doppler shift caused by the planet's radial velocity around the star. It is also used to study molecular clouds in which stars form.[15]

Material Science

In this zone, NIR has many applications in nanoscience for measuring the thickness of thin films and optical properties of nanoparticles. It is extensively used in the telecommunication manufacturing for optical coating purpose.[16]

Remote Monitoring

NIR spectroscopic imaging techniques have been developed. Hyperspectral imaging has been utilised for a wide range of applications, including remote plant and soil study. Data can be gathered from aircraft or satellite tools to evaluate ground cover and soil chemistry.[9]

Medical uses

The potential of NIRS to offer information on oxygen and haemoglobin saturation in the microcirculation makes it useful in the medical area. NIRS can be utilised for non-destructive evaluation of brain function via intact human skulls by detecting variations in blood haemoglobin concentrations.The advantage of NIR spectroscopy in medical applications are it has no side effect after uses and it is less in cost, time effective and easily portable.[17]

Pharmaceuticals

The primary use of NIR spectroscopy in pharmaceuticals is to determine the active pharmaceutical compounds present in drugs or tablets. NIR spectroscopy also employed to determine the excipients. Samples were used for development of calibration model to assess the effect of different moisture contents on drug content. The NIR spectroscopy were used to predict the ascorbic acid in bilayer tablets.[18]

1.8 Millets

1.8.1 International year of Millets (IYOM) 2023

- The Indian government suggested to the United Nations that 2023 be recognized as the International Year of Millets (IYOM). The Indian proposal was endorsed by 72 countries, and the United Nations General Assembly (UNGA) announced 2023 to be the International Year of Millets on March 5, 2021.
- The Government of India has now decided to celebrate IYOM, 2023 for popularizing Indian millets and recipes at global level.

Millets are a kind of incredibly nutritious small grain food plants grown in poor soils with minimal inputs such as fertilizers and insecticides. Different varieties of the millets i.e. Pearl millet, Sorghum and Finger millet are the main millets. Whereas, foxtail millet, small millet, kodo millet, barnyard millet, proso millet and brown top millet are classified as small millets shown in Figure 1.13. Millets require less water than rice and wheat and plants that are deemed resistant to drought.[19–23] Millets are earliest foods known to human civilization, but owing to urbanization and industrialization, their significance and production decreased. Whereas, pearl millet is very prominent in terms of cropped area and it accounts for half of global millet production. After that, foxtail millet, proso millet and finger millet are important crop of Africa and Asia, it is mostly cultivated in cooler and high-altitude areas. Proso millet is important for making bird seed in industrialized countries and for use as food in some regions of Asia. Other millets like fonio and tef are cultivated in some African nations or individual countries. Most millets are plants from the Kharif season (sown during May-June) and ripen from September to October. In the rabi season (October-March) and winter season (January-April), most of these plants yield returns.

Small millets are grown in China, India, Russia and several African countries. In India they grow as a single crop or as a mixed crop in fertility-depleted soils. In tribal areas millet is cultivated on slopes of hills. The consumption and production of millets in Eastern Europe

and Russia have persisted to a greater extent, where they are still utilizing for cooking, baking, brewing or other purposes in certain parts. They are basic foods for millions in Africa, Asia, including India. The physical traits, qualitative attributes, soil and climate needs, as well as the length of growth, vary amongst the various species as shown in Figure 1.9.

Pearl Millet (Bajra)

Sorghum Millet (Jowar)

Finger Millet (Ragi)

Proso Millet (Cheena)

Kodo Millet (Kodra) **Foxtail Millet (Kangani)**

Barnyard millet (Sama ke chawal) **Little Millet (Kutki)**

Figure 1.9 Various species of Millets

1.9 Worldwide Production and distribution of Millets

Global distribution

Millets have an annual cultivation region of approximately 2.5 million hectares, and small millets currently account for less than 1% of the world's food crops (ICAR, 2010). The cultivated region in India has decreased from 46.77 in 1961-66 to 9.05 lakh hectares in 2008-2009 under small millet. Small millet production decreased from 18.89 to 4.45 tons of lakh during the same period. The millets are globally distributed in various countries with large area of production as represented in Table 1.2. (FAO stat 2021)

Table 1.2 Cultivation of millets on Global Level

Regions	**Production (lakh ton)**	**Area (lakh ha)**
India	173 (20%)	138 (20%)
Africa	423 (49%)	489 (68%)
Europe	20 (2%)	8 (1%)
Americas	193 (23%)	53 (7%)
Asia	215 (25%)	162 (23%)
Australia & New Zealand	12 (1%)	6 (1%)
WORLD	863	718

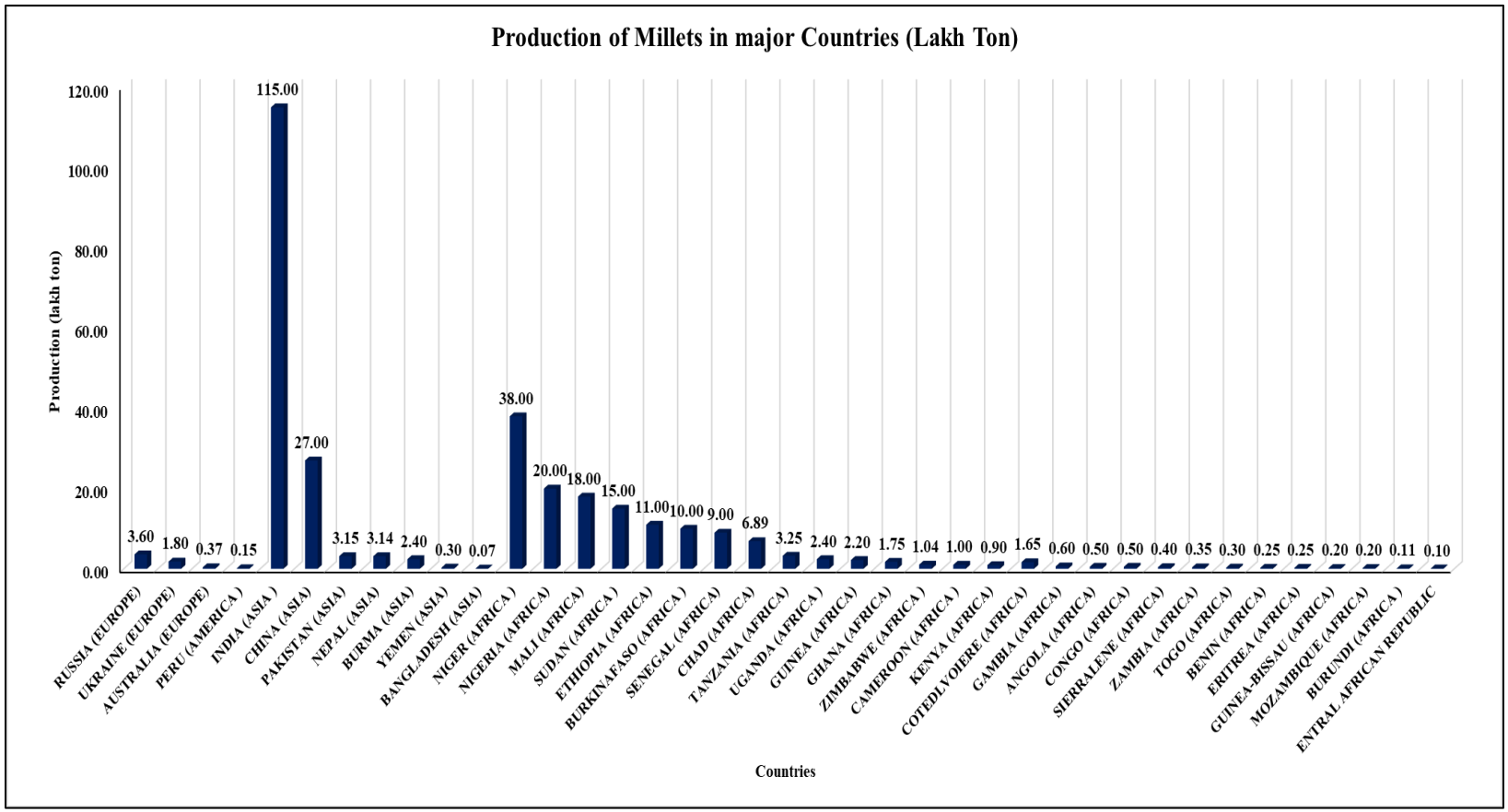

Figure 1.10 Global production of Millets

(Source: FAO Stat 2021)

Millets Production in India

The production of Millets is limited as compare to other cereals in India. The Figure 1.12 present the data of production of millets since last 10 years (FAO Stat 2021). The Jowar crop are highly produced at the year of 2011-2012 with 59.8 lakh ton and lower in 2015-2016 and that was 42.4 lakh ton. In contrast, the Bajra crop is India's most significant crop that is utilized mostly in form of food and highly nutritious in nature. Therefore, the Pearl millet (Bajra) highly produced as compared to other species of millets as shown in Figure 1.10. (FAO stat 2021). The millets cover the 138-lakh ha area as well as 173 lakh ton of production in India. Figure1.11 shows the production of various spices of millets in India.

Figure 1.11 Millet production in India

Source: https://agricoop.nic.in/sites/default/files/Crops.pdf

Millets are highly cultivated in the top five states of India as shown in Table 1.3. Millets are mainly originated in dry, tropical and sub-tropical regions. Pearl and sorghum millets are mostly cultivated in state Rajasthan because to the dry region. Whereas, Jowar and ragi originated in Karnataka and Maharashtra states. Because of high consumption of Pearl millet, it is also cultivated in Uttar Pradesh and Haryana state.

Table 1.3 Millet cultivation in top five states of India

Top 5 States	**Millets**
Rajasthan	Bajra/Sorghum
Karnataka	Jowar/Ragi
Maharashtra	Ragi/Jowar
Uttar Pradesh	Bajra
Haryana	Bajra

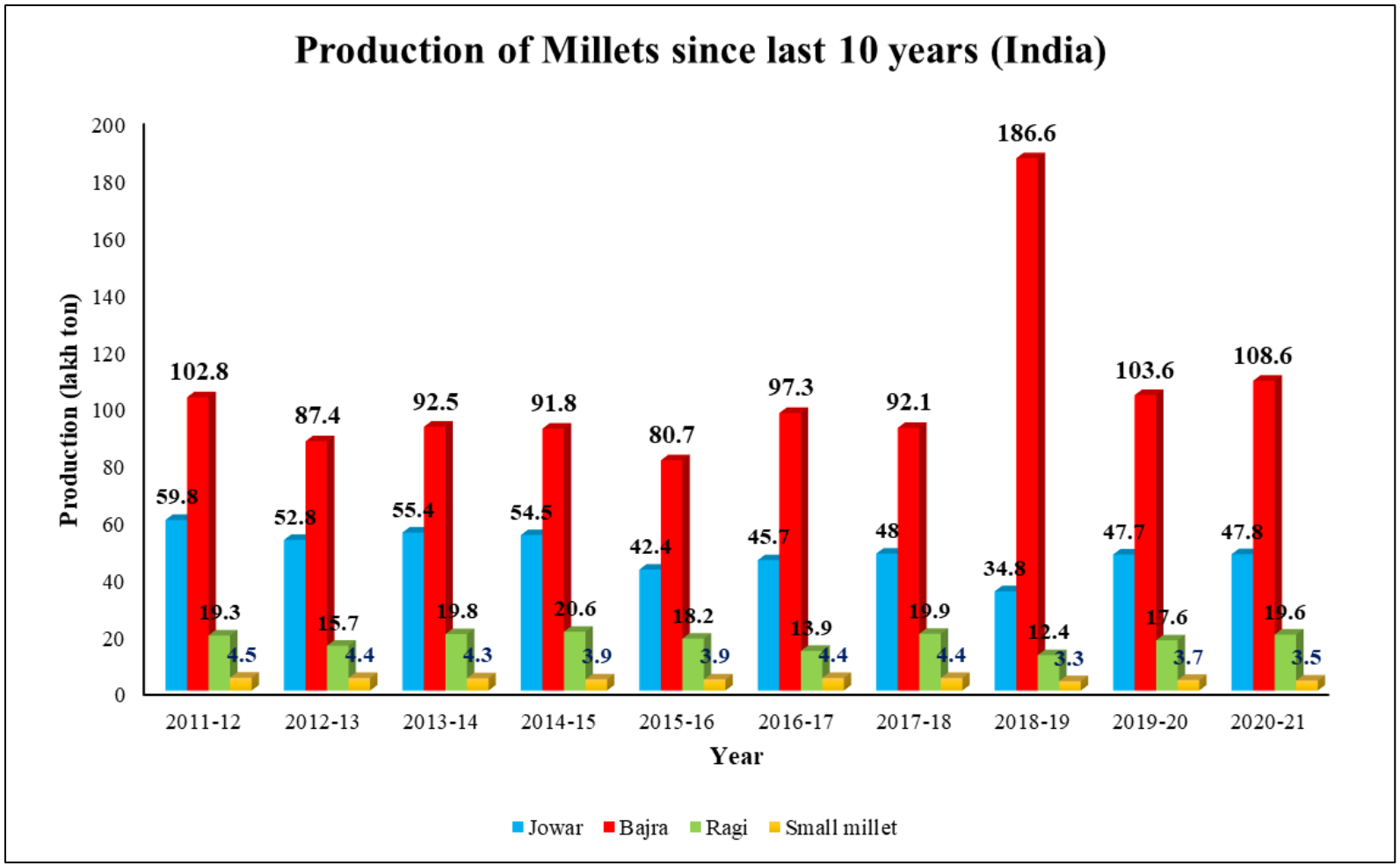

Figure 1.12 Millet production from last 10 years

Source: https://agricoop.nic.in/sites/default/files/Crops.pdf

1.10 Major Millets

Pearl millet (Bajra)

Pearl millet has traditionally been a significant crop for grain, forage, and store mainly in many emerging nations and subtropical areas. Its origins may indeed be related back to Central Tropical Africa and has since spread throughout the tropics and India. It was launched in the West in the 1850s and was founded in the Southeast and Gulf Coast countries as a minor forage. As pearl millet cultivation in temperate and developed countries extends into non-traditional fields, disease-related manufacturing limitations are becoming more important. Pearl millet grows in areas characterised by dryness, minimal soil fertility, and extreme temperatures. It is particularly effective works in soils with high salt and low ph. It may be grown in places where other cereal crops like maize or wheat would not suffer due to its tolerance to hard increasing circumstances.[20]

Sorghum millet

Sorghum is a warm-season crop that tolerates low temperatures yet is responsible for resistance to severe pests and pathogens. It is regarded by multitude of names (in Asia, West Africa and sections of the Middle East, such as excellent millet and rice maize). The majority of sorghum harvested in Northern south America, and Oceania is utilised for animal feed.

Finger millet

Although finger millet is predominantly utilised as a food grain, it is not frequently used for livestock since it is of lower quality to that of sorghum, maize, and pearl millet. It is occasionally used in India to feed growing animals, ill and convalescing animals, as well as new-born calves.

1.11 Properties of millets

Millets are similar to major cereals in nutrition and function as a useful source of protein and micronutrients. Millets are not only similar to significant cereals in terms of dietary characteristics, but they are also very excellent sources of nutraceutical carbohydrates and micronutrients. The millets comprise 7 to 12% crude protein, 2 to 5% crude fat, 65 to 75% carbohydrate, and 15 to 20% nutritional fiber. Among them, pearl millet has significantly elevated percentage of proteins (12 to 16%) and lipids (4 to 6%), while finger millet includes lower protein (6 to 8%) and crude fat (1.5 to 2%) concentrations.

Sorghum has a moisture content of 11.9% and a protein content of about 10.4% and a fat content of 1.9%. The seed sorghum's fiber and mineral content is substantially comparable and is 1.6%. It is a good energy source, providing about 349 kcal and 72.6% of carbohydrates. Starch is the grain's major carbohydrate. Simple sugars, cellulose, and hemicellulose are the other carbohydrates current. The starch amylose content ranges from 21-28%. Sorghum also has high nutritional fiber content (14.3%). The sorghum contains calcium, phosphorus, and iron as 25 mg, 222 mg and 4.1 mg/100g respectively. Furthermore, black finger millet contains 8.71 mg/g fatty acid dry weight and 8.47 g/dry protein weight. The dietary fiber has been reported in Kodo millet and little millet about 37% to 38%. Whereas, Pearl millet contains moisture, ash, protein, fat, fiber and carbohydrates in 8.97, 1.37, 10.96, 5.43, 11.49 and 61.78 respectively as shown in shown in Figure 1.13.On a larger scale, it also plays an important role in increasing food and nutrition security in both established and developing countries.[24]

Millets and Cereals		Moisture (g)	Protein (g)	Ash (g)	Total Fat (g)	Dietary Fibre (g)			Carbo hydrates (g)	Energy (KJ)
						Total	Insoluble	Soluble		
Bajra (*Pennisetum typhoideum)*		08.97 ± 0.60	10.96 ± 0.26	1.37 ± 0.17	5.43 ± 0.64	11.49 ± 0.62	9.14 ± 0.58	2.34 ± 0.42	61.78 ± 0.85	1456 ± 18
Sorghum (*Sorghum vulgare)*		09.01 ± 0.77	09.97 ± 0.43	1.39 ± 0.34	1.73±0.31	10.22± 0.49	8.49 ± 0.40	1.73 ± 0.40	67.68 ± 1.03	1398 ± 13
Ragi (*Eleusine coracana)*		10.89 ± 0.61	07.16 ± 0.63	2.04 ± 0.34	1.92 ± 0.14	11.18 ± 1.14	9.51 ± 0.65	1.67 ± 0.55	66.82 ± 0.73	1342 ± 10
Little Millet (*Panicum miliare)*		14.23 ± 0.45	08.92 ± 1.09	1.72 ± 0.27	2.55± 0.13	06.39 ± 0.60	5.45 ± 0.48	2.27 ± 0.52	65.55 ± 1.29	1449 ± 19
Kodo Millet (*Setaria italica)*		14.23 ± 0.45	08.92 ± 1.09	1.72 ± 0.27	2.55 ± 0.13	06.39 ± 0.60	4.29 ± 0.82	2.11 ± 0.34	66.19 ± 1.19	1388 ± 10
Foxtail Millet *		-	12.30	-	4.30	-	-	-	60.09	331
Barnyard Millet *		-	06.20	-	2.20	-	-	-	65.55	307
Proso Millet *		-	12.50	-	1.10	-	-	-	70.04	341
Wheat	Whole	10.58 ± 1.11	10.59 ± 0.60	1.42 ± 0.19	1.47 ± 0.05	11.23 ± 0.77	9.63 ± 0.19	1.60 ± 075	64.72 ± 1.74	1347 ± 23
	Refined flour	11.34 ± 0.93	10.36 ± 0.29	0.51 ± 0.07	0.76± 0.07	02.76 ± 0.29	2.14 ± 0.30	0.62 ± 0.14	74.27 ± 0.92	1472 ± 16
	Atita	11.10 ± 0.35	10.57 ± 0.37	1.28 ± 0.19	1.53 ± 0.12	11.36 ± 0.29	9.73 ± 0.47	1.63 ± 0.64	64.17 ± 0.32	1340 ± 07
	Semolina	08.94 ± 0.68	11.38 ± 0.37	0.80 ± 0.17	0.74 ± 0.10	09.72 ± 0.74	8.16 ± 0.58	1.55 ± 0.18	68.43 ± 0.99	1396 ± 18
Rice	Raw Brown	09.33 ± 0.39	09.16 ± 0.75	1.04 ± 0.18	1.24 ± 0.08	04.43 ± 0.54	3.60 ± 0.55	0.82 ± 0.15	74.80 ± 0.85	1480 ± 10
	Raw milled	09.93 ± 0.75	07.94 ± 0.58	0.56 ± 0.08	0.52 ± 0.05	02.81 ± 0.42	1.99 ± 0.39	0.82 ± 0.22	78.24 ± 0.68	1491 ± 15
	Parboiled	10.09 ±_0.43	07.89 ± 0.63	0.65 ± 0.8	0.55 ± 0.08	03.74 ± 0.36	2.98 ± 0.35	0.76 ± 0.09	77.16 ± 0.76	1471 ± 8
Quinoa (*Cheno podium quinoa)*		10.43	13.11	02.65	5.50	14.66	10.21	4.46	53.65	1374
Amaranth Seed	Black	09.89	14.59	02.78	5.74	07.02	5.76	1.26	59.98	1490
	Pale Brown	09.20 ± 0.40	13.27 ± 0.34	3.05 ± 0.30	5.56 ± 0.3	07.47 ± 0.09	5.80 ± 0.17	1.67 ± 0.21	61.46 ± 0.60	1489 ± 10
Maize	Dry	09.26 ± 0.55	08.80 ± 0.49	1.17 ± 0.16	3.77 ± 0.48	12.24 ± 0.93	11.29 ± 0.85	0.94 ± 0.18	64.77 ± 1.58	1398 ± 25

Figure 1.13 Properties of Millets and other cereals

(Source-Indian Food Composition Tables, NIN – 2017 and *Nutritive value of Indian foods, NIN – 2007)

1.12 Pearl millet consumption

Pearl millet grains gain the importance due to its highly nutritious values. Pearl millet is mostly consumed in India by traditional customers as roti and unleavened pancakes, which use whole flour. Although it was formerly only consumed by a small portion of the population, its nutritional value has recently increased. In Africa, traditional foods like ogi (a cereal-based cuisine), fura (a traditional foodstuff in Nigeria), and bensaalga are frequently made using pearl millet (a fermented gruel in Burkina Faso). The overall acceptability chapati prepared from bleached or acid-treated or heat-treated pearl millet flour, enhanced when compared to the chapati made from the raw, untreated grains[22].

1.13 Health Benefits

Millets may have beneficial health effects and observational studies have shown that consuming them lowers the risk of heart disease, helps the digestion, guards against diabetes, reduces cancer threat, boosts immunity in the respiratory system, detoxifies the body, enhance energy, as well as being protective against several degenerative diseases.[25,26] Resistant starch (RS), lipids, antioxidants named as phenolic acids, avenanthr amides, phytosterols and lignans, are thought to be responsible for numerous health benefits, are among the essential elements included within millets.[27]

1.14 Thesis Outline

Thesis work is divided into various Chapters and explained below.

Chapter 1 explain the introduction part of spectroscopy, it comprises the Near Infrared spectroscopy, Principle, Instrumentation, NIR spectra collection, Quantitative and Qualitative multivariate analysis for calibration model development, Application of NIR in agriculture, food and non-food areas. It discussed brief about Millets, types of millets, Production of millets at global level and health benefits.

Chapter 2 explains the review of the literature to review the work done so far in this. Literature review describe the application part of NIR spectroscopy in the mainly agriculture field and food industry along with the association of various regression technique and chemometric approach by using quantitative (PLS, iPLS, SiPLS, MLR and PCR) as well as qualitative approach (PCA, PLS-DA, ANN), prediction of quality attributes, proximate compositions of various cereals (wheat, rice, millets, legumes and lentils), origin/region discrimination. The research gap and objectives were found after completion of literature survey and it will be discussed in this chapter.

Chapter 3 discuss about some physical and rheological properties of Pearl millet. This chapter include the introduction and required background, methods, results and discussions and conclusion of the proposed work.

Chapter 4 describes the chemical quality attributes i.e. moisture, ash, crude fat, crude protein and carbohydrates content present in the pearl millet by using AOAC standards. This chapter include the principal component analysis (PCA) method applied by using NIR, MIR spectroscopy for classification of pearl millet samples according to their regions.

Chapter 5 explains the development of calibration model of pearl millet to predict the quality attributes named as moisture, carbohydrate, crude protein, crude fiber and crude fat, by using NIR and MIR spectroscopy with association of chemometrics. This chapter covers the prediction of quality parameters of pearl millet using selective wavelengths in NIR region. In this chapter, PLS, iPLS and SiPLS regression, numerous pre-processing, regression coefficients, coefficient of determination and root mean square were explained

in detail. The PLS models were built by using full wavelength and selective wavelengths of NIR and MIR spectroscopy and results were compared.

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CHAPTER-2

REVIEW OF LITERATURE AND OBJECTIVES

2 Literature Review

In comparison to other vibrational spectroscopic methods, the use of lower wavelengths in the NIR region increases penetration depth. This makes it possible to analyse solid materials directly with little to no sample preparation. NIR spectrophotometers could now be used at-line, on-line, or in-line for quality control reasons in the production setting due to this, as well as their advantages of being chemical-free, quick, non-destructive, and non-invasive.

There are several applications of NIR spectroscopy for the development of calibration model to predict the physical as well as chemical properties of the sample. The review of literature covers the use of infrared spectroscopy for determination of parameters in cereals and legumes. In cereals (wheat, rice and millets) review includes the research papers where the model has been developed to determine parameters of wheat and rice. Spectroscopic application for property determination of lentils and peas has also been listed. In case of millets, review revealed that research has been carried to determine the physical and chemical parameters, but no or very few literature has been obtained for determination of properties of millets using spectroscopy.

2.1 Application of spectroscopy in quantitative analysis of Cereals

2.1.1 Wheat

A Ibrahim et al.[1] reported the quality attributes of various varieties of wheat using rapid and non-destructive near infrared spectroscopic technique. They determined the protein, gluten, moisture content and starch attributes by employing AACC methods for the three wheat varieties named as Alfold (QA), Mv Suba (QS), and Mv Toborzo (QT) They have employed the rapid NIR technology to develop the statistical calibration model. For checking the accuracy of calibration model, the external validation sets have been used. They have taken 120 wheat samples for development of calibration model. The model was developed using partial least square (PLS) regression and they noticed that protein, gluten and starch in 1000-2500 nm region and moisture in 680-2500 nm NIR region. They observed the coefficient of external prediction (R^2_P) as 0.96, 0.98, 0.98 and 0.94 for protein, gluten, moisture content and starch respectively. They concluded that the precise results of

prediction model can be obtained from PLS calibration model using NIR technique and it was confirmed from the accuracy of model that NIR approach is rapid, safe and non-destructive method to determine the wheat quality attributes non-destructively.

B Foroozani et al.[2] reported the development of non-destructive calibration model and classification for protein content of three varieties (Mihman, Gaskojhen and Pishgam) of wheat samples. They have taken the 54 wheat samples for the model development of protein content. They used discriminant analysis techniques PLS-DA and LDA with various preprocessing to classify the wheat samples on the basis of protein and identification of protein content in wheat. They observed the parameters coefficient of determination (R^2) as 0.82 and 0.79 and root mean square error (RMSE) as 0.73 and 0.79 for calibration and validation sets for protein content respectively. They concluded that PLS-DA gives the excellent performance as compare to PCA-LDA for classification of varieties of wheat samples. Hence, they pointed that NIR techniques are useful for classification and identification of protein content of wheat samples.

Y Bao et al.[3] reported the classification of the five different varieties named as Zhenmai9, Annong1124, Shannong102, Weilong169 and Longpingmai6 of wheat grains collected from China, by implementation of hyperspectral imaging technique with the combination of chemometrics. They have used the principal component analysis (PCA) and linear discrimination analysis (LDA) technique for development of classification models. Overall, the findings indicated that hyperspectral imaging was a promising method for the quick and precise identification of wheat varieties, which might be carried out in large-scale seeds classification and quality detection in the contemporary seed sector.

R Wang et al.[4] reported the development of calibration model for resistant starch (RS) with the help of PLS regression and four preprocessing by ATR-MIR and NIR spectroscopy. They observed that the maximum normalization association with Savitzky-Golay smoothing (MN + SGS) preprocessing in the NIR technique exhibited a lower root mean square error value (RMSE-0.385, RMSEP -0.459) and a highest coefficient of determination (R^2_C= 0.67, R^2_P= 0.55). In terms of the ATR-MIR technique coefficient of

determination represented as R^2_C= 0.92, R^2_P= 0.82; and RMSEC as 0.15, and RMSEP as 0.28, and they also observed that the baseline preprocessing approach performed better. They have concluded that rigorous validation of the constructed calibration models and indicated that the ATR-MIR calibration model has a superior resistant starch prediction performance than the NIR optimum model.

A Ibrahim et al.[5] reported the use of non-destructive NIR technique for determining the hardness of the wheat grains. They used PLS regression method to build the calibration model of wheat samples. They measured the hardness from Single-Kernel Characterization System (SKCS) and Instron Universal Testing Machine (IUTM) and then used reference values for development the calibration model. The quality of model completely depends upon the prediction of unknown samples. Therefore, they observed the coefficient of determination of prediction (R^2_P = 0.91) and prediction deviation as 3.35 for hardness of wheat. They concluded that the NIR spectroscopy can be used to measure the hardness of wheat samples nondestructively.

W Tian et al.[6] investigated the phenolic compound in wheat flour non-destructively by using NIR technique and chemometrics. They observed that the coefficient of determination of calibration and validation sets were 0.92 and 0.90 and prediction deviation was 3.4 for phenolic compounds. They concluded that the total phenolic compounds were present in wheat flour. They reported that NIR techniques with the help of chemometric give the quality attributes in rapid and eco-friendly manner and more efficient than wet chemistry.

K Liang et al.[7] compared the Vis-NIR and SWIR hyperspectral imaging techniques to determine the deoxynivalenol content in wheat flour and wheat kernel. The authors have taken Vis-NIR within range from 400-1000 nm and SWIR from 1000-2500 nm. They used the spectral data with various preprocessing named as MSC and SNV. They used genetic algorithm (GA), support vector machines (SVM) and sparse auto encoder (SAE), neural network methods to construct classifiers. They concluded that models based on the wavelengths selected by the genetic algorithm out performed those based on the entire

spectrum. They observed that in the 400-1000 nm range, the results of the models built with wheat flour samples equivalent to those observed with wheat kernel samples. However, they observed in the 1000-2000 nm range, the results of the models established with wheat flour samples were superior to those found with wheat kernel samples.

U Kamboj et al.[8] reported the comparison of multivariate chemometric methods with NIR technique to determine the protein and carbohydrates content of stored wheat grains. They used the PLS, PCR, MLR and SVM Regression methods to develop the model. They observed that the coefficient of determination of protein content from PLS model as 0.955, 0.997 for wavelength set I and II at 4-degree Celsius storage of grains and 0.903, 0.989 for 21 degrees Celsius respectively. They also observed that R^2 values for prediction of carbohydrate content of wheat samples stored at 4 and 21 degrees Celsius as 0.978, 0.951, and 0.946, 0.974 at Set I and Set II, respectively. They concluded that MLR and PLS predict the properties with high accuracy and PCR predict with low accuracy ($R^2 = 0.4$). They also concluded that the wheat grains can be distinguished from one other when preserved under different conditions using near infrared spectroscopy.

M Cocchi et al.[9] investigated the adulteration of durum wheat flour with bread wheat flour using near-infrared spectroscopy. They used the PLS and the newly generated wavelet-based WILMA calibration algorithm, multivariate calibration methods to accomplish this purpose. They observed both methods produced acceptable outcomes, with the amount of adulterant in the samples being measured with less uncertainty than that implied by the official Italian approach.

T Guo et al.[10] reported the investigation of chemical compositions of wheat and corn straw non-destructively. They have investigated the chemical compositions such as hemicelluloses, neutral detergent fiber (NDF), crude protein (CP), acid detergent lignin (ADL), acid detergent fiber (ADF) and moisture for 156 corn and 135 wheat straw samples by using NIR technique. They developed the calibration model by employing the modified partial least square (MPLS) regression with good accuracy. They observed that the coefficient of determination for crude protein (CP), neutral detergent fiber (NDF) and acid

detergent fiber (ADF), as 0.962, 0.834 and 0.874 for both straws because moisture, CP and NDF gave good prediction in corn straw and only moisture as well as CP was predicted in wheat straw, therefore the author mixed the samples to achieve the accurate prediction models. They have noticed the coefficient of determination of calibration model for moisture and ADF was 0.793 and 0.834 respectively. Thus, they concluded that the advance non-destructive NIR technique useful to determine the nutritional parameters of forage crops.

P Pandey et al.[11] reported the comparison in between NIR and FTNIR spectroscopy techniques in term of physico-chemical compositions of wheat samples. They observed that the coefficient of determination for moisture, protein, ash, fat, thousand kernel weight and hardness as 0.97, 0.95, 0.87, 0.90, 0.95 and 0.82 respectively using FTNIR technique and as 0.96, 0.90, 0.87, 0.75, 0.97 and 0.88 respectively using NIR technique. They concluded that the FTNIR spectroscopic technique shows the precise results in terms of proximate composition of wheat samples.

N Caporaso et al.[12] reported the review on the combination of near infrared (NIR) spectroscopy and hyperspectral imaging (HSI) for non-destructively assessment of quality attributes in cereals and grains. They concluded that the capability of multi-component analysis using HSI, such as the determination of protein content, moisture as well as other minor compounds or more complex properties, is another clear advantage. For instance, by imaging wheat grains, one can predict the viscoelastic behavior of dough or bread. When employing NIR-based equipment and specifically HSI, crucial considerations to bear in mind include the accuracy of the models and the requirement for reliable data processing, especially when using this technology for practical applications.

D Ye et al.[13] reported the development of calibration model for protein present in wheat samples by employing NIR and PLS regression method. They used Improved simulated annealing (ISA) combined with PLSR to select the effective wavelengths from spectral data. They observed the standard error of prediction values decreased from 0.071 to 0.056, and the coefficient of determination between the predicted and reference values of protein

content present in wheat increased from 0.998 to 0.999. They concluded that by enhancing data analysis methods, this study significantly increased the prediction performance of the model and established a quick, and accurate approach for the online assessment of wheat protein content.

H Shi et al.[14] reported the comparison of MIR and NIR spectroscopic technique with chemometrics in terms of determination the crude protein and intestinal protein digestibility of wheat samples. The authors predicted the crude protein and protein digestibility with the help of chemometrics and spectroscopic techniques. They have used the various types of preprocessing on the spectral raw data to develop the calibration model. They observed that coefficient of determination of prediction performance at the effective variables were defined as 0.98 and 0.96 for crude protein and 0.68 and 0.67 for protein digestibility using NIR and MIR spectroscopy respectively.

R M Amir et al.[15] reported the quality characteristics by AACC standards and characterization of wheat samples by using FTIR spectroscopy. The authors analyzed the physical, chemical and rheological properties of various varieties of wheat flours. The FTIR spectroscopy contain the fundamental bands within MIR region and functional groups present in the organic samples. They observed that on the basis of functional group H and OH, water peaks were seen in the range of 1640 cm^{-1} and 3300 cm^{-1}. On the basis of bond amide, I and amide II, protein was found in the ranges of 1600 to 1700 cm^{-1} and 1550 to 1570 cm^{-1} respectively. Starch and fat were both detected within these ranges, whereas starch was detected based on the C-H bond between 2800 and 3000 cm^{-1} (C-H stretch area) and between 3000 and 3600 cm^{-1} (O-H stretch region). Therefore, they concluded that the FTIR technique is a rapid, dependable, and less expensive analytical method that is very successful for assessment of several wheat quality parameters and provides a great way to analyze the chemical composition of various wheat varieties.

J Hell et al.[16] reported the study of calibration model for moisture, ash, protein, starch, soluble as well as insoluble dietary fibers and lipids of wheat bran samples with PLS regression method and using NIR and MIR spectroscopic techniques. The authors

compared the NIR and MIR spectroscopic results and they reported that in contrast to MIR, that was shown to be superior just for protein, NIR was found to perform better for ash, starch, and dietary fiber. Moisture and fat received about equal scores. Ash, insoluble dietary fiber and fat prediction outcomes were acceptable. They concluded that, the NIR outcomes of prediction give the better results as compared to MIR spectroscopic technique.

P F Ndlovu et al.[17] reported the detection of adulteration and quantitative analysis of unripe banana flour (UBF) with wheat adulteration using NIR spectroscopic technique. They were prepared the samples by adding 0-800 g/kg of wheat flour in unripe banana flour in gap of 20 g intervals. They observed that the PCA successfully classify the adulteration in samples. They have done the PLS regression to construct the calibration model for detection of the quantity of adulteration present in the samples with good accuracy. Therefore, they concluded that the NIR technique can be used for detection of adulteration in organic samples in rapid and nondestructive manner.

C Miralbes[18] analyzed the protein, moisture, dry gluten, wet gluten, starch damage, and ash levels of wheat flour samples nondestructively. Additionally, the authors used the farinograph and alveograph evaluate the rheological testing of wheat flour dough. For each component or attribute, modified partial least squares (MPLS) analyses using NIR transmittance spectroscopy were constructed. They concluded that some models were accurate enough to be used in regular analysis to estimate chemical or physical properties using NIR transmittance spectroscopy.

2.1.2 Rice

M Makky et al.[19] reported moisture content of rice samples and develop the non-destructively calibration model to predict the moisture using NIR spectroscopic technique. They procured two varieties of rice samples namely Junjuangan and Mundam rice and used for spectral analysis. They used the principal component analysis to determine the correlation in between moisture contents for both rice samples and PLS regression used for calibration and validation sets. They observed that calibration coefficient of determination of moisture as 0.826 and 0.955 for Junjuangan and Mundam rice respectively and

validation coefficient of determination as 0.788 and 0.968 for Junjuangan and Mundam rice samples respectively. They concluded that NIR techniques successfully used to predict the moisture content of rice samples non-destructively.

S Fan et al.[20] reported the non-destructive method to estimate the amylose and fat content in various rice varieties using NIR techniques. They develop the calibration model to predict the fat and amylose content in rice with good accuracy. They observed that coefficient of determination for calibration and prediction of fat and amylose was greater than 0.6. They concluded that the NIR techniques might be used for the quick, non-destructive identification and classification of individual rice seeds with various amylose and fat content.

L Lin et al.[21] reported the moisture prediction model of the moisture content for rice grains using NIR techniques and also constructed sensor. They chose light source, an LED with a wavelength of 1450 nm and the designed the circuit and software for construction. They observed that the rice grain within 13–30% moisture range have the sum of squares for error 25.47 and the coefficient of determination 0.936. As a consequence, their suggested approach for measuring the moisture content of rice grains using a single NIR band produced good results and suited the requirements of manufacture of products.

D L N Doan et al.[22] reported a quick and non-destructive method for spotting contaminated rice samples. The authors have taken the total 128 adulterated rice samples and 72 original rice samples for detection of adulteration in rice samples and analysis were done using near infrared spectroscopy in association with soft independent modelling of class analogies (SIMCA) and partial least squares-discriminant analysis (PLS-DA). They concluded that while PLS-DA approach was more appropriate as compared to SIMCA. They also concluded that the accuracy of the technique was even greater when focusing on original or 10%-contaminated samples.

D Wu et al.[23] reported the geographical discrimination of Chines rice samples by using PCA and PLS-DA with NIR technique. In this study, the authors used 231 rice samples according to different regions of China and they reported that the classification was not

possible using PCA. Therefore, PLS-DA method successfully classifies the samples with the help of various preprocessing applied on the NIR spectral data. Thus, it was concluded that as a consequence, NIR in combination with PLS-DA, NSD, or MSC preprocessing can offer an effective tool to differentiate the various agricultural techniques and the origin of Chinese rice.

H L Wang et al.[24] have constructed the four calibration models to quantitatively determine the amount of fat in brown rice grain, flour and milled rice and flours using the NIRS technology and the PLS algorithm. The coefficient of determination for calibration models were 0.79, 0.84, 0.89, and 0.91 for validation 0.73, 0.81, 0.81, and 0.89 and external validation models were 0.62, 0.80, 0.81, and 0.87 respectively for rice samples. They reported that in terms of predicting the fat content of rice, NIRS is comparatively accurate. The four calibration models developed in this study should be helpful for rice breeding to increase nutritional quality.

Q Zhang et al.[25] have determined the γ-aminobutyric acid content in germinated brown rice by using NIR technique with wavelet de-noising pretreatment method. They observed that the coefficient of determination of calibration and prediction as 0.931 and 0.916 respectively. They concluded that NIR spectroscopic techniques is rapid, hazardous and is non-destructive approach to detect the γ-aminobutyric acid content in brown rice sample.

L H Lin et al.[26] reported the prediction of protein content in rice samples using MLR, PLS and ANN methods with the help of NIR approach. In this study the 180 rice samples were used for analysis. The authors observed that the coefficient of determination of prediction as 0.769 using MLR, 0.782 using PLSR and 0.806 using ANN methods. They concluded that the PLSR and ANN gives the better results as compared to MLR method and the protein content in rice samples was predicted successfully with good accuracy using the association of NIR and chemometrics.

R John et al.[27] reported the detection of chemical compositions named as total protein, starch, amylose, dietary fiber, and oil content in rice samples using NIR spectroscopic technique. They observed that the coefficient of determination for dietary fiber and protein

content was 0.945 and 0.917 respectively, give the best model performance with high accuracy. Therefore, they reported that the development of whole grain models that are really non-destructive, directly useful to plant breeders, and do not require homogenization of a sample.

J Zhang et al.[28] investigated the adulteration in Y Liangyou 900 rice samples with other different four varieties using NIR spectroscopy and then develop a calibration model to detect the adulteration in rice samples. They observed that the coefficient of determination for prediction and validation in modeling was 0.920 and 0.845 respectively using PLS method. In long NIR region coefficient of determination for prediction and validation was 0.930 and 0.894 respectively. They reported that with the help of NIR and chemometric the good correlation was achieved in between actual and predicted values.

P Mishra et al.[29] reported the identification of protein content in rice kernel, rice powder and brown rice using NIR with the help of PLSR methods. They observed the good prediction for the brown rice with low prediction error i.e., 0.349%. Thus, they concluded that there is no requirement of making the flour of rice sample for protein analysis. Only 12 important wavelengths (960.1, 978.9, 986.8, 1001.6, 1006.6, 1051.1, 1086.7, 1155.0, 1194.6, 1202.5, 1218.4 and 1235.2 nm) were required for prediction of protein content in rice sample.

T B Bagchi et al.[30] compared the calibration models constructed by various chemometric approach for the grain protein and amylose content of brown rice and nutritional parameters rice bran. In the study, the author used 173 samples of brown rice and 86 samples of rice bran. They observed that modified partial least squares model correlating with the optimal mathematical treatments. Further, they used the pair t-tests and correlation regression analysis in between reference and predicted data to confirm the accuracy of models.

C Ma et al.[31] reported the prediction of protein content in rice samples with husk by using hyperspectral imaging (HSI) in range of 938-2215 nm and PLS, PCR and LS-SVR chemometric technique. PLSR model was developed for protein in rice with husk samples at full wavelength with R^2_C 0.8894 and R^2_P 0.8434. Also, PCR and LS-SVR prediction

model with R^2_C = 0.8416, R^2_P = 0.8357 and R^2_C = 0.8676, R^2_P =0.8292 respectively was developed. They concluded that the PLS prediction model gives the better performance as compared to PCR and LS-SVR prediction models. Also, for the 17 effective wavelengths the PLSR model gave excellent performance with the R^2_P = 0.8011, RMSEP = 0.52.

Z Qiang et al.[32] determine the moulds colony of paddy rice with the help of NIR and PCR and MLR chemometrics. The authors stored the 121 paddy rice samples at various temperatures 5℃, 15℃ and 25℃ and NIR spectra for all samples were collected within the range of 918 to 1045 nm to develop the model based on PCR and MLR method with maximum value of R^2 for calibration as 0.943 and prediction as 0.897. Therefore, they concluded that the NIR spectroscopy is most important tool for determination of colony of moulds in paddy rice.

S Wongsaipun et al.[33] reported the detection of adulteration in Jasmin Thai rice samples by using NIR and chemometrics by using 423 adulterated samples for analysis with 39 pure rice samples and others mixture of 4 samples of Oryzasativa L. ssp. indica cv. Pathumthani 1 (PT1), 16 samples of Thai rice, 19 samples of Oryza sativa and KDML105 rice. PLS regression model was used to determine quantity of KDML105 in the mixed rice samples using Orthogonal projection (OP) and Piecewise direct standardization (PDS) algorithm to achieve the accuracy of model. Therefore, they concluded that OP and PDS give the excellent results as compare to PLS.

F Shen et al.[34] reported the MIR and NIR spectroscopy technique with combination of chemometrics for classification and quantification of aflatoxins content within range of 0-2435.8 lg/kg in 132 brown rice. LDA method was used for classification of aflatoxins content with the accuracy rate of 96.9 and 90.6% for NIR and MIR approach respectively. PLS regression was used for calibration model development with good accuracy in terms of high R^2 =0.936 to 0.973 and 0.922 to 0.970 for NIR and MIR spectroscopy respectively. Results showed that IR spectroscopy technique is a rapid and non-destructive tool for detection of aflatoxin adulteration in grains.

2.1.3 Millets

D N Chilkunda et al.[35] investigated the carbohydrate profile of different types of wheat, sorghum and bajra to make the good quality roti using an isolation process for polysaccharide fractions from these cereals and wheat brans. They have studied about the bajra which had almost same ratio of arabinose and xylose and observed that the major polysaccharides arabinose has major part, whereas starch and cellulose have minor.

J Chen et al.[36] used the near infrared method to determine the crude fat, total carbohydrate and protein and crude fat contents in foxtail millet. They used SPA, iPLS and MLR method for the variable selection. As a result, they have determined coefficient of validation, the ratio of standard error of prediction to standard deviation (RPD) and the root mean square error of prediction of the obtained optimum models were 0.94, 0.28 and 4.07, for protein content. For total carbohydrate r^2, RPD and RMSE parameters were 0.92, 0.40 and 3.28, and for crude fat were 0.70, 0.17 and 1.76, respectively. They concluded that, the near infrared spectroscopy is the most appropriate approach for predicting the crude fat, total carbohydrate and protein contents of foxtail millet particularly.

C D Nandini et al.[37] investigated the structures of arabinoxylans from pearl millet analyzed the quality of the millet for making the roti and unleavened bread. They have done the qualitative analysis by using FTIR, ^{13}C NMR, methylation analysis. An isolation process was done arabinoxylans from hemicellulose B and the barium hydroxide-extracted polysaccharides. Research discovered that the arabinoxylans from bajra has large no. of branches which is responsible for crispiness of roti.

A Sumathi et al.[38] have done the characterization of the pearl millet for investigating the physico-chemical properties and shelf-life and nutritional quality. They observed that the millet grits were the size of less than 355 μm and the moisture content is almost 18%. They also investigated the melt energy, the carbohydrate digestibility, and viscosity of the cold and cooked paste of precooked products. It was concluded that the technology of extrusion cooking of pearl millet can have the great applications for preparing the food products from the millet which can have some values added over there and can also have some diversity.

W C Qing et al.[39] have discussed near infrared spectroscopy technology for variety identification and composition extraction of the millet - DUN millet, JIN 21 millet, and 5 other types of millet. Principal component analysis, discriminant analysis and neural network technology were used to build BP neural network prediction model for millet. As a result, they concluded that the prediction model was best to be found.

X S Yang et al.[40] have determined fat, protein, starch and amino acids in Foxtail millet using FT-NIR spectroscopy. They have done the spectral data lineralized from chemical analysis and PLS regression for building the calibration model and testing of optimized model by external validation set samples. As a result, It was concluded that for crude protein, glutamic acid, leucine, alanine, aspartic acid, isoleucine, and serine the RPD values were approximately greater than equal to 2.5 whereas the RPD value for proline, threonine, glycine, histidine, phenylalanine, tyrosine, and valine was greater than equal to 2.0 and R^2_{val} values were greater than 0.83 and for arginine and lysine, crude fat, total starch, RPD values were greater than 1.5 and R^2_{val} values were greater than 0.70.

E A Baryeh[41] has discussed about the physical as well as mechanical properties of millet and other grains. They have determined the grain length, geometric diameter, and thickness of millet within moisture content having range between 5%-22.5% and observed grain moisture increased from 3.522 to 4.163 mm, the grain length increased from 2.735 to 3.211 mm and the thickness increased from 2.180 to 2.788 mm and the geometric diameter increased from 2.759 to 3.340 mm. The grain surface area, volume and sphericity also have been defined as increased from 23.91 to 35.05 mm^2 and 8.2 to 14.65 mm^3 and 0.783 to 0.802 respectively. The coefficient of static friction also has changed from 0.42 to 0.79, 0.39 to 0.75, 0.36 to 0.69 respectively for plywood, mild steel and galvanized iron.

R K Jain et al.[42] have discussed some properties of millet. They have taken three different types of pearl millet seeds named as GHB30 and Bajra 28-15, and one Babapuri variety as a sample and average three principal dimensions which are 3.12, 1.94, 1.70 mm for GHB30 and 2.98, 1.86 and 1.82 mm for Bajra 28-15 and 3.36,2.24 and 2.01 mm for Babapuri varieties. They found the volume and surface area parameter for a single grain . For hybrid

varieties its 3.8 mm^3 and 12.5 mm^2 and for traditional variety its 5.8 mm^3 and 16.4 mm^2 respectively. They also focused other parameters like sphericity, bulk density, grain density and the shape factor for different varieties.

N G Malleshi et al.[43] have discussed about the physicochemical properties of those starches which are isolated from native and malted finger millet, pearl millet and foxtail millet. The authors compared the native starches to malt starches and that have small granules, more amylase, greater gelatinization temperature, lesser swelling power and lesser intrinsic viscosity. They concluded that pearl millet starches are more frequently capable for amylolysis as compare to finger millet starch.

F Zhu[44] has discussed about the various physicochemical properties, the structure and the uses of millet starch. Author has focused on the physicochemical properties, modifications, isolations, structures, chemical compositions, enzyme susceptibilities and uses of millet starch.

F O Sade[45] have discussed the effect of germination, roasting and fermentation on composition, anti-nutritional parameters and functional properties of pearl millet. They have determined the protein, fat, ash, crude fiber, carbohydrates and energy content for processed pearl millet flour and also determined the water absorption capacity and oil absorption capacity in functional properties as well as tannin and total phenol in anti-nutritional properties for processed pearl millet flour. They observed that the protein content, water absorption capacity and oil absorption capacity significantly increase in germination process and anti-nutritional properties were decreased in roasted process of pearl millet.

S Balasubramanian et al.[46] have discussed the characterization of modified pearl millet starch. The authors have determined the physicochemical, freeze thaw stability, paste clarity and pasting characteristics from native and modified pearl millet starch. It was observed that the acidic and enzymatic modified starch shows high values of color, freeze thaw stability, paste clarity and pasting characteristics, whereas high values of swelling power, solubility and water binding capacity for hydrothermally modified starch of pearl millet.

G W Burton et al.[47] have determined the proximate composition of pearl millet. In the chemical compositions they investigated moisture, protein, oil, ash and calcium (Ca), phosphorus (P) contents. They observed the moisture content (10.01%), protein (16.0%), oil (4.5%), ash (2.2%), Ca (46 mg) and P (314 mg) in pearl millet.

M A Abd Allah et al.[48] have discussed the physical properties of starches extracted from yellow corn, sorghum, sordan and pearl millet. They have calculated the different physical properties likewise swelling power, solubility, amylograph, water binding capacity and intrinsic viscosities and predicted that the yellow corn and pearl millet have higher binding capacity

S Rathore et al.[49] have discussed about the effect of natural and pure culture fermentation on nutritive, physicochemical, functional and anti-nutrient compositions of pearl flour. Pearl millet naturally fermented at 20, 25, and 30 degrees Celsius whereas, in pure culture fermentation they used saccharomyces cerevisiae, saccharomyces diasticus, Lactobacillus fermentum and Lactobacillus brevis methods. They observed that moisture and fat content increased and protein decreased in both fermentations. In the natural fermentation thiamine content significantly decreased with increase in temperature but it was increased in pure culture fermentations. Total soluble sugar content increased in natural fermentation and decreased in pure culture fermentation whereas oil absorption capacities were increased in all fermentations. Whereas pH, least gelation concentration, and anti-nutritional factors like tannins and phenolic compounds were decreased in all types of fermentations.

K O Falade[50] has discussed the effect of γ-Irradiation on color, physicochemical and functional properties of two pearl millet varieties namely SOSAT and ZATIV. It was observed the γ-irradiation treatment was within range from 2 and 4 kGy and no significant change in color and functional properties whereas a little effect of radiation was observed on the pasting characteristics of the grains.

A K Siroha et al.[51] have discussed about the physicochemical, functional and antioxidant properties of flour from various cultivars namely HC-10, HHB-223, HHB-67, W-445,HHB-226and GHB-732 of pearl millet that grown in India. In the physicochemical

properties they determined moisture, ash, fat, protein, fiber content bulk density and color. Therefore, in functional properties, water absorption capacity, oil absorption capacity, foaming capacity, emulsion capacity and in antioxidant properties were measured. They used the PCA to determine the variations in between pearl millet varieties and observed the good correlation in between physicochemical, functional, and antioxidant properties.

M Tomar et al.[52] have developed the calibration model for the biological nutritional parameters such as starch, amylose, resistant starch, protein, oil, phenolics, total dietary fiber, total soluble sugars and phytic acids for pearl millet by using NIR and modified partial least square regression method.

J O Ojediran et al.[53] have discussed the moisture effect on physical properties of two variety of pearl millet grains namely Ex-Borno and SOSAT C88. In the physical properties, they measured the parameters such as axial dimensions (length, width and thickness), bulk density, solid density, sphericity, porosity, angle of repose, static coefficient of friction on five structural surfaces and thousand seeds mass in 10 to 20% moisture range. It was observed that the axial dimensions, porosity, thousand mass and angle of repose increases as well as bulk density and solid density decreased with increase in moisture content. They concluded that the seed from Ex-Borno were smallest as compared to SOSAT C88.

L D Goneli et al.[54] have discussed the water sorption isotherms and thermodynamic properties of pearl millet. In the water sorption isotherms, they observed the equilibrium moisture content at various temperatures and relative humidity conditions. In thermodynamic properties, the determination of the parameters named as integral isosteric heat of sorption, enthalpy–entropy compensation theory and differential entropy was also done.

K S Sandhu et al.[55] have discussed the physicochemical, thermal, rheological, morphological and in vitro starch digestibility properties from various cultivars of pearl millet starches. They observed the correlation between these properties of millets.

M Sharma et al.[56] have discussed about the effect of heat moisture treatment (HMT) of starches from pearl millet on rheological and functional properties. They have determined the rheological, functional, and morphological characteristics of pearl millet starch. Furthermore, they have done the heat moisture treatment (HMT) of starches at various moisture levels for 8 hours at 110 °C. They observed during HMT, peak, breakdown, cold paste, and setback viscosities all reduced while pasting temperature rose. Also observed, HMT-30 sample has the highest level of shear stability. The native sample had the highest (33.5 Pa) storage modulus value while the HMT-25 sample had the lowest. Starch gel yield and flow point reduced during HMT, indicating softer gels with greater spread ability. They have also noticed the gelatinization temperatures were raised by HMT from native to HMT-30. In the HMT-30 sample compared to the native, the amount of resistant starch rose by almost three times. After HMT, swelling power and solubility reduced.

M Sharma et al.[57] have discussed the heat moisture treatment (HMT) effect on resistant starch (RS) content, heat and shear stability of pearl millet starch. They used HMT to modify starch for 4 hours at 110°C with various moisture content, namely HMT-20, HMT-25, and HMT-30. It was observed that the content of resistant starch (RS) rose considerably and dextrose equivalent (DE) value increased as well. The starch's solubility dropped, whereas swelling power and oil absorption capacity increased.

A M N Azhari et al.[58] addressed the impact of fermenting pearl millet flour (with the use of defatted moringa flour and defatted fenugreek flour supplements) on the basis of cooking on chemical composition and total energy.

A K Siroha et al.[59] have discussed the physicochemical, pasting and rheological properties of starches from several pearl millet cultivars namely RHB-173, GHB-538, RHB-177, GHB-558, GHB-905, GHB-719, RHB-58, GHB-744, RHB-30, RHB-121.

P P Pawar et al.[60] have discussed about the physicochemical properties of the pearl and finger millet. In the physicochemical properties the authors described the physical as well as chemical properties of millets. They have determined the physical parameters like bulk

density, true density, porosity, thousand kernel weight and angle of repose and chemical properties named as moisture, ash, protein, fat and carbohydrate contents.

A A Kulthe et al.[61] have discussed the proximate compositions, minerals and anti -nutrients parameters of pearl millet cultivars namely Shanti (RHRBH 9808), Dhanshakti (ICTP 8203 Fe10-2) and Pioneer 86M64. They determined the proximate compositions that were moisture, ash, fat, protein, fiber and carbohydrates, minerals contents were calcium, phosphorus and iron and anti-nutritional properties like phytic acid, tannin and polyphenol content of pearl millet.

D Patni et al.[62] have reported proximate compositions, mineral compositions, anti-nutritional factors from raw and germinated pearl millet flour as well as sensory properties like texture, taste, appearance and flavor from homesteads products of pearl millet were determined. They observed that the crude protein content of millet flour was high as compared to raw millet flour and concluded that the germination process increased bio availability of nutrients.

A A Abdalla et al.[63] have measured the proximate content, starch, phytate and mineral contents from ten genotypes of pearl millet. They have found proximate composition like ash, crude protein, crude fiber, oil, starch and phytic acid, minerals like Ca, Mg, K, Zn, Mn, Na, Cu and Fe as well as the relationship between phytate and total phosphorus content. They observed that Kabti genotype is the maximum value of crude protein.

J A Adebiyi et al.[64] have reported the proximate compositions, minerals, amino acid and total phenolic content from fermented and malted pearl millet flour as well as biscuits. They observed that fermentation and malting process improved the proximate compositions of pearl millet flour and high value for moisture, crude fiber, protein and energy as well as lower values of fat and ash for fermented and malted biscuits samples and also observed that the essential and nonessential amino acids were increased in fermentation and malting pearl millet flour.

M A M Ali et al.[65] have discussed about the fermentation effect on pH, protein and vitro protein digestibility from two cultivars of pearl millet namely Madelkawya and Population 1/Shambat. They have determined the physicochemical properties for two cultivars of pearl millet. They observed reduced value of pH, minor change in protein and enhancement in protein digestibility with increased the time of fermentation from 0 to 14 h for both cultivars and all measurements were taken within 2 h of intervals.

N Dangi et al.[66] have reported the effect of gaur gum and acid hydrolysate on physicochemical, pasting, rheological, thermal and texture properties of native pearl millet starch within various concentrations.

S H A Elyas et al.[67] have discuss the fermentation effect on nutritive values and protein digestibility of the two cultivars of pearl millet (Composite Population III and Baladi). The pearl millet was fermented for 36 h and observations taken within 4 h interval. They observed that the polyphenols and phytic acid reduced and tannin content remains constant with natural fermentation of two pearl millet cultivars.

J O Owheruo et al.[68] have discussed about the physicochemical (total titratable acidity, pH, proximate compositions, minerals), phytochemical and antioxidant properties within effect of germination of pearl as well as finger millets. They observed that during the germination, total titratable acidity, protein, fiber, alkaloid and saponin contents increased and reduction in pH, fat, tannin and phytate contents.

D Panda et al.[69] have reported the nutritional, physicochemical and function properties of sprouted and raw millets. The authors have reported the comparison of various types of raw and sprouted millet flours on the basis of their proximate compositions and functional properties. They have concluded that the nutrients, vitamins and functional properties led to superior by sprouting except crude fiber and protein in all the investigated millets.

2.2 Application of spectroscopy in quantitative analysis of Legumes

2.2.1 Dried Beans and lentils

R Hayati et al.[70] employed the NIR spectroscopy to estimate the quality attributes such as fat and moisture for intact cocoa beans. They have taken total of 110 raw cocoa bean samples and near-infrared spectral data have been collected at wavelengths between 1000 and 2500 nm and constructed the prediction models using PCR and PLSR, two regression techniques. They concluded that PLSR was more accurate than PCR at predicting both quality metrics.

J Akhir et al.[71] reported the development of calibration model using NIR and ANN technique for caffeine content in green coffee beans. They have used various types of preprocessing to reduce the noise of NIR spectra. They concluded that the combination of ANN and NIR gives the good accuracy in prediction of caffeine content in green coffee beans.

Chris Heil et al.[72] developed the calibration model to predict the moisture (8.9–14.6), protein (32.5–39.6), oil (16.0–20.8) and lenolenic acid (1.0–9.5) in soyabean by using FT-NIR and PLS regression. They observed that the coefficient of determination of moisture, protein, oil and lenolenic acid as 0.98, 0.87 0.85 and 0.94 respectively. They summarized, that the FT-NIR approach can rapidly and precisely determine the number of important constituents in soybeans.

R Wiradinata et al.[73] reported the prediction model of moisture content with the help of NIR and MLR techniques of Java Preanger coffee bean samples. They constructed the calibration model for 50 samples by using 17 effective wavelengths. Therefore, the coefficient of determination for moisture content 0.902 and low prediction error shows the good accuracy of model.

M A Berhow et al.[74] determine the isoflavone and saponin content in ground soybean by using MLR and NIR spectroscopic technique. They have taken total 3200 samples and used various preprocessing in analysis of soybean samples. They have collected the chemical data by HPLC technique and used as a reference for model requirement. They established

reliable verified predictions for isoflavones and less accurate calibrations for total saponins and concluded that it is possible to rapidly and nondestructively extraction of isoflavone content of ground soybeans using NIRS.

M Meenu et al.[75] reported the phenolic compound in mung bean by using NIR and PLSR chemometric approach. They have used the various type of preprocessing named as standard normal variate (SNV) and linear baseline correction (LBC) to develop the calibration model with high accuracy ($R^2 > 0.987$) and root mean square (RMSE < 1.82%). They concluded that the NIR is a rapid, non-hazardous and non-destructive technique that predict the phenolic compound in mung beans in green way.

B Carbas et al.[76] reported the study of phenolic compounds in mung beans using MIR and NIR spectroscopic technique and chemometric approach. They have used HPLC-DAD chemical approach for determination of phenolic compounds. They have determined the accuracy of model by coefficient of determination of calibration and validation sets and that were $R^2_C = 0.86\text{-}0.99$ and $R^2_V = 0.75\text{-}0.94$ respectively. Therefore, they concluded that the NIR technique led over the MIR in prediction of phenolic compounds.

C Xie et al.[77] reported the study of classification of varieties of mung beans by using vis-NIR hyperspectral imaging. They concluded that, the NIR hyperspectral imaging approach can be employed to classify the mung bean varieties with the help of Modified gram-schmidt (MGS) algorithm in non-destructive manner.

C Lastras et al.[78] reported the prediction of fatty acid and mineral compositions of brown, black, red and green lentils by using NIR and modified partial least square regression approach. They obtained the coefficient of determination higher than 0.9 with low root mean square error. They concluded that NIR technique with association of chemometric is able to predict the compositions in lentils.

F A Mendoza et al.[79] reported the prediction of five canning quality traits named as visual appearance (APP), color (COL), hydration coefficient (HC), texture (TXT) and washed drained coefficient (WDC) in dry beans by using VIS-NIR (400-2500 nm) and PLSR

approach. They observed that the coefficient of determination of prediction for APP and TXT as low i.e., R^2 = 0.275-0.566 and R^2 = 0.270-0.681 respectively and fine results for HC, WDC and COL and that was R^2 = 0.517-0.810, R^2 = 0.420-0.796 and R^2 = 0.796-0.907 respectively. They concluded that, NIR has an appropriate approach to evaluate the canning properties in dry beans non-destructively.

A Giraudo et al.[80] reported the geographical classification of green coffee beans by using rapid and non-destructive FT-NIR technique and models were developed on the basis of origin of green coffee beans samples. They have used PCA and PLS-DA to classify and model development of the 191 samples on the basis of continent and countries. They observed that, in comparison to the country-based classification model, which correctly predicted 100% of them and the continent-based classification model was capable of identifying more than 98% of them with accuracy. They concluded that classification of green coffee bean samples using NIR spectroscopy and chemometric was considered as a rapid and efficient approach.

I Revilla et al.[81] reported the development of calibration model and geographical classification of ground lentils by using NIR and multivariate analysis. They have predicted the compositions named as weight, size, moisture, ash, crude protein, total fat and total fiber content of ground and whole lentils by calibration models. On the basis of classification, the 42 samples used and collected from Protected Geographical Indication (PGI). They observed that the 95% classification rate of accuracy belong to PGI. They concluded that the calibration model gives the excellent coefficient of determination with good accuracy.

J Hang et al.[82] reported the prediction of protein and 18 types of amino acid content in lentils by employing NIR and PLSR method. They have used two types of NIR spectrophotometer i.e. Perkin Elmer DA 7250 and FT 9700 for data acquisition and model development. They observed from both spectrophotometers, the calibration model for protein and 14 amino acids showed the better coefficient of determination (R^2 > 0.652). Therefore, they concluded that no significant difference in accuracy of models were

observed for ground lentils and NIRS exhibited an incredible potential for efficient, precise, and instantaneous prediction of the protein and majority of amino acid levels in lentils.

V Innamorato et al.[83] reported the geographical classification of lentils by using FT-NIR and FT-MIR spectroscopic technique with PLS-DA and LDA approach. They have collected the total 83 samples from Italy and Canada for classification. They observed that the FT-MIR shows the more discrimination (98-100%) prediction as compare to FT-NIR (91-100%) techniques. They concluded the spectroscopy technique classifies the lentils on the basis of geographical origin with excellent outcomes.

2.2.2 Peas

G Hacisalihoglu et al.[84] reported the calibration model development for weight, protein and oil content of 96 pea samples with help of NIR and PLS regression techniques. They observed that the prediction of weight and protein give the high accuracy with coefficient of determination (R^2=0.94) for both and prediction of oil content was observed with less accuracy (R^2=0.74). Thus, they concluded that the NIR approach could able to predict the nutritional parameters of pea seed non-destructively.

U Saha et al.[85] reported the various constituents of winter peas by using NIR and chemometric approach. They developed the calibration models for the constituents named as moisture, dry-matter, nitrogen content, crude protein, ADF, NDF, AD-lignin cellulose, and non-fibrous carbohydrate of winter peas. They observed that the quality constituents predicted have good coefficient of determination and low predictions errors. They concluded that the NIR and multivariate technique predict the various quality constituents of pea in green way.

U Kamboj et al.[86] determined the quality attributes of chickpea flour by using NIR and chemometrics approach. They determined the moisture, fat, protein and carbohydrates content chemically first and then reference data were used to develop the calibration model. They used PLS, iPLS and synergy iPLS regression in development of model. The accuracy

of model was determined the higher coefficient of determination and it was higher than 0.96 for all parameters. They concluded that the NIR is a cheap methodology for detection of quality attributes in chickpea flour.

S Kaliramesh et al.[87] reported the determination of moisture, protein and starch content in green grams using NIR approach. They developed PLS and PCR models to predict the main constituents in green gram samples. They observed that the PLS model give the more accurate outcomes as compare to PCR model. They concluded that the moisture, protein and starch content gracefully predicted using NIR and chemometric approach in non-destructive manner.

M Bala et al.[88] have determined the adulteration (1-90%) of maize flour in chickpea flour and developed the calibration model by using NIR and modified partial least square (MPLS), PLS and PCR methods. They concluded MPLS regression show best results as compare to other with high accuracy.

T Alemu et al.[89] reported use of the NIR technique to estimate the nutritional content of chickpea straw. Firstly, they analyzed 190 samples using standard techniques for dry matter, ash, crude protein, acid detergent fiber (ADF), neutral detergent lignin (NDF), Zn, Mn, Ca, Mg, Fe, and P content as well as metabolizable energy for in vitro gas generation. Further they used multiple regression analysis to construct the prediction equations. They concluded that the nutritional value of chickpea straw was correctly predicted by the NIR prediction equations with good coefficient of determination.

D Cozzolino[90] has reviewed the prediction of functional properties namely moisture, protein fat and fiber contents in food and its products by using NIR spectroscopy techniques. NIR spectroscopy highly used in the diary and its products as well as in cereals to predict the nutritional parameters in non-destructive manner.

U Kamboj et al.[91] reported the chemical composition prediction within 700-2500 nm NIR range with help of PLS and PCR regression methods. They obtained the coefficient of determination of prediction for moisture, fat, protein and carbohydrates as 0.985, 0.986,

0.988 and 0.991 respectively using PLS and 0.965, 0.979, 0.973 and 0.983 respectively from PCR method. They concluded that, the NIR approach able to predict the chemical composition in chickpea flour.

C Sivakumar et al.[92] reported the classification of chickpea, yellow pea, navy bean, and green lentil flours were studied using hyperspectral imaging in the visible near infrared (Vis-NIR) within range of 400-1000 nm and SWIR range from 1000-2500 nm regions. They used PCA and PLS-DA to construct classification models. They observed that the PLS-DA models were developed by employing SWIR regions of 1370–1500 nm and 1700–2000 nm for achieving 95% classification accuracy.

J Wang et al.[93] reported the development of calibration model to predict the protein, starch, oil and total polyphenol contents present in faba bean (Viciafaba L.) seeds by employing the NIR spectroscopy and PLS regression method. They observed that the coefficient of determination for protein, starch and polyphenol were 0.97, 0.93 and 0.89 respectively. They concluded that results of powder form of beans were more superior to intact seed. Therefore, NIR approach able to predict the composition of faba beans.

2.3 Research Gap

Near-infrared spectroscopy is well established technique for determining properties of many agricultural products because of its non-destructive, green, fast technique. Millets are important because of their nutritional properties, high protein and fiber content. Work has been carried out for the determination of its properties using chemical methods which are hazardous to the environment and are destructive in nature. Thus, there is need to develop a regression method for determining their properties in a non-destructive, rapid and green method.

Literature review shows that very few research has been carried out for property determination of millets using Near-infrared spectroscopy. Thus, research can be carried out for development of a regression model using near infrared spectroscopy for quantitative and qualitative analysis of pearl millets.

2.4 Research Objectives

1. Qualitative and quantitative determination of physical & chemical composition and rheological properties of pearl millets.
2. Classification of millets using Near Infrared Spectroscopy based on region.
3. Development of green, rapid and non-destructive statistical model with selective near infrared wavelengths using multivariate analysis for simultaneous prediction of properties of millets.

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CHAPTER-3

MOISTURE EFFECT ON PHYSICAL PARAMETERS & RHEOLOGICAL STUDIES OF MILLET

3 Moisture effect on Physical parameters and study of rheological properties of millet

3.1 Introduction

Millets belong to a category of small-grained cereal crop, they are cultivated on low-fertility soil and are used as food, fodder, and forage. Millets of several species are farmed and consumed in India, including small millet, foxtail, kodo, barnyard, common, finger, sorghum and pearl millet. Pearl millet (Pennisetum glaucum), also known as Bajra, is a kind of millet that originated in tropical and subtropical regions of the world and is mostly grown in semi-arid areas of Africa and India. Pearl millet is cultivated in semi-arid areas with high temperatures and light soils situations because it has the best heat and drought tolerance capacity of any crop. Pearl millet grains can be elliptical, hexagonal, or ovoid in shape, with a length of 3-4 mm and a color of yellow, brown, grey, pale blue, purple, greyish white, or cream. It is a tall plant that may reach a height of 6 to 15 feet.[1] The physical properties of pearl millets are essential for designing the equipment required for its cleaning, grading, processing and storing the grains.[2,3] Consequently, it is essential to determine the physical characteristics named as shape, size, geometric mean diameter, surface area, volume, true density, bulk density, porosity, sphericity, angle of repose and coefficient of static friction of the millets at different moisture levels were determined by researchers for handling the storage of sample.[1–16]

The nutritional parameters of millets make it important from other cereals. Millets are good source of fiber, protein and carbohydrates[17–20] and are abundant in minerals and proteins than wheat, rice and sorghum, and they also provide numerous health benefits like help to control diabetes by digesting slowly and releasing glucose into the bloodstream at a slower pace than other foods. It is beneficial for heart health and helps to reduce migraine attacks and cancer risks due to its high magnesium content. It also has anti-allergic qualities and is beneficial for immune illnesses such as celiac disease.[21–23]

Other than the nutritional properties, rheological properties also prominent to understand the nature of the dough. The study of rheology examines both materials and forces. More

specifically, the area of physics known as rheology deals with the flow of liquids and the deformation of solid objects. For engineering calculations and processes, machine designs, and product quality analysis as well as for the purpose of determining the flow properties of food items, rheological data is necessary.[24] Many researchers have conducted the study of rheology on cereals grains powder,[25,26] starch,[27,28] liquids and gels[29] to understand the viscoelastic behaviour of the samples.

Due to the importance of these properties for health benefits, present research has the objective of this work is to study the physical properties at various moisture levels and rheological properties to determine the viscosity of pearl millet samples.

3.2 Materials and Methods

3.2.1 Physical characteristics

Sample collection and preparation

The Bajra-1827 and Pioneer 84M88 grains were procured from farmers of Nanuana and Balasar village, Sirsa, Haryana, India as shown in Figure 3.1(a) and 3.1(b).

Figure 3.1 (a) Bajra-1827 variety

Figure 3.1 (b) Pioneer 84M88 variety

Figure 3.1 Pearl millet Samples

Grains were cleaned from foreign materials and immature grains removed manually. Physical properties of pearl millet were determined in 11.55 to 18.44% moisture range. The desired moisture content was obtained by adding the calculated amount of water. Grains at different moisture levels were sealed in polythene zip-lock bags and all samples were placed in refrigerator (4°C) for 15 days to reach uniform moisture content. Before each experiment, samples were equilibrated for 2 hours at room temperature to maintain the moisture and uniform temperature.

Moisture analysis

Sample was grounded and sieved through 1.0mm sieve and weighted 5g. The grounded sample in the previous dried moisture dish was dried in the hot air oven for 2 hours at 130-135°C. After 2 hours, dish was removed from oven, cooled in desiccator and weighted. In the half-hour interval, the dish was placed in the oven to reduce moisture, the process was repeated until the weight remained consistent. The moisture content was determined using the following formula:

$$\text{Moisture } \% = \frac{(W_1 - W_2)}{(W_1 - W)} \times 100 \quad (3.1)$$

Where, W_1 represents for weight of the dish before drying in grams (g), W_2 defined as weight of the dish with the material after drying in grams (g), W shows the weight of the empty dish in grams (g).

Dimension Analysis

Ten grains were chosen randomly from each variety to determine the average grain size of pearl millet at each of the four moisture levels. The dimensions namely Length (L), width (W) and thickness (T) were measured by Vernier calliper with 0.001mm least count. The geometric mean diameter (D_g) was measured by using the equation below

$$Dg = (L * W * T)^{1/3} \quad (3.2)$$

Jain & Bal.[30] have stated the grain volume V and surface area S given as below,

$$S = \frac{\pi B L^2}{2L - B} \tag{3.3}$$

$$V = \frac{\pi B^2 L^2}{6(2L - B)} \tag{3.4}$$

Where $B = (WT)^{1/2}$

The sphericity (ø) value of grains were calculated by equation written as follows[31–35]

$$\emptyset = \frac{(LWT)^{1/3}}{L} \tag{3.5}$$

Bulk density Measurements

The weighed amount of 50 g millet sample was placed in a 100 ml measuring cylinder to evaluate the grain's bulk density. For achieving the consistent volume, cylinder was tapped on a laboratory bench multiple time. The experiment was repeated ten times, and the bulk density of millet was calculated using the following formula

$$\text{Bulk density } \left(\frac{g}{ml}\right) = \frac{\text{weight of the sample (g)}}{\text{Volume of the grains including void space (ml)}} \tag{3.6}$$

3.2.2 Viscosity

Sample Preparation

The pearl millet samples having varieties 9422+Dhamal, 9444+ROAGARO, Jodha+Dhamal, Bajra-1827, Bajra-222 and 9444+Jodha were collected from the local farmers of Sirsa, Haryana. Pearl millet samples were prepared by grinding, using Aero mixer grinder 500W. Dough of the flour was prepared by mixing equal amount of water and flour w/v (20 g of millet flour with 20 ml of water) and keep 10 minutes for mixing accordingly. The Labman 200 rotating viscometer was used to determine the viscosity of samples. The analysis of viscosity was performed at 18°C temperature with 100-200 rotation per minute speed as shown in Figure 3.2.

Figure 3.2 Labman -200 Viscometer

3.3 Results and Discussion

3.3.1 Physical parameters

Axial Dimensions

The physical properties of pearl millet grains namely size, geometric mean diameter, volume, surface area, sphericity and bulk density were calculated by using standard methods and results are shown in Table 3.1. It was observed that the length of the pearl millet grains within moisture content of 11.55, 12.95, 16.35 and 17.29% are 3.672, 3.754, 3.920, 3.826 mm respectively for Bajra-1827 variety and length within moisture content of 11.85, 13.96, 16.18 and 18.44% are presented 3.171, 3.337, 3.505 and 3.671 mm for the Pioneer 84M88 variety of pearl millet grains respectively. The width was 2.087-2.672 mm for Bajra-1827 variety and 2.504-2.838 mm for Pioneer84M88. Similarly, their thickness was within range from 2.004-2.838 mm and 2.002-2.171 mm for Bajra-1827 and Pioneer84M88 variety. All observations were considered by using hot air oven method in desired moisture contents. Length, width and thickness of the pearl millet seems to be

increased linearly. The enhancement in dimensions with increase in moisture may be due to hygroscopic nature of the grains. Similar results for the length were obtained for the pearl millet Ex-Borno and SOSAT C88 varieties.[36] It was observed from the literature, length of the pearl millet for Ex-Borno and SOSAT C88 varieties are polynomial increased from 3.17 to 3.80 mm and 3.87 to 4.46 mm, width was increased from 2.93-3.39 mm and 2.30- 2.66 mm for SOSAT C88 and Ex-Borno variety within 10-20% moisture ranged. Similarly, their thickness was increased 2.05-2.51 mm and 1.54-1.66 mm respectively. The geometric mean diameter, for both varieties Bajra-1827 and Pioneer 84M88 are within ranged from 2.671 to 2.906 mm and 2.582 to 2.828 mm within different moisture levels. Hence, it can be concluded that the grain from Bajra-1827 variety is maximum in size as compared to Pioneer 84M88. For both varieties, the relationships between axial dimensions and moisture content were revealed to be linear equations as represented in Table 3.2. In this table, x shows the moisture content and y represents the property and coefficient of determination (R^2) reveal the relationship between the two variables. The averaged value of surface area, volume and sphericity of the variety Bajra-1827 and Pioneer84M88 of pearl millet grain summarized in Table 3.1. It was observed that the surface area and volume of both varieties of pearl millet increased with increase in moisture content. The range of sphericity was in between 0.713-0.762 for bajra-1827 variety and 0.770-0.814 for Pioneer 84M88 variety of pearl millet. The sphericity of pearl millet reported as 0.937-0.942[30] and 0.667-0.744.[36] It was observed that the sphericity of pearl millet grain was decreased for variety bajra-1827 and Pioneer 84M88 with enhancement of moisture levels.

Bulk Density

The density of a substance when packed or piled in bulk is known as bulk density and is depends upon the geometry, size, and surface properties of grains. The principle of cleaning the grains is based on the grain densities. Breakage susceptibility and hardness analyses have indicated grain densities to be essential. A decreasing trend of bulk density was observed within 11.55 to 18.44% moisture levels (Figure 3.3 & Figure 3.4). At a lower moisture level, the bulk density was higher for both varieties of pearl millet. The relationship between moisture and bulk density was linear with coefficient of determination

(R^2) (Table 3.2). Similar trends was observed for the barnyard millet, proso millet, kodo millet, finger millet, little millet and foxtail millet reported by S. Balasubramanian.[10] However, J.O. Ojediran et.al.[36] 2010 reported the quadratic relationship with moisture and bulk density of pearl millet grains.

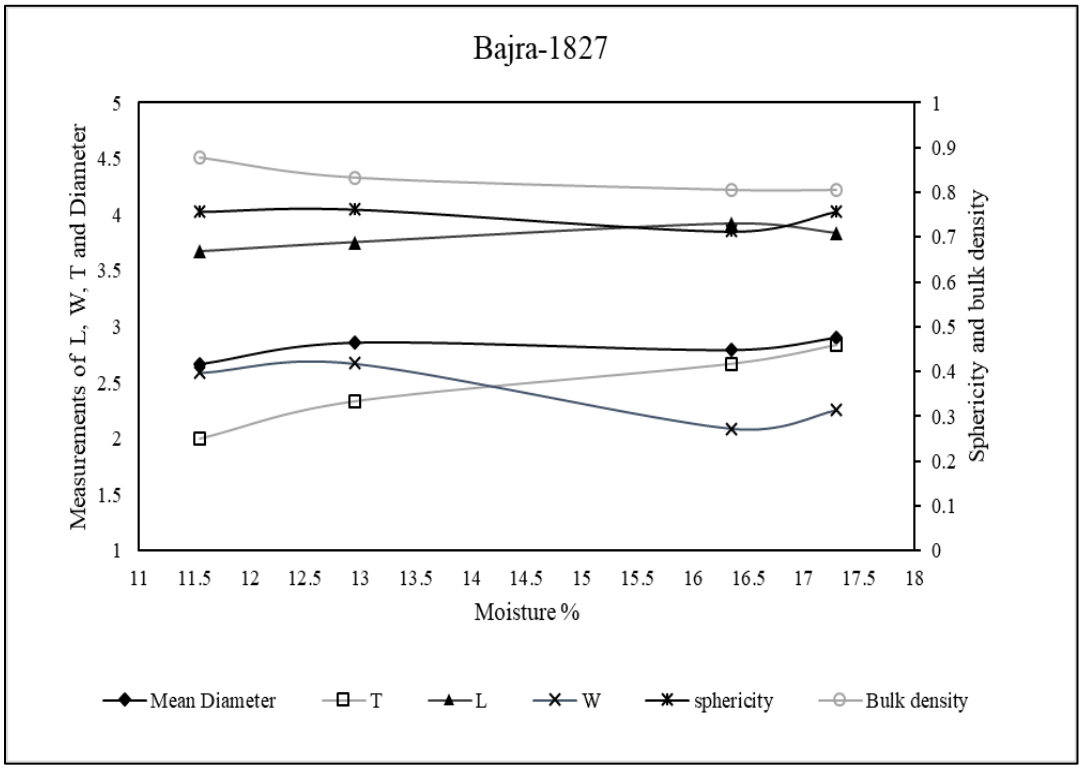

Figure 3.3 Moisture effect on pearl millet variety (Bajra-1827)

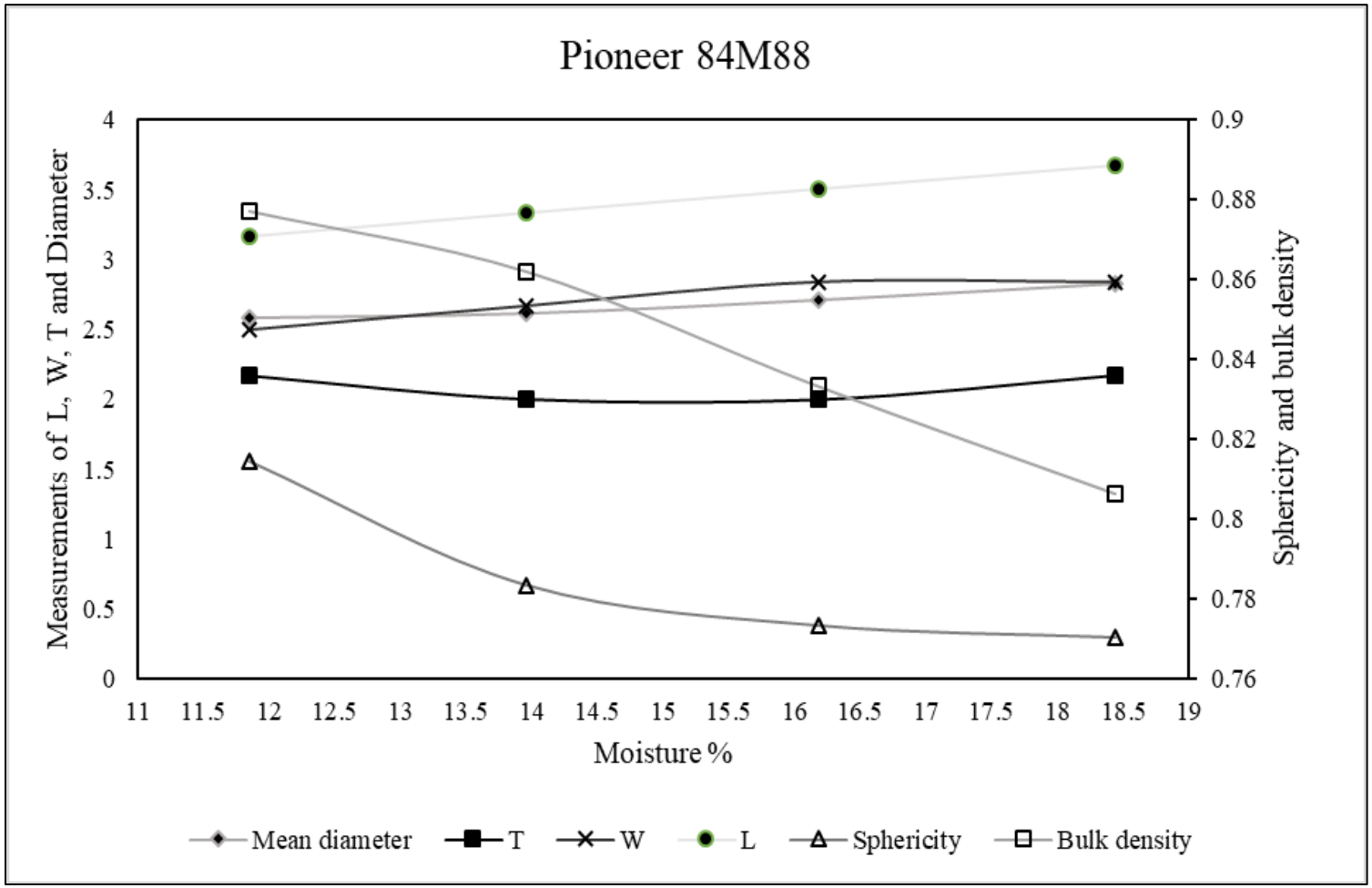

Figure 3.4 Moisture effect on pearl millet variety Pioneer 84M88

Table 3.1 The Physical properties of Bajra-1827 and Pioneer 84M88 pearl millet.

Variety								
Parameters	Bajra-1827				Pioneer 84M88			
Moisture content (%) d.b	11.550	12.950	16.350	17.290	11.850	13.960	16.180	18.440
Length (L), mm	3.672	3.754	3.920	3.836	3.171	3.337	3.505	3.671
Width (W),mm	2.589	2.672	2.087	2.255	2.504	2.671	2.838	2.838
Thickness (T),mm	2.004	2.337	2.671	2.838	2.169	2.005	2.002	2.171
Mean diameter, mm	2.671	2.862	2.796	2.906	2.582	2.614	2.711	2.828
Surface area (S), mm^2	23.136	31.521	26.634	33.053	23.656	23.431	26.305	30.604
Volume (V), mm^3	10.003	16.404	12.374	17.621	10.707	10.455	12.457	15.712
Bulk density (g/ml)	0.877	0.833	0.806	0.806	0.877	0.862	0.833	0.806
Sphericity	0.757	0.762	0.713	0.758	0.814	0.783	0.773	0.770

Table 3.2 Linear and polynomial correlation equations of physical parameters of pearl millet with moisture

Properties	Bajra-1827	Pioneer 84M88
Length	$y = 0.0347x + 3.2914$ $R^2 = 0.7835$	$y = 0.0758x + 2.2749$ $R^2 = 0.9998$
Width	$y = -0.0881x + 3.6811$ $R^2 = 0.7563$	$y = 0.0529x + 1.9129$ $R^2 = 0.8824$
Thickness	$y = 0.1336x + 0.5209$ $R^2 = 0.9702$	$y = 0.0173x^2 - 0.5242x + 5.9486$ $R^2 = 0.9999$
Mean Diameter	$y = 0.0111x^3 - 0.4878x^2 + 7.0643x - 31.024$ $R^2 = 1$	$y = 0.038x + 2.1095$ $R^2 = 0.9543$
Bulk density	$y = -0.0114x + 0.9962$ $R^2 = 0.8632$	$y = -0.011x + 1.0106$ $R^2 = 0.9877$
Sphericity	$y = 0.0031x^3 - 0.1316x^2 + 1.8176x - 7.5007$ $R^2 = 1$	$y = -0.0064x + 0.8824$ $R^2 = 0.8193$

Therefore, it was observed that the physical parameters namely length, width, thickness, geometric mean diameter, surface area and volume increased and bulk density decreased for both varieties. Also observed that the physical parameters linearly correlated with moisture content. It was concluded that the grain of variety Bajra-1827 had more effect on size as compared to Pioneer 84M88 for same moisture content. The findings of this study can be the basis for engineers to design the apparatus for cleaning, grading, processing and storing of pearl millet grains with effect of moistures and also provide the nutritious proximate compositions for various health benefits.

3.3.2 Viscosity

In the present study, the viscosity of samples with the verities 9422+Dhamal, 9444+ROAGARO, Jodha+Dhamal, Bajra-1827, Bajra-222 and 9444+Jodha of pearl millets are determined at the 100 and 200 rotation per minuets at ambient temperature. The viscosity of slurry or liquids are varied with the temperature and as the temperature increases the viscosity of the most of the liquids are decreased. To determine the viscous nature of the various verities of pearl millet samples, the viscosity was determined and shown in Table 3.3. As a result, it was observed that there was no variation in the viscosity of the samples at 100 or 200 RPM at 18°C temperature.

Table 3.3 Parameters of rheological properties at various rotations of pearl millet

Samples	Viscosity (mPa-Sec) at 100RPM (with 18°C)	Viscosity (mPa-Sec) at 200RPM (with 18°C)
9422+Dhamal	1376.24	688.44
9444+ROAGARO	1367.16	687.52
Bajra- 1827	1375.87	687.78
Bajra- 222	1375.48	688.18
9444+Jodha	1366.66	685.52
Jodha+Dhamal	1377.57	688.89

3.4 Summary

In this chapter, the study of physical properties of pearl millet at different moisture levels have been evaluated as they are essential to design the equipment for cleaning, grading, processing and handling the storage of grains. The pearl millet with variety Bajra-1827 and Pioneer 84M88 were collected from Haryana, India. The moisture effect on pearl millet was also determined for both varieties. Therefore, it was observed that the physical parameters namely length, width, thickness, geometric mean diameter, surface area and volume increased and bulk density decreased for both varieties and physical parameters linearly correlated with moisture content. It was concluded that the grain of variety Bajra-1827 had more effect on size as compared to Pioneer 84M88 for same moisture content. On the other side, the samples for the rheological properties were collected from the Haryana. The varieties of pearl millet named as 9422+Dhamal, 9444+ROAGARO, Bajra-1827, Bajra- 222, 9444+Jodha and Jodha+Dhamal were used in the analysis. The viscosity of samples was determined at the 100 and 200 rotation per minuets at ambient temperature. The viscosity of the samples was lying in between 1366.66-1377.57 mPa-Sec at 100RPMand 685.52-688.89 mPa-Sec at 200 RPM. The results indicate that there was no variation in the viscosity of the samples at 100 or 200RPM at 18°C temperature.

The findings of this study can be the basis for engineers to design the apparatus for cleaning, grading, processing and storing of pearl millet grains with effect of moistures and also provide the viscosity parameters for various varieties to determine the viscous nature of pearl millet.

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CHAPTER-4

GEOGRAPHICAL CLASSIFICATION OF PEARL MILLETS ON BASIS OF PROXIMATE COMPOSITION & SPECTRAL DATA

4 Geographical classification of pearl millet on basis of proximate composition and spectral data

4.1 Introduction

Pearl millet (*Pennisetum glucum*) is widely cultivated and consumed in arid and semi-arid tropics of Asia and Africa. The pearl millet crop can survive in high temperature and dry conditions in the soil with low water holding capacity.[1,2] Pearl millet is recognized as sixth cereal crop in terms of the world agriculture production. As reported by Directorate of Millets Development, during 2018-2020 the annual production of pearl millet in India was 8.61 million tons and around 6.93 million hectares of land was covered for pearl millet production.[3,4] It is mostly grown in the Indian states of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, and Haryana. The nutritional value of pearl millet is compatible to the other major cereals grains in terms of proteins, fiber, carbohydrates, ash, fat content and minerals. The carbohydrates content of pearl millet is less than rice, wheat and sorghum millet but higher than maize.[5] Pearl millets are a rich source of minerals such as magnesium, phosphorus**,** zinc and proteins that are responsible for the potent health benefits of pearl millets such as antidiabetic and anti-carcinogenic activity.[4] Pearl millets also contain high amount of fiber content that leads to slow digestion and low rate of release of glucose into blood compared to other food grains with low content of fiber.[6] Pearl millets efficiently helps diabetic patients in maintaining a constant blood sugar level over a long period of time. Pearl millet is type of grain that do not contain any type of gluten. The gluten-free grain is low in calories but high in nutrients that prevent people from the celiac diseases helps to weight loss, blood sugar control, and other health issues.[7,8] To analyze and interpret the large amount of data generated by modern analytical methods, multivariate approaches have been frequently used. Furthermore, multivariate data analysis examines relationships between samples and variables. Among the available multivariate data analysis techniques, PCA is a most common multivariate statistical approaches employed for the exploratory data analysis.[9–11] PCA transform and represent large data sets into a new perspectives that emphasizes the essential valuable information. PCA is a

technique for identifying groups of similar objects in data.The conventional methods employed to analyze nutritional composition of pearl millets are laborious, time consuming and require harmful chemicals. To overcome these shortcomings, infrared spectroscopy along with multivariate analysis is gaining researcher's interest to predict chemical composition, classify various food product and detecting adulteration of agricultural and food products.[12–15]

Literature reports that pearl millet contain significant amount of protein (13.84%), ash (2.03%), carbohydrates (79.92%) and moisture content (9.61%).[16] The carbohydrates content of various varieties of pearl millets was reported to vary from 69.6 to 72.5%, moisture varied from 6.5 to 7.7%, protein content ranged from 9.7 to 11.3, fat content was about 5.1 to 7.2%, ash content varied from 1.65 to 1.90% and fiber was ranged from 2.9 to 3.8%.[17] Pearl millet varieties from Hisar, Haryana also reported to contain a significant amount of protein (11.81-12.48%), moisture (7.41-8.6%), ash (1.79-1.92), crude fiber (1.27-1.67%) and crude fat (5.44-6.19%) [18]. The flour of raw pearl millet was also reported to contain 4.5% moisture, 10.57% protein, 7.69% ash and 74.41% carbohydrates.[19]

Achten Elisabeth[20] used attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) as a non-destructive technique to classify the 73 maize grains samples collected from Ukraine, USA and Peru. Li, Gang[21] applied PCA, discriminant function analysis (DFA), and Fibonacci index analysis (FIA) for discrimination of rice samples (n=206) collected from various geographical regions of China. The rice samples from Thailand, France, India, Italy, Japan and Pakistan were also classified based on the data collected from high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) by employing discriminate analysis (DA) and PCA.[22] In addition, PCA was employed for classification of adulterated milk and honey samples.[23–25]

Previously, Fourier transform infrared (FTIR) and near infrared (NIR) spectroscopy associated with machine learning techniques was successfully employed to examine the variations among various cultivars of pearl millet grains collected from China using PCA.[26] Literature review presents that very little research had been carried out to characterize or classify pearl millet samples according their spectral and chemical information.

Consequently, this research was aimed to categories a sample of pearl millet collected from various geographical locations of Haryana and Punjab regions of India based on their NIR and FTIR spectral data, proximate composition, by employing PCA. This research demonstrates the application of the proposed technique to interpret, characterize, and classify pearl millet flour in a nondestructive manner.

4.2 Materials and Methods

4.2.1 Collection of pearl millet samples

The pearl millet samples were collected from local farmers of 50 different locations of Haryana and Punjab states of India in year 2021. Figure 4.1 represents the geographical location of collection of samples. The samples were cleaned manually to remove broken grains and foreign materials. The cleaned samples were stored in air-tight polythene bags and stored at 4°C until further analysis.

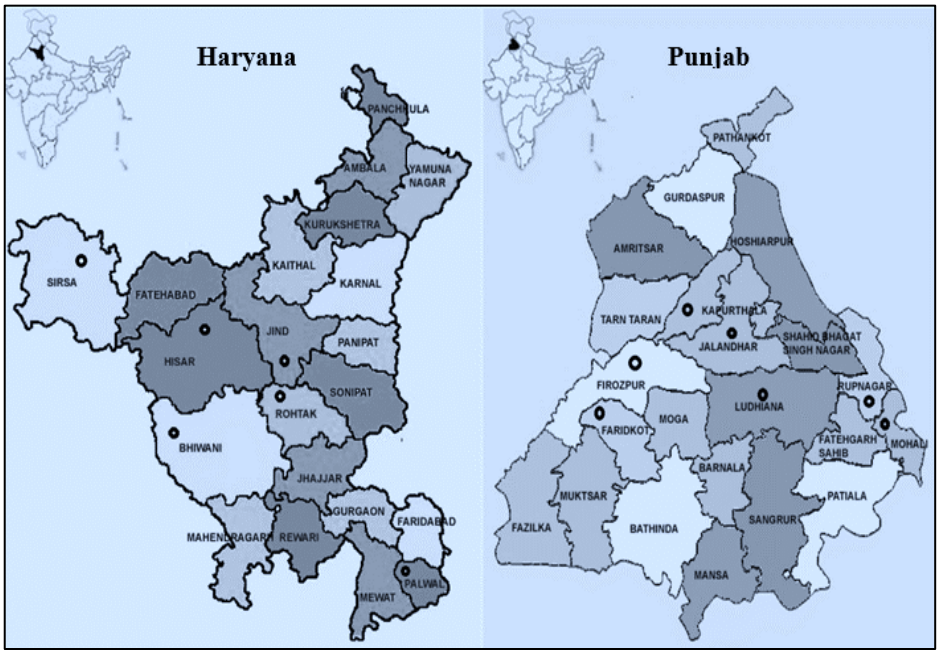

Figure 4.1 The geological map of Haryana and Punjab, India (dotes showed the sample collection from districts of Haryana and Punjab)

4.2.2 Acquisition of Near Infra-Red spectra

The NIR spectra of pearl millet samples of flour were collected using FOSS NIR DS-2500 spectrometer with VISION software (Nils Foss Alle 1, 3400 Hilleroed, Denmark) using circular sample holder in reflection mode from 750 to 2500 nm using three scans at 0.5 nm intervals (Figure 4.2). Pearl millet samples were kept at room temperature for 24 h to obtain uniform temperature, before spectra acquisition. The absorbance of the spectra from average number of scans was calculated using Lambert Beer law and further employed for analysis.

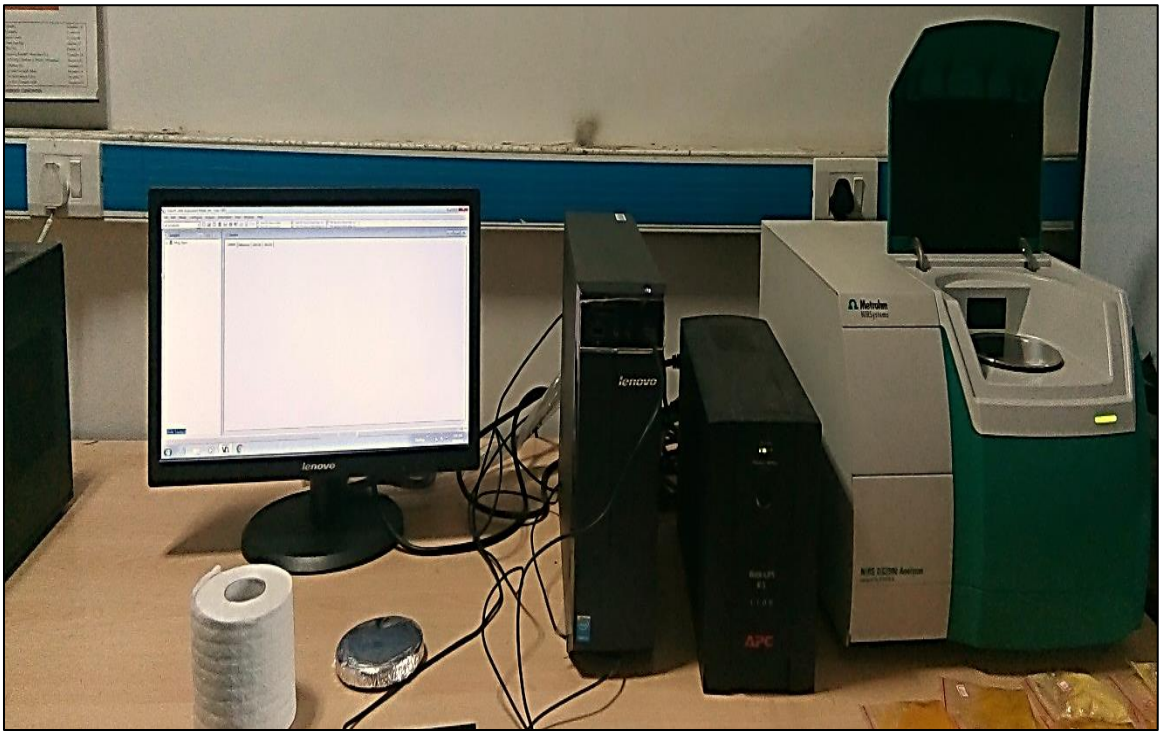

Figure 4.2 FOSS NIR DS-2500 spectrometer with VISION software (Nils Foss Alle 1, 3400 Hilleroed, Denmark)

4.2.3 Acquisition of Fourier Transform Infrared spectra

The Fourier transform infrared (FTIR) spectrum of pearl millet samples were collected by using Shimazu 8400S spectrophotometer using diamond ATR and IRSOLUTION software (Shimazu, Japan) (Figure 4.3). The spectra were collected in transmittance mode from 4000 cm^{-1} to 500 cm^{-1} at 100 cm^{-1} intervals using 10 scans for each sample. All spectra were recorded at room temperature and mean of 10 scans was further employed for analysis.

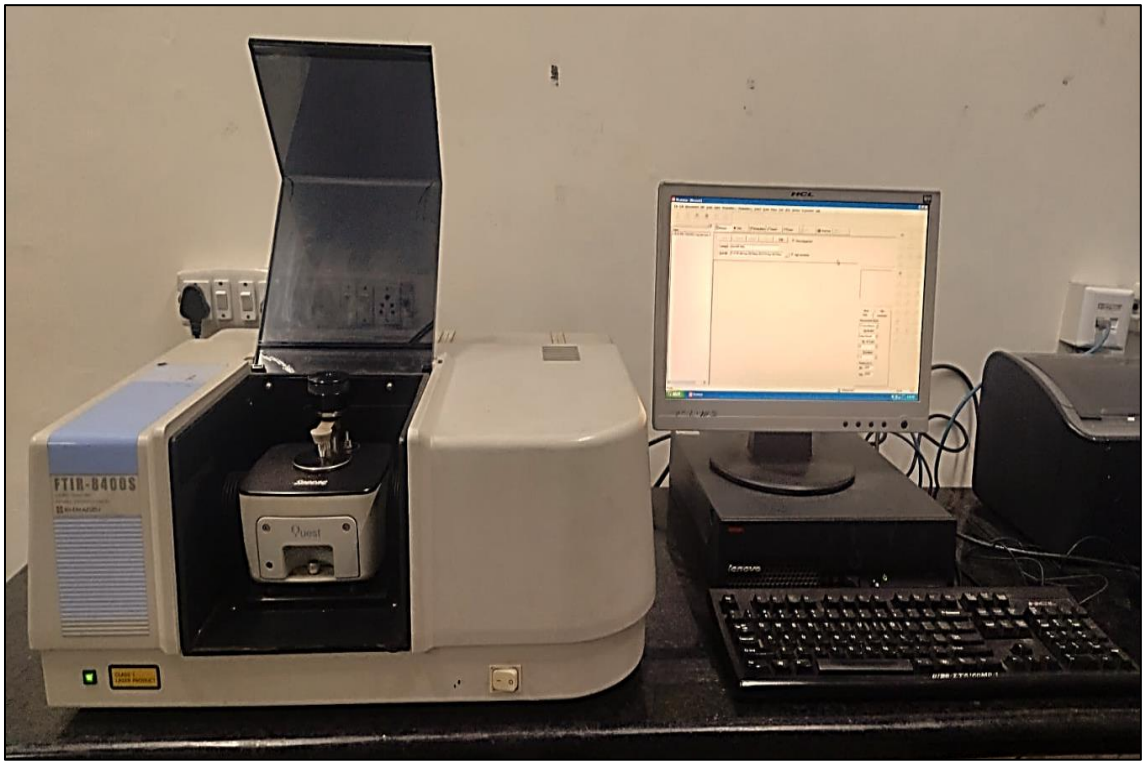

Figure 4.3 Shimazu 8400S spectrophotometer with diamond ATR and IRSOLUTION software (Shimazu, Japan).

4.2.4 Chemicals

The analytical grade sulfuric acid (98%), sodium hydroxide solution (45%), boric acid, and petroleum ether (60-80°C) were purchased from Loba Chemie (Jehangir Villa, 107, Wodehouse Rd, Colaba, Mumbai, Maharashtra 400005, India) for the reference analysis.

4.2.5 Physicochemical parameters

The proximate compositions of pearl millet samples were evaluated via Association of Official Analytical Chemists (AOAC) method. All the data were collected in triplicate. The parameters are explained in brief as below.

Moisture

Sample was grounded and sieved through 1.0 mm sieve and weighted 5 g. The grounded sample in the previous dried moisture dish was dried in the hot air oven for 2 hours at 130-135°C. After 2 hours, dish was removed from oven, cooled in desiccator and weighted. At the half-hour interval, the dish was placed in the oven to reduce moisture, and the process was repeated till weight remained consistent. The moisture content was determined using the following formula:

$$\text{Moisture } \% = \frac{W_1 - W_2}{W_1 - W} \times 100 \quad (4.1)$$

Where, W_1 denotes the weight of the dish prior to drying in grams, W_2 is the weight in grams of the dish containing the material after drying, W shows the weight of the empty dish in grams.

Ash

About 5g ground sample placed in the pre-weighed silica crucible that was heated for 3h at 600°C in muffle furnace. After that crucible was placed in desiccator to reduce the temperature to normal and then weighed the crucible until constant weight was achieved. The ash content was calculated using the formula below:

$$\text{Ash content } \% = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \quad (4.2)$$

Crude Fat

SOCS PLUS was used to extract the fat from the sample using solvent. SOCS PLUS apparatus work on the principle of Soxhelt chemistry. About 2.5g (W) of sample were transfer to the thimble and placed in thimble holder (Figure 4.4). In a beaker, add 50-75 ml petroleum ether as a solvent, set the thimble in the beaker, turn on the assembly, and keep temperature at boiling point of the solvent. Boiling stage was performed and leave for half an hour undisturbed. At the end of boiling stage, increase the temperature to 150 to 200°C. Notice the condensation of solvent after few minutes. Allow to condense till the solvent gets condensed. Open the value and allow the condensed solvent to flow through the thimble to perform reclamation stage. After the stage of reclamation, evaporate all the solvent in the beaker.[5,27]

Crude fat content was extracted by using the formula shown in below:

$$\text{Crude Fat } \% = \frac{W_2 - W_1}{W} \times 100 \quad (4.3)$$

Where, weigh the beaker with fat (W_2) and without fat (W_1).

Figure 4.4 The SOCS PLUS assembly

Crude protein

KEL PLUS Nitrogen estimation system was used to find the nitrogen content from the sample. Take the 0.3g of sample in powder form and placed in 250 ml Macro DTL tube within pre-heated digestion unit up to 350 degree Celsius (Figure 4.5). Now add 10 ml concentrated H_2SO_4 and 3 gm mixture of catalyst within 5:1 of Potassium and Copper Sulphate. Load the sample in digestion unit and it will take 1 to 1.30 hour till clean green color obtained (Figure 4.6). After cooling the sample dilute it with 20 ml distilled water. Prepare the solutions by using boric acid, alkali and HCL before checking the distilled water reservoir. Load the Alkali to the distillation unit while wait for READY signal (Figure 4.7). Fill a 250 ml conical flask with 10 ml boric acid with indicator and set it at the receiver end. Fill the sample side of the distillation unit with the diluted sample tube. Liquid ammonia will gather in boric acid during the procedure, and the color will change depending on the indicator used. After distillation process was completed, the conical flask was detached from receiver and titrated with 0.1N H_2SO_4.The same procedure was employed for the calculation of blank value. The nitrogen content calculated by using

$$\mathrm{N\%} = \frac{[(\mathrm{S} - \mathrm{B}) \times \mathrm{N} \times 1.4007]}{\mathrm{W}} \tag{4.4}$$

Where, S defined for titrated value and B represents blank value, N, normality of H_2SO_4 and 1.4007 was nitrogen's molecular weight. Whereas W shows the weight of sample.

Figure 4.5 KEL PLUS assembly for digestion the sample

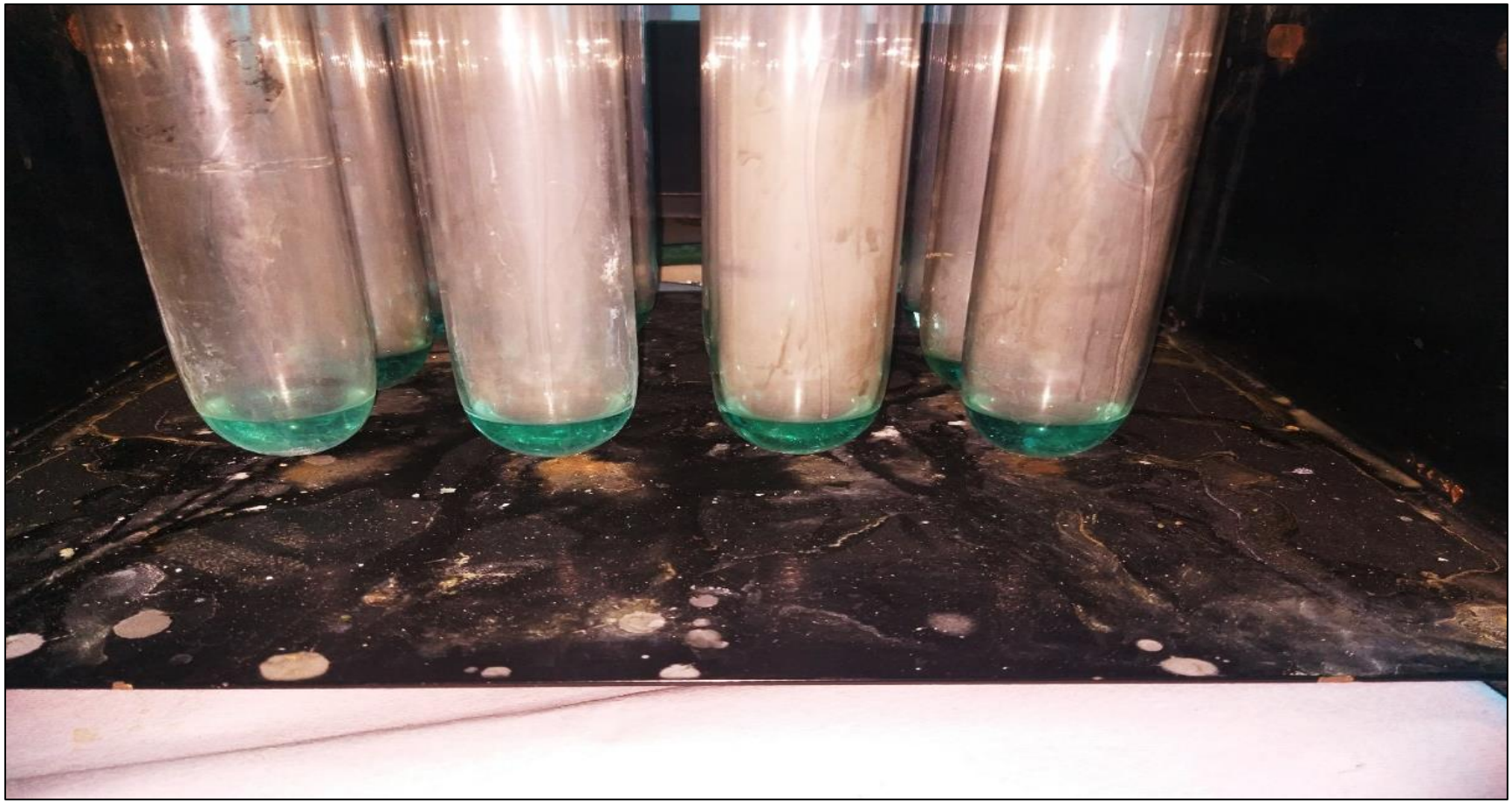

Figure 4.6 The samples after digestion

Figure 4.7 shows the KELPLUS distillation assembly

By multiplying the nitrogen content by the 5.95 conversion factor, the protein content was calculated.[4,8,16]

$$\text{Crude protein (\%)} = \text{N\%} \times 5.95 \quad (4.5)$$

Crude fiber

FIBRA PLUS apparatus (Pelican equipment, Chennai, India) was employed to determine the crude fiber content from the sample. A sample of around 2 g was placed in oven-dried crucibles. The crucibles were placed in the Fibra plus hot extraction equipment and 150 ml of H_2SO_4 (1.25%) are placed in extractors. Switch on the setup and boiling starts. Allow samples to boil in acid for 45 minutes before draining and washing with distilled water (Figure 4.8). After that 150 ml NaOH (1.25%) was placed in extractors. The identical process was repeated till washing with distilled water. The crucibles were dried completely in a hot air oven after an alkali wash. With the help of a desiccator cool the crucibles to room temperature. Calculate the crucibles weight and ash them at 550°C in a muffle furnace. Cool the heated crucibles after ashing and record the reading. The following formula was employed to determine crude fiber:

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{W} \times 100 \quad (4.6)$$

Whereas W_1 is the weight of crucible before ashing, W_2 is weight of crucible after ashing and W is weight of the sample.

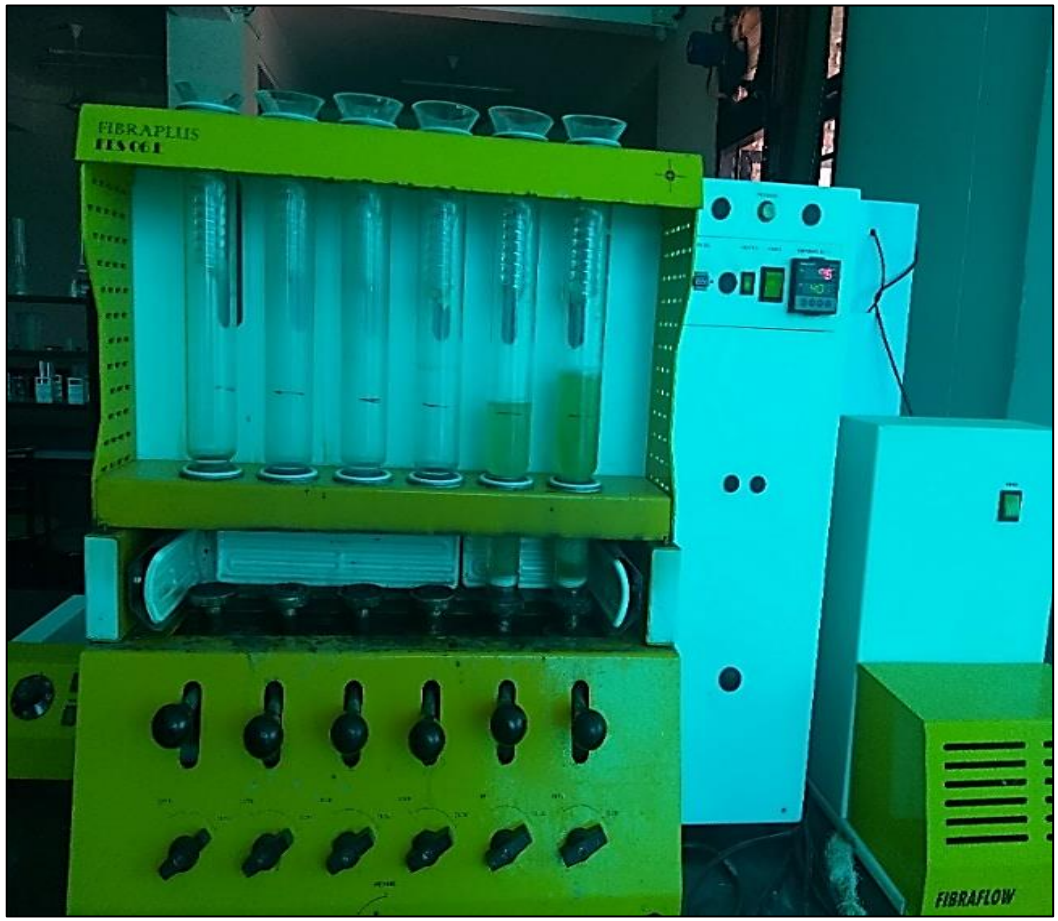

Figure 4.8 FIBRA PLUS assembly

Carbohydrates

The total carbohydrate content was calculated employing the formula below.

Carbohydrates (%) = 100 – [moisture (%) + ash (%) + crude fat (%) + crude protein (%) + crude fiber (%)].[28]

The proximate analysis of pearl millet samples was carried out in triplicate and data was represented as mean of three independent values.

4.2.6 Statistical Analysis

Descriptive statistics was employed for the proximate composition namely moisture, ash, crude fat, crude protein, crude fiber and carbohydrates of pearl millet samples using MS Excel version 2016. Furthermore, the correlation between carbohydrates, moisture, and protein content as well as regression analysis for the corresponding parameters were also investigated using maximum normalized data from samples.

4.2.7 Principal component analysis (PCA)

The principal component analysis was carried out using a web application framework ClustVis, (http://biit.cs.ut.ee/clustvis/). Principal components (PCs) can be computed by employing two default methods that was Singular Value Decomposition (SVD) and nonlinear iterative partial least square (NIPALS). In this study, NIPALS (PCA) method was chosen for further analysis because while calculating inner products, it will iteratively find the components by leaving out missing values. The group of similar objects in the data were obtained by using score plot in PCA.[29] The data was inserted in the matrix form for classification of pearl millet samples from different locations.

4.3 Results and Discussion

4.3.1 Proximate composition of pearl millet

The proximate composition named as moisture, ash, crude fat, crude fiber, crude protein, carbohydrates and energy of fifty pearl millet samples are shown in Table 4.1. All the experiments were performed in triplicate and the average of all individuals were considered for further analysis. Descriptive statistics of chemical parameters in the pearl millet samples are shown in Table 4.2- Table 4.4. The minimum and maximum value of moisture content was 5.187% and 14.833% respectively. The ash content lying in between 1.480-1.880% and crude fat was 3.640-6.560%. Similarly, the crude fiber is observed in between 1.550-4.500% and crude protein in between 5.556-11.946% whereas carbohydrate content lying in between 66.529 to 79.461% and energy in between 350.640-386.604 kcal. The standard deviation for moisture (1.755%), ash (0.099%), crude fat (0.565%), crude fiber (0.663), crude protein (1.400%), carbohydrates (2.343%) and in energy (8.226) states that the less variation in the experimental measurements. It is observed that the proximate composition namely moisture, ash, crude fat, crude protein, carbohydrates and energy shows the maximum value such as 14.833%, 1.880%, 6.560%, 11.946%, 79.461% and 386.804 kcal respectively of the pearl millet samples collected from Palwal, Adampur (Sirsa), Dhotar (Sirsa), of Haryana region as shown in Table 4.3. In comparison of another studies, it was observed that the pearl millet of Haryana region contain 7.44% moisture, 2.11% ash, 5.53% crude fat, 9.81% crude protein, 3.41% crude fiber and 71.70% carbohydrates.[30] Similarly, According to the literature various varieties of pearl millet samples originated in Haryana contains moisture, ash, fat, protein, fiber and carbohydrate content ranged from 6.5 - 7.7, 1.65 - 1.90, 5.1 - 7.2, 9.7 - 11.3, 2.9 - 3.8 and 69.6 - 72.5%, respectively.[17] The composition of pearl millet samples collected from Haryana is comparable with proximate composition of pearl millet samples explored previously. As per the literature, all the samples obtained from Sirsa, Haryana, India, as well as the parameters of the present study provides compatible results. Whereas, the samples collected from Punjab region present lowest values for crude fat and crude protein as

compare to Haryana region samples shown in Table 4.3 and Table 4.4. Overall, the pearl millet samples collected from Haryana are rich on the basis of nutrients compared to the samples collected from Punjab region of India.

Pearl millet is good source of protein, carbohydrates and fiber as compare to other cereal grains and it has protein content of 11.95%, that is greater than the 7.2% reported in rice, 11.1% in maize, 11.5% in barley, and 10.4% in sorghum millet.[31]

Table 4.1 Proximate composition of all pearl millet samples (n=50) obtained from Haryana and Punjab region

ID	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude protein (%)	Carbohydrates (%)	Energy (Kcal)
H	9.56	1.65	4.85	2.33	10.28	71.33	370.11
H	10.30	1.75	4.51	4.25	7.50	71.69	357.32
H	9.02	1.67	4.89	1.57	8.61	74.23	375.39
H	10.61	1.88	5.31	4.50	6.95	70.76	358.59
H	9.49	1.69	5.79	1.55	6.53	74.95	378.01
H	10.08	1.53	5.77	1.92	8.06	72.64	374.74
H	7.97	1.75	6.56	2.17	8.75	72.80	385.25
H	9.62	1.73	5.41	2.48	7.08	73.67	371.75
H	10.63	1.85	4.93	2.95	6.39	73.25	362.95
H	10.34	1.69	3.87	2.15	7.92	74.04	362.63

H	10.73	1.73	4.27	2.30	5.83	75.13	362.27
H	10.78	1.74	4.93	3.35	7.50	71.70	361.19
H	12.35	1.87	5.95	2.62	6.81	70.40	362.33
H	9.83	1.70	5.27	1.90	8.33	72.97	372.63
H	8.91	1.86	5.45	1.98	9.72	72.08	376.27
H	10.17	1.77	4.65	2.07	8.61	72.71	367.18
H	12.13	1.48	5.20	1.65	8.89	70.65	364.97
H	10.86	1.76	5.21	1.67	10.56	69.94	368.89
H	14.83	1.61	4.84	1.95	8.75	68.02	350.64
H	8.64	1.68	5.25	2.28	8.61	73.54	375.89
H	5.19	1.69	4.80	2.47	6.39	79.46	386.60
H	6.93	1.49	5.48	1.87	7.36	76.86	386.22
H	8.00	1.69	5.51	2.88	5.83	76.10	377.29
H	8.64	1.72	4.88	2.45	9.72	72.59	373.16
H	10.30	1.83	5.27	4.12	11.95	66.53	361.30
P	11.70	1.84	3.64	1.77	8.06	72.99	356.94
P	8.95	1.84	4.87	2.62	8.20	73.53	370.69

P	10.52	1.74	5.13	3.65	7.22	71.73	362.03
P	11.29	1.87	5.01	2.67	6.81	72.34	361.70
P	11.85	1.76	5.68	1.68	8.61	70.43	367.27
P	10.60	1.79	5.27	3.03	7.92	71.40	364.66
P	9.89	1.61	5.27	1.85	6.53	74.85	372.93
P	9.55	1.71	6.52	2.77	8.47	70.97	376.46
P	9.11	1.72	6.03	2.72	7.78	72.64	375.89
P	10.33	1.73	5.87	2.85	7.50	71.72	369.69
P	11.27	1.57	5.57	2.17	9.72	69.68	367.78
P	10.13	1.62	5.15	2.30	7.22	73.58	369.52
P	9.47	1.77	5.71	1.87	5.83	75.34	376.07
P	11.90	1.53	5.95	2.45	5.56	72.62	366.23
P	11.96	1.80	5.15	2.40	7.08	71.61	361.09
P	11.37	1.71	4.80	2.13	9.17	70.83	363.21
P	11.25	1.75	5.04	2.28	6.53	73.16	364.13
P	10.92	1.67	5.28	2.75	7.92	71.46	365.05
P	7.99	1.59	4.75	2.15	10.56	72.97	376.81

P	9.85	1.63	5.87	2.43	6.25	73.97	373.69
P	8.54	1.63	5.52	2.90	6.39	75.02	375.31
P	7.97	1.69	4.72	2.58	6.81	76.24	374.66
P	7.23	1.62	4.96	2.35	8.20	75.65	380.01
P	6.41	1.65	5.65	3.23	6.39	76.67	383.13
P	6.37	1.80	5.43	2.98	7.64	75.79	382.54

Table 4.2 Descriptive statistical analysis of chemically processed pearl millet samples (n=50).

	Moisture (%)	Ash (%)	Fat (%)	Crude Fiber (%)	Crude protein (%)	Carbohydrates (%)	Energy (Kcal)
Mean	9.847	1.709	5.233	2.480	7.826	72.904	370.021
Standard Error	0.248	0.014	0.080	0.094	0.198	0.331	1.163
Median	10.107	1.713	5.260	2.375	7.709	72.757	369.903
Standard Deviation	1.755	0.099	0.565	0.663	1.400	2.343	8.226
Minimum	5.187	1.480	3.640	1.550	5.556	66.529	350.640
Maximum	14.833	1.880	6.560	4.500	11.946	79.461	386.604
Count	50.000	50.000	50.000	50.000	50.000	50.000	50.000

Table 4.3 Descriptive statistical analysis of chemically processed pearl millet samples of only Haryana region

	Moisture (%)	Ash (%)	Fat (%)	Crude Fiber (%)	Crude protein (%)	Carbohydrates (%)	Energy (Kcal)
Mean	9.836	1.713	5.154	2.458	8.117	72.721	369.742
Standard Error	0.372	0.021	0.112	0.164	0.308	0.543	1.875
Median	10.080	1.720	5.213	2.275	8.056	72.713	370.113
Standard Deviation	1.859	0.107	0.559	0.819	1.542	2.715	9.374
Minimum	5.187	1.480	3.867	1.550	5.834	66.529	350.640
Maximum	14.833	1.880	6.560	4.500	11.946	79.461	386.604
Sum	245.901	42.827	128.853	61.450	202.937	1818.032	9243.557
Count	25.000	25.000	25.000	25.000	25.000	25.000	25.000

Table 4.4 Descriptive statistical analysis of chemically processed pearl millet samples of Punjab region

	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude protein (%)	Carbohydrates (%)	Energy (Kcal)
Mean	9.857	1.706	5.313	2.503	7.534	73.088	370.299
Standard Error	0.337	0.018	0.114	0.095	0.240	0.388	1.416
Median	10.133	1.707	5.267	2.450	7.501	72.967	369.693
Standard Deviation	1.683	0.092	0.571	0.474	1.202	1.941	7.080
Minimum	6.373	1.527	3.640	1.675	5.556	69.682	356.940
Maximum	11.960	1.873	6.520	3.650	10.557	76.672	383.127
Sum	246.430	42.640	132.813	62.575	188.352	1827.190	9257.487
Count	25.000	25.000	25.000	25.000	25.000	25.000	25.000

The arithmetic mean and standard deviation in the descriptive statistics express the central tendency and variation of data. The low value of standard deviation indicates the data was more symmetric and higher value shows the variation in data. The standard deviation was highest for carbohydrates, energy and lowest for ash content

4.3.2 Correlation and regression analysis of proximate components of pearl millets

The results of correlation analysis among various proximate components are mentioned in Table 4.5- Table 4.7. The maximum normalized data for all the chemical parameters was employed for the correlation and regression analysis. The correlation analysis was used to perceive the relation among the variables. It was observed that the carbohydrate content of pearl millets is negatively correlated with the moisture content, protein and carbohydrates content negatively correlated with each other and moisture and energy also negatively correlated (Figure 4.9- Figure 4.11). Similarly, crude fat, energy and carbohydrates positively correlated as shown Figure 4.12- Figure 4.13. Whereas Haryana and Punjab region samples were correlated within same manner as shown in Table 4.6 &Table 4.7. Therefore, further linear regression analysis has performed to evaluate the relation among moisture, carbohydrates, fat, protein and energy content as follow.

$$M = -0.562C + 50.871 \ (R^2 = 0.564) \qquad (4.7)$$

$$M = -0.186E + 78.97; \ (R^2 = 0.766) \qquad (4.8)$$

$$P = -0.3581C + 33.934 \ (R^2 = 0.359) \qquad (4.9)$$

$$F = 0.030E - 6.083 \ (R^2 = 0.198) \qquad (4.10)$$

$$E = 2.3834C + 196.26 \ (R^2 = 0.460) \qquad (4.11)$$

Where, C represents carbohydrates and M denotes for moisture, E represent for energy, F represents for crude fat and P represents crude protein. It is observed from equation 4.7, 4.8 and 4.9 has negative slope and equation 4.10 and 4.11 having positive slope with

different intercepts. Consequently, it is concluded that if any one of these parameters was determined by chemical approach, then the other parameters can be predicted by using these equations but this prediction of parameters will be poor because of coefficient of determination (R^2) stands below 0.5.

Table 4.5 Correlation analysis of proximate compositions of all samples.

	Moisture	Ash	Fat	Crude Fiber	Crude protein	Carbohydrates	Energy
Moisture	1.000						
Ash	0.117	1.000					
Fat	-0.123	-0.133	1.000				
Crude Fiber	-0.080	0.380	0.029	1.000			
Crude protein	0.082	0.002	-0.112	-0.116	1.000		
Carbohydrates	**-0.751**	-0.206	-0.085	-0.177	**-0.599**	1.000	
Energy	**-0.876**	-0.317	0.445	-0.263	-0.072	**0.679**	1.000

Table 4.6 Correlation analysis of proximate compositions of Haryana region samples.

	Moisture	Ash	Fat	Crude Fiber	Crude protein	Carbohydrates	Energy
Moisture	1.000						
Ash	0.083	1.000					
Fat	-0.161	0.023	1.000				
Crude Fiber	0.061	0.536	-0.121	1.000			
Crude protein	0.142	0.005	0.030	-0.077	1.000		
Carbohydrates	**-0.754**	-0.266	-0.077	-0.295	**-0.648**	1.000	
Energy	**-0.866**	-0.292	0.467	-0.458	-0.077	**0.690**	1.000

Table 4.7 Correlation analysis of proximate compositions of Punjab region samples.

	Moisture	Ash	Fat	Crude Fiber	Crude protein	Carbohydrates	Energy
Moisture	1.000						
Ash	0.162	1.000					
Fat	-0.086	-0.305	1.000				
Crude Fiber	-0.353	0.091	0.272	1.000			
Crude protein	0.006	-0.024	-0.232	-0.190	1.000		
Carbohydrates	**-0.767**	-0.105	-0.128	0.096	**-0.509**	1.000	
Energy	**-0.900**	-0.353	0.428	0.173	-0.047	**0.658**	1.000

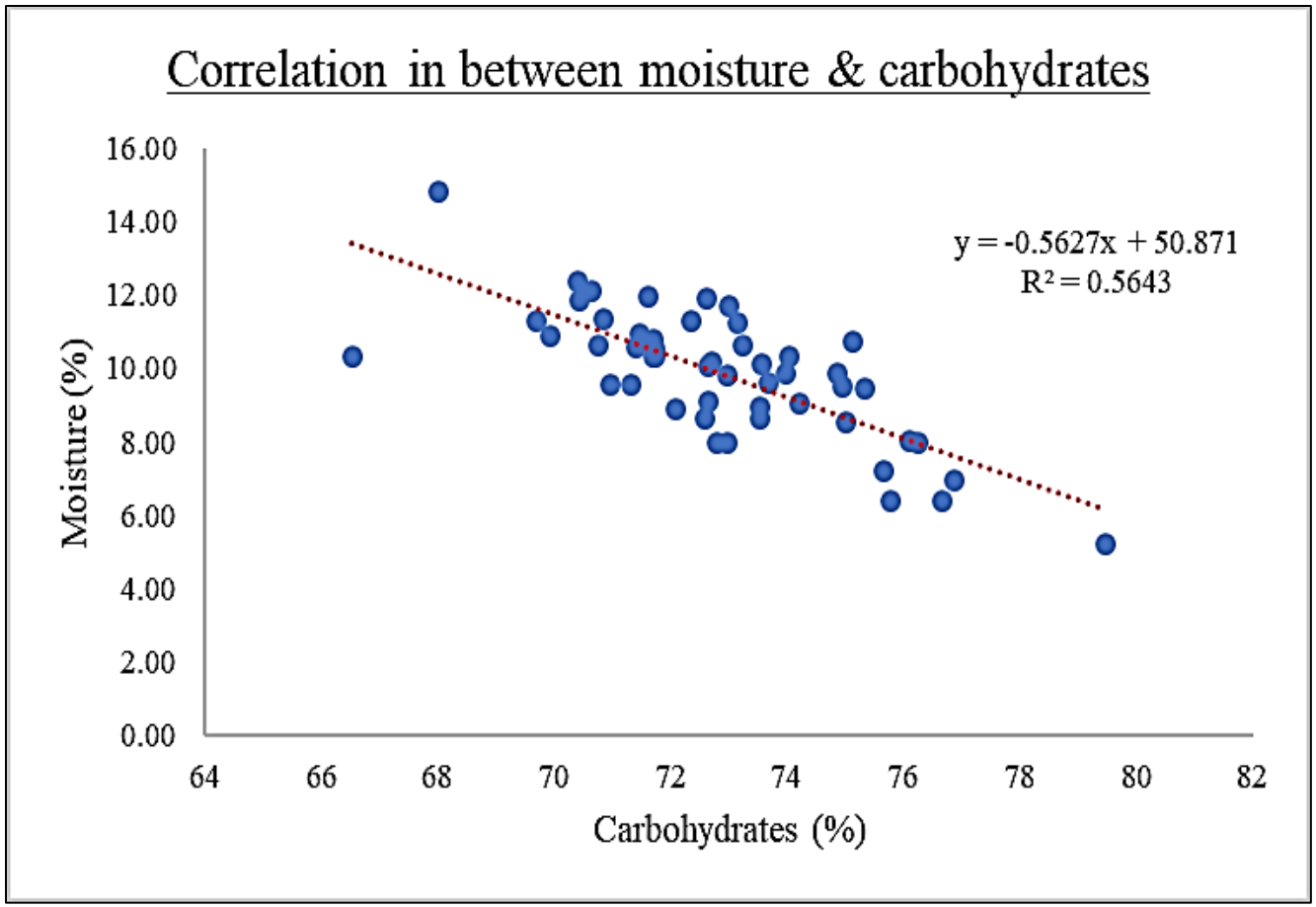

Figure 4.9 Negative correlation in between moisture and carbohydrates

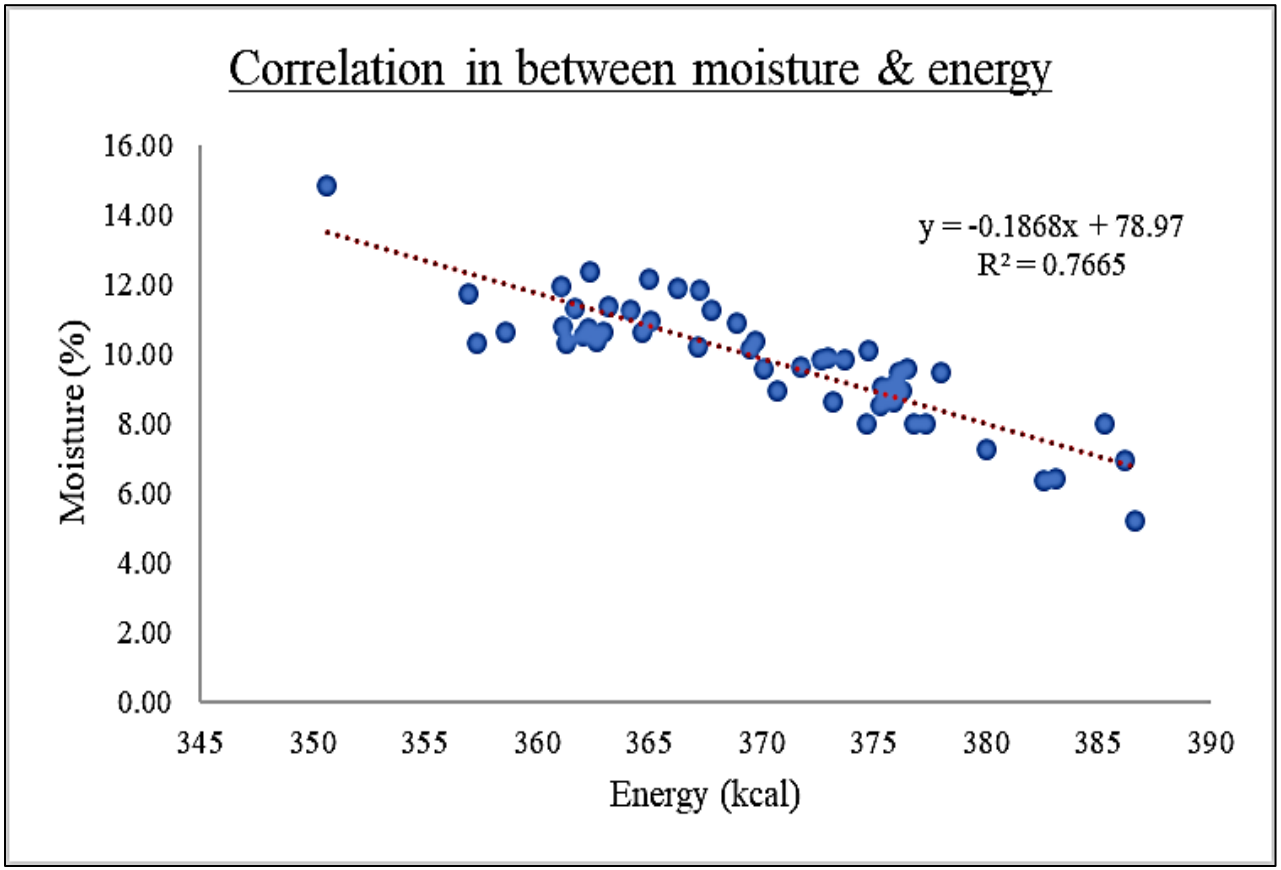

Figure 4.10 Negative correlation in between moisture and energy

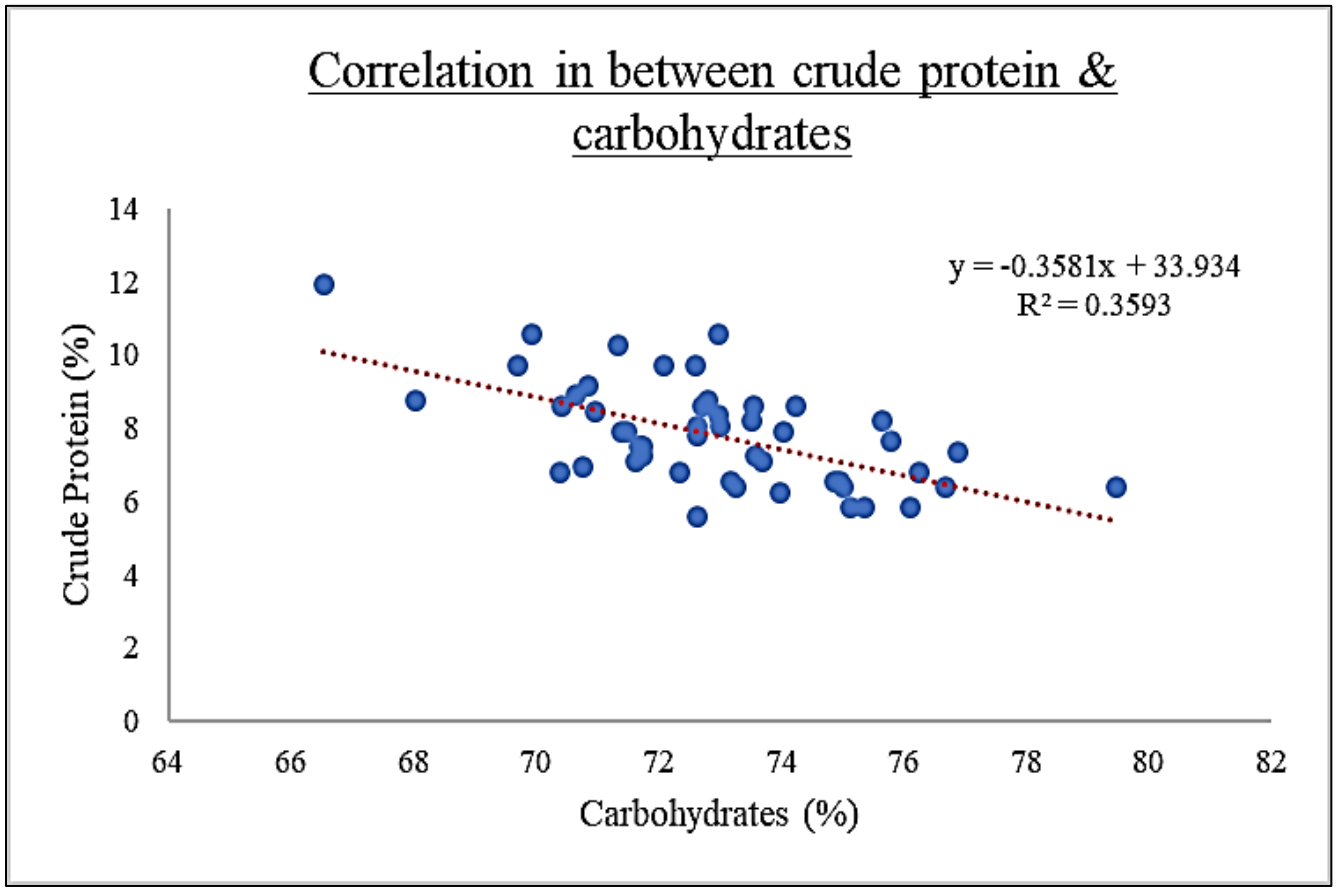

Figure 4.11 Negative correlation in between crude protein and carbohydrates

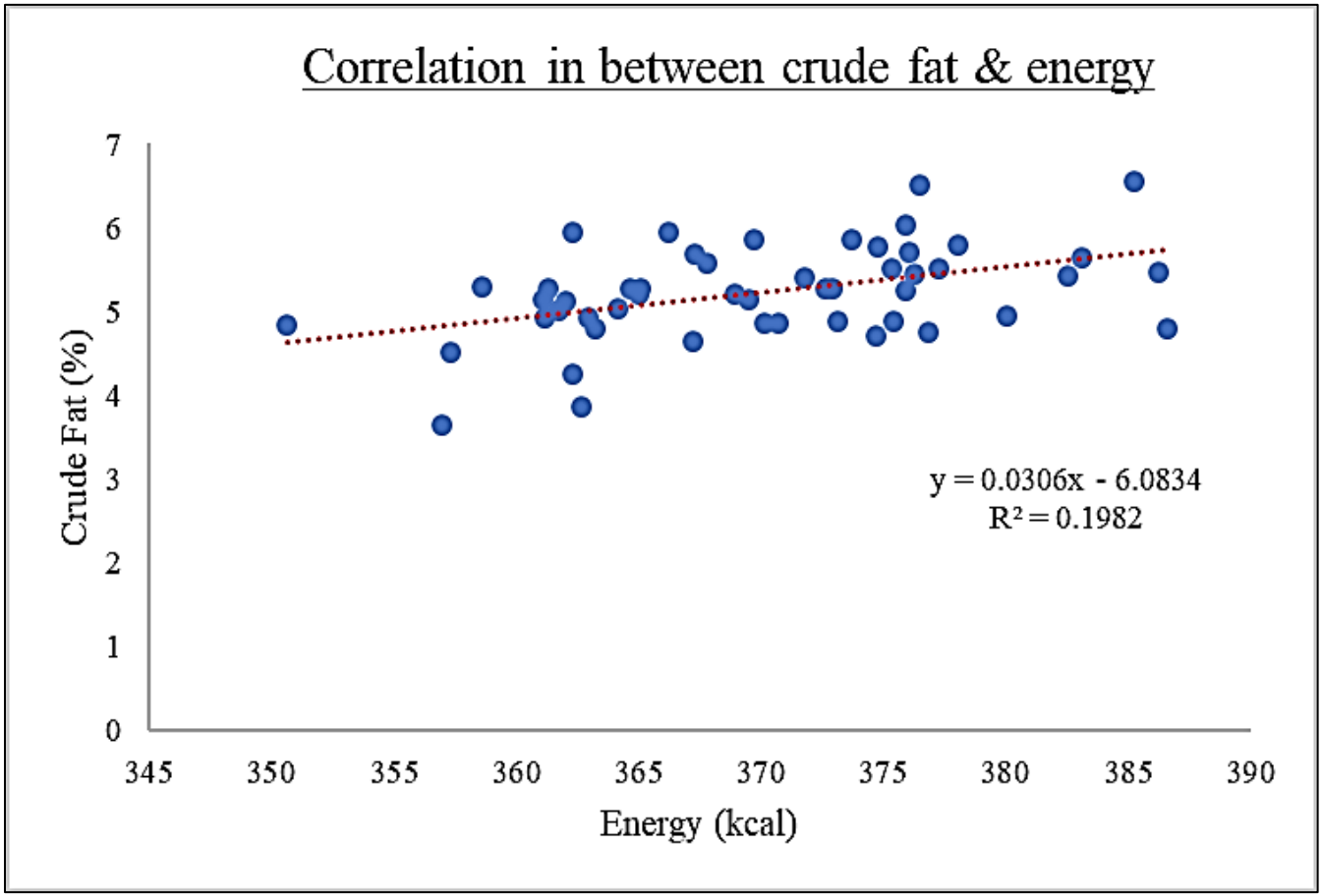

Figure 4.12 Positive correlation in between crude fat and energy

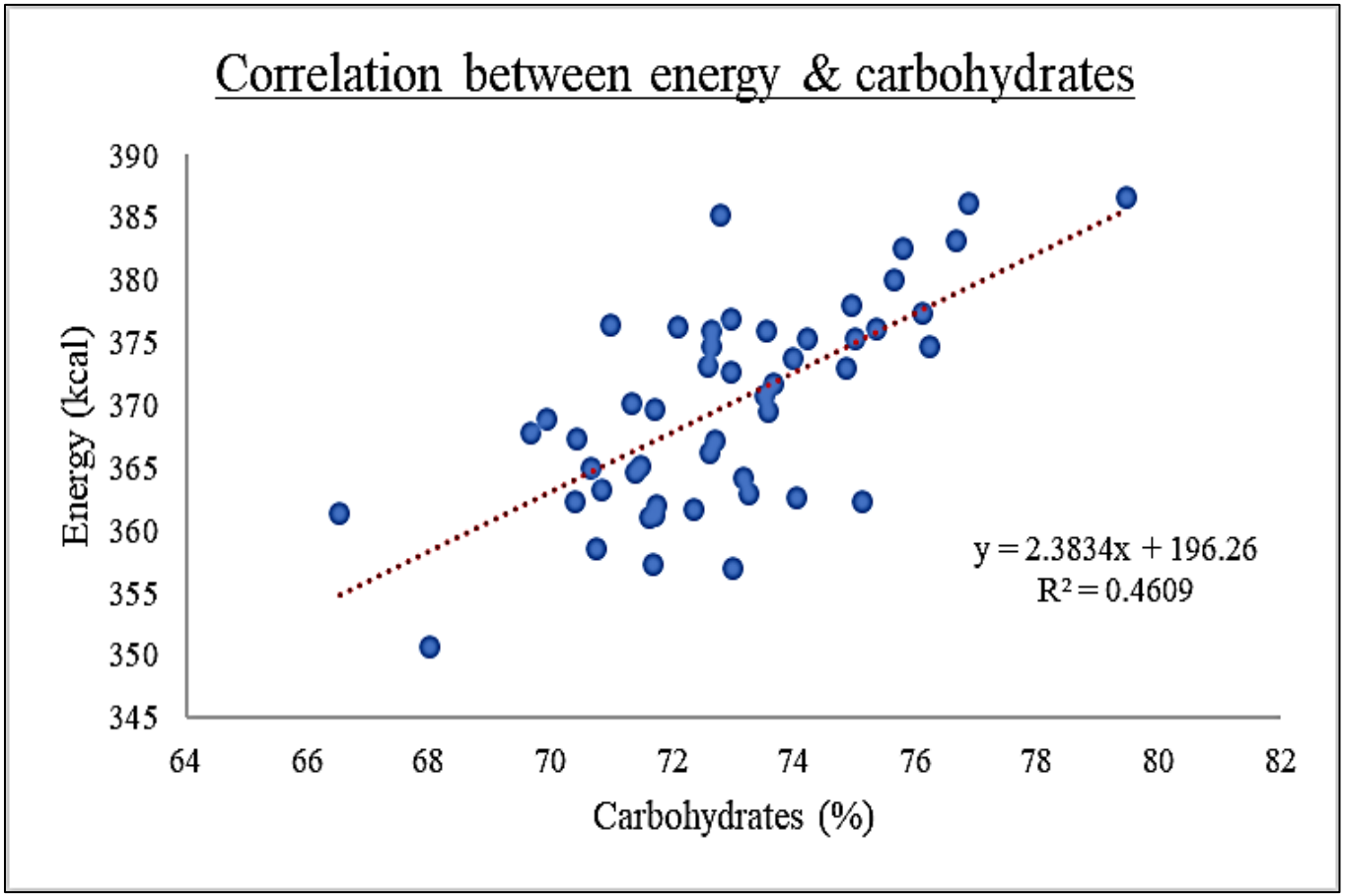

Figure 4.13 Positive correlation in between carbohydrates and energy

4.3.3 PCA Analysis of proximate composition and IR spectral data

The PCA applied to the proximate composition of pear millet samples revealed the negative correlation between carbohydrates and moisture content, as well as protein and carbohydrates content also in negative correlation shown in Figure 4.14. These finding are in accord with the results observed in previous section as mentioned in correlation and regression equations (4.7) & (4.8) respectively. PCA was applied to all chemical and spectral data set that in turn, explain the classification of pearl millet samples according to their region as shown in Figure 4.15- Figure 4.17. It was observed that the PC1 and PC2 explain 85.5 and 11.5% of total variance for FTIR spectral data and 85 and 11.4% for NIR data, as well as 40.3 and 20.4% of variation for chemical data respectively. The variance showed the maximum data covered in PC1 and PC2 but it was clearly seen that all the samples by chemical, fourier transfer infrared and near infrared spectral approach on the basis of region are not classify. After seen this the few samples were removed named as outliers and then PCA applied on 41 samples as shown in Figure4.18- Figure 4.20. Based on the FTIR spectral data, the PC1, PC2 accounted for 82.9% and 13.3% of the total variance (Figure 4.18). Whereas, based on the NIR spectral data, PC1 explains 84.3% and PC2 explains 11.8% of total variance. (Figure 4.19) It is important to note that the PCA applied on chemical data of various samples presented least variance compared to the spectral data (Figure 4.20).

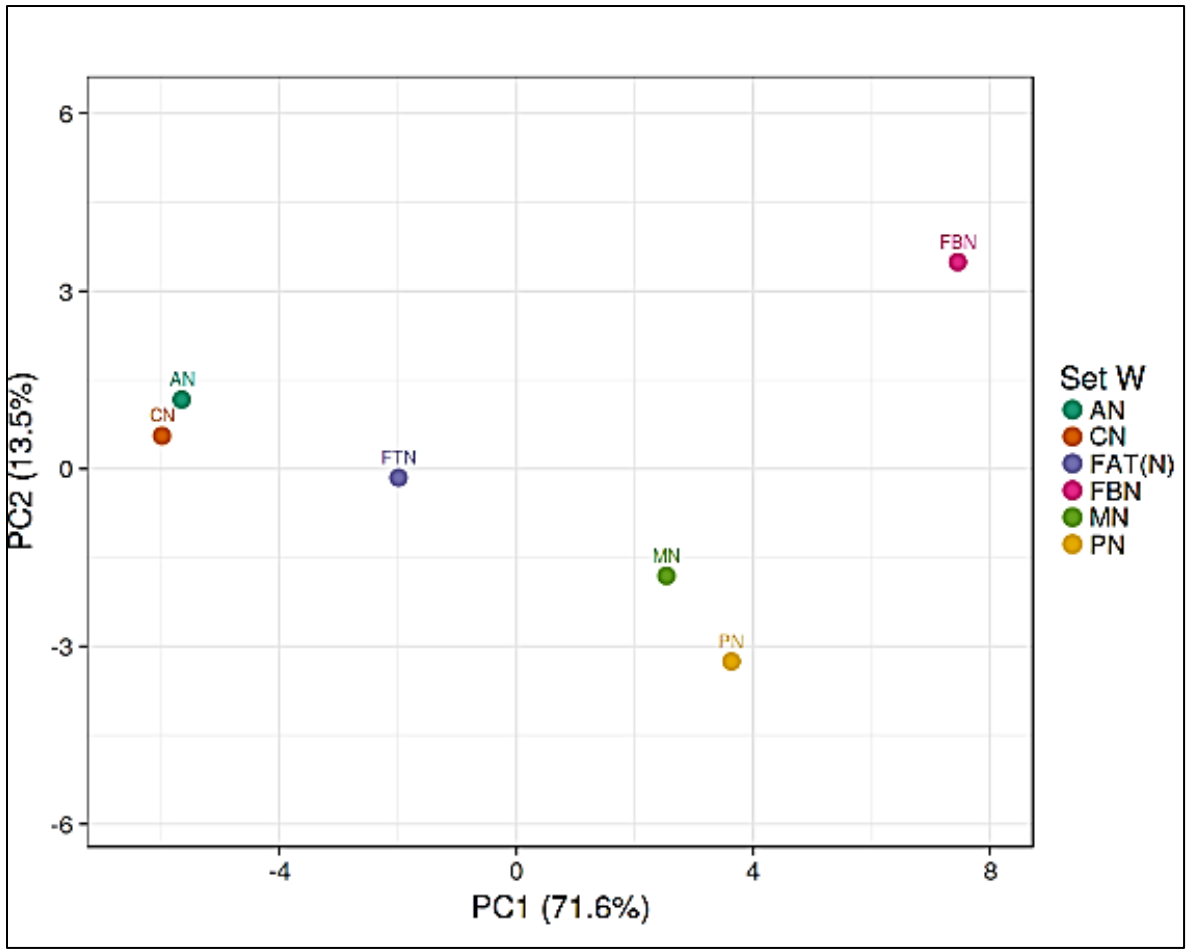

Figure 4.14 Correlation analysis in between chemical properties using Principal component analysis (PCA). Where, MN: moisture normalized; AN: ash normalized; FBN: fiber normalized; FN: fat normalized; PN: protein normalized; CN: carbohydrates normalized.

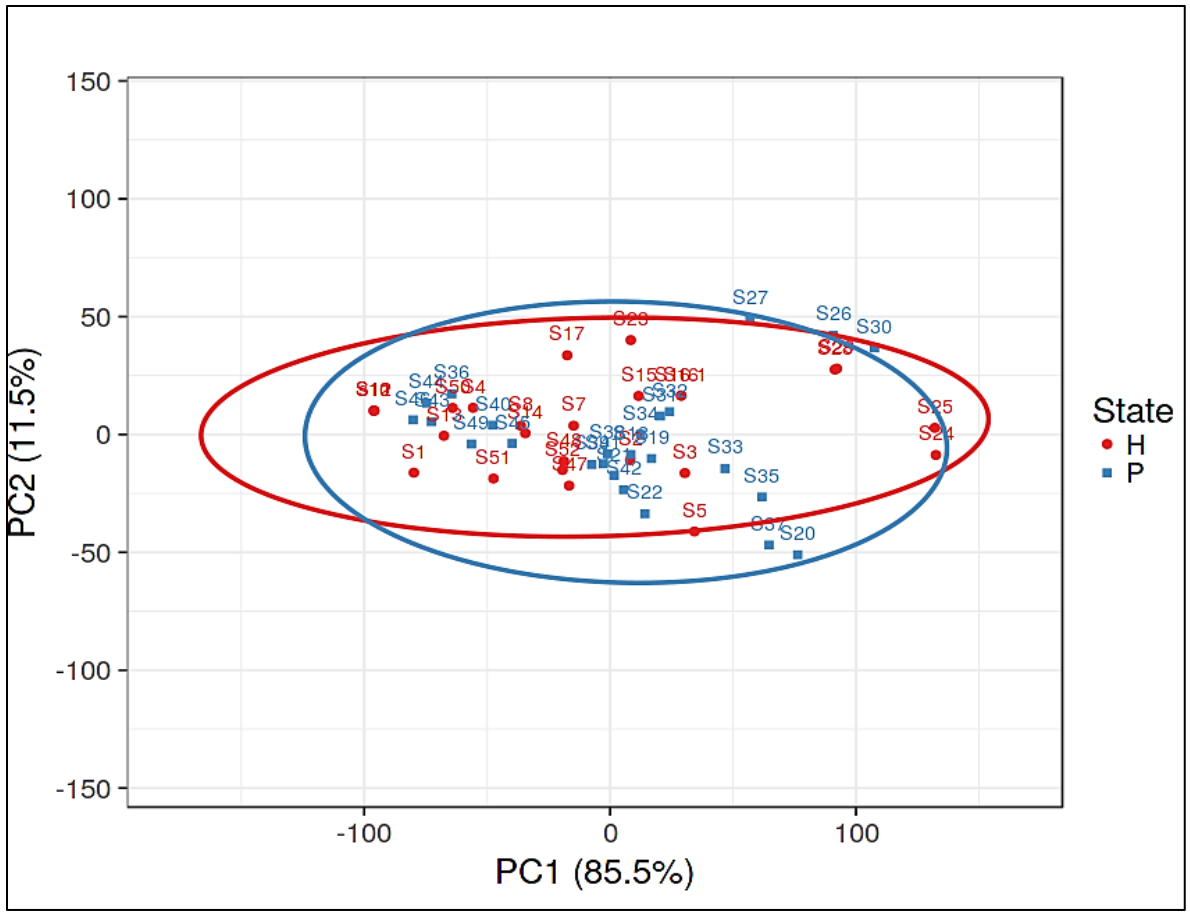

Figure 4.15 Score plot of PCA to classify the samples using FTIR spectral data

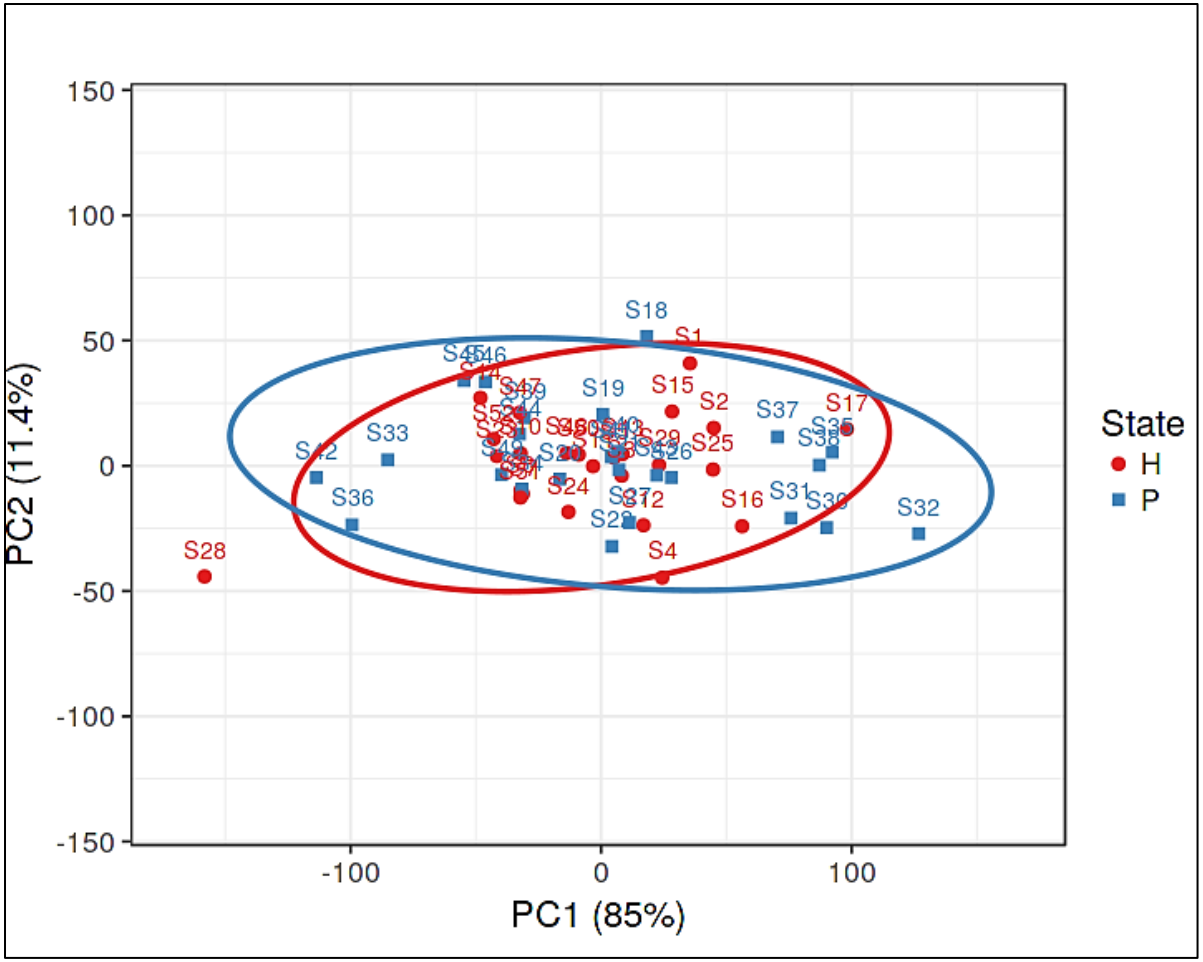

Figure 4.16 Score plot of Principal components shows the differentiation of all (n=50) pearl millet samples from regions using of near infrared (NIR) spectral data.

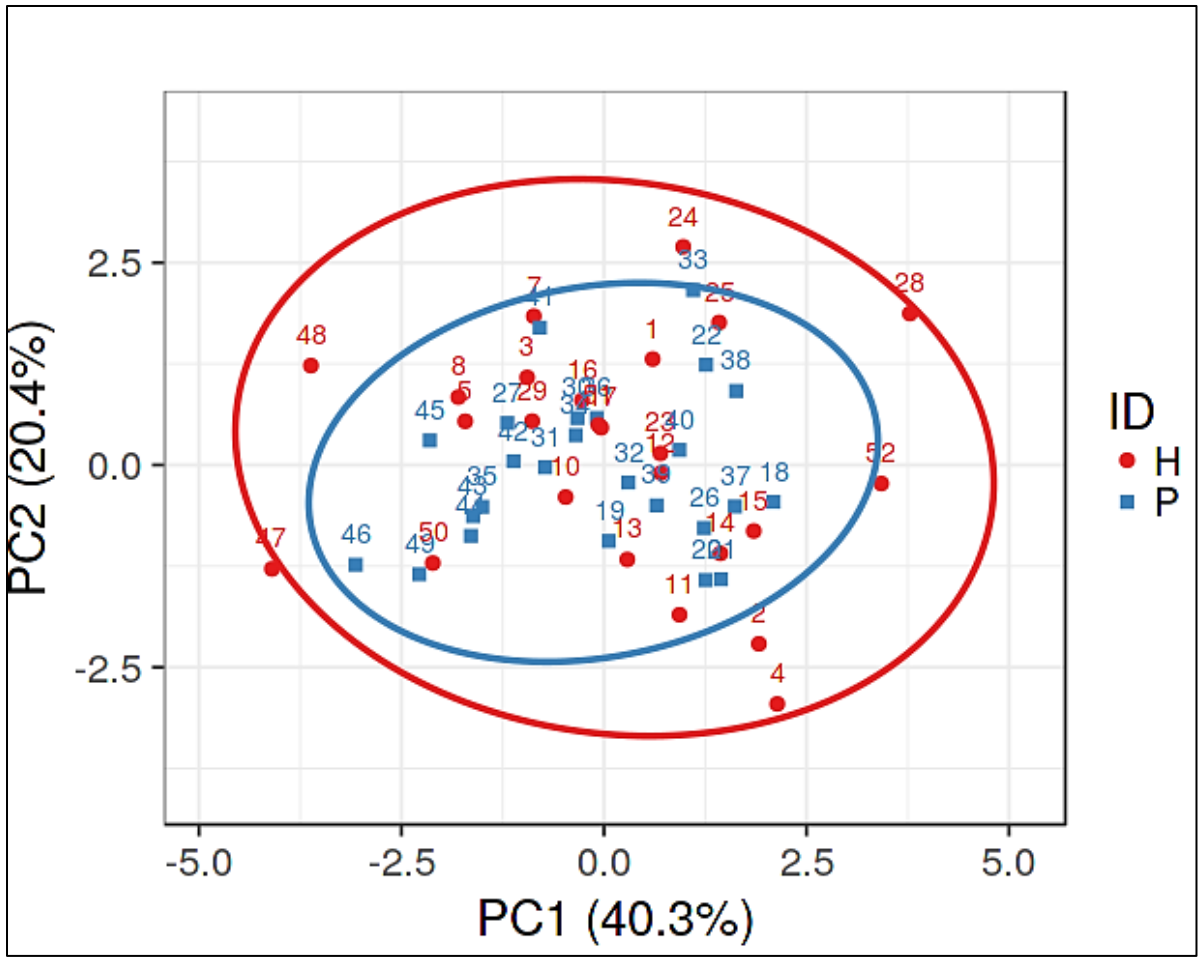

Figure 4.17 PCA on chemical data to classify the all-pearl millet samples according to their regions

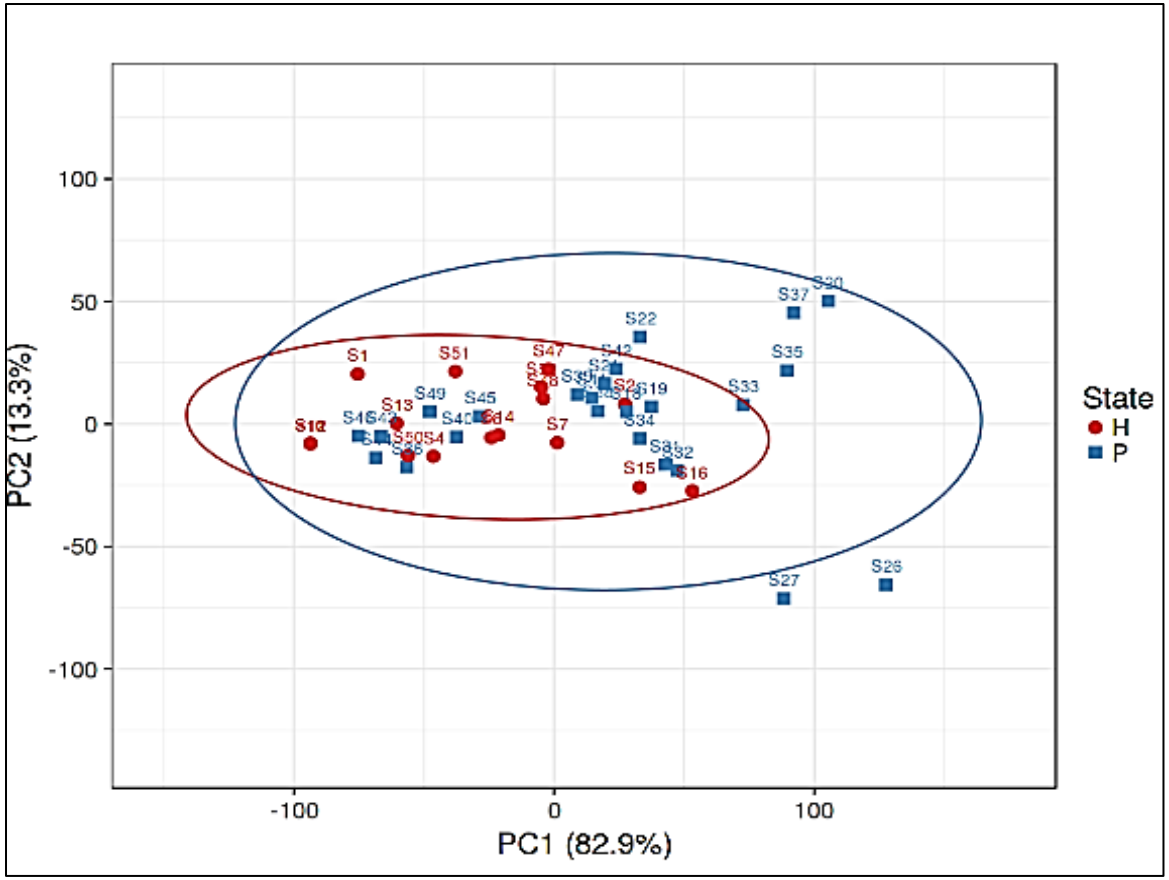

Figure 4.18 Score plot of principal components to classify the samples using FTIR spectral data on the basis of region Haryana (H) and Punjab (P).

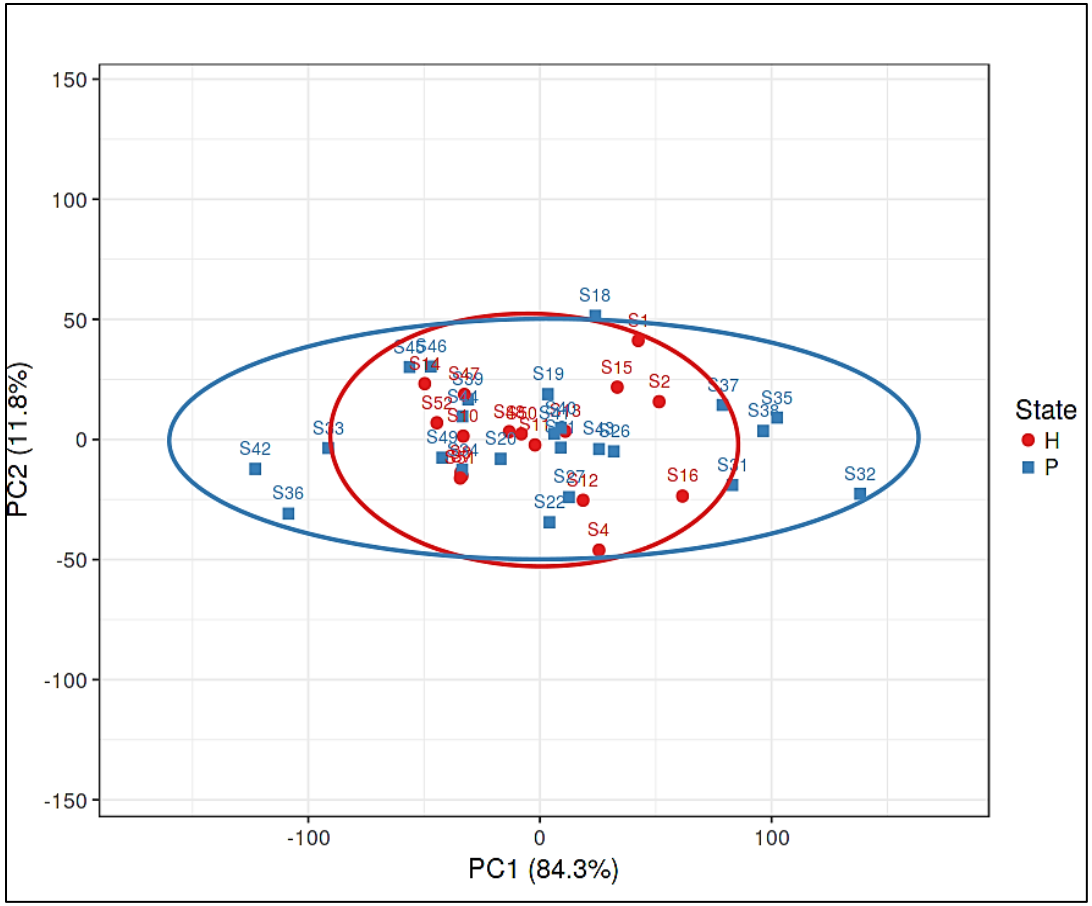

Figure 4.19 Score plot of PCs illustrating the differentiation of pearl millet samples from regions using of near infrared (NIR) spectral data.

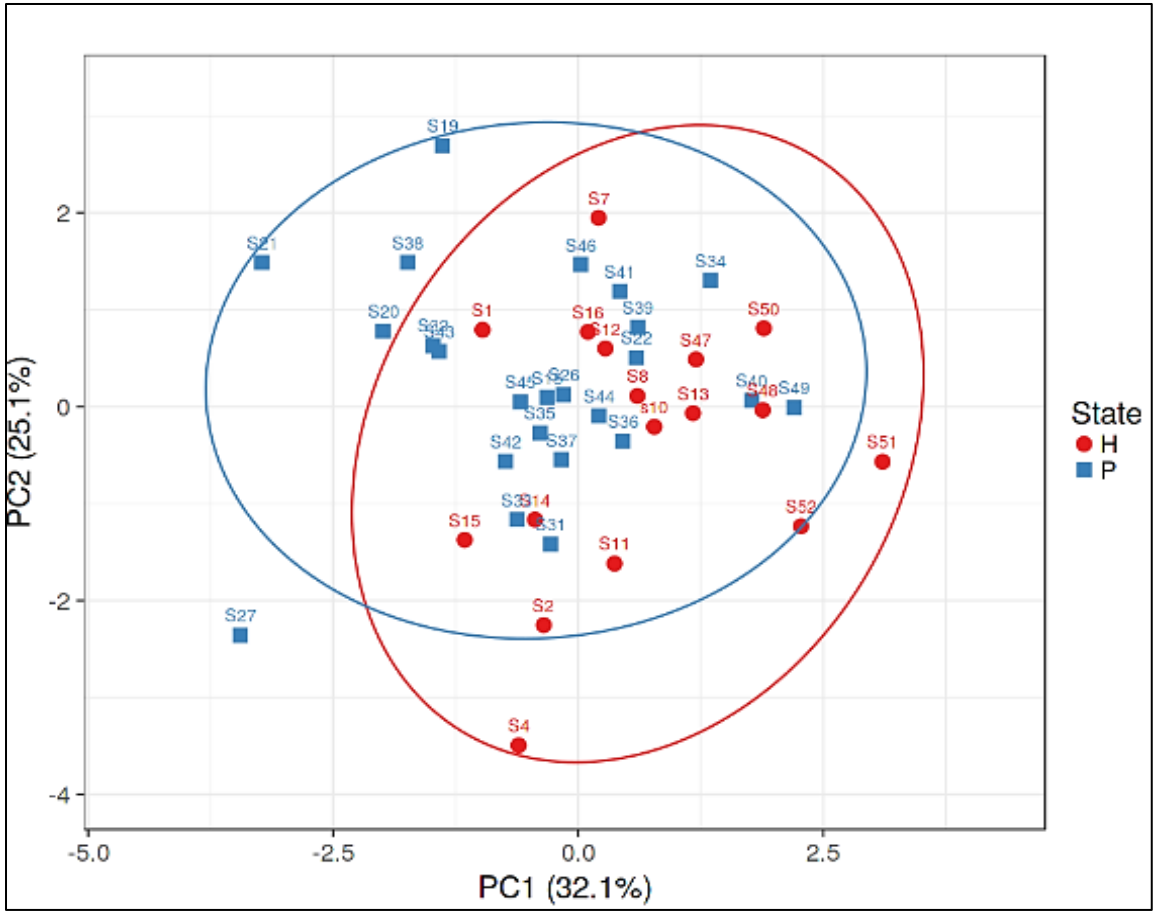

Figure 4.20 PCA on chemical-based data to classify the few (n=41) pearl millet samples based on region.

The classification of pearl millet samples using NIR spectral data is shown in Figure4.19. It was observed that samples from Punjab region exhibit PC1 values from negative to positive, this may be attributed to the large variation in the chemical data of the samples, whereas all the samples from Haryana region are clustered near the origin. The samples from Haryana region present negative values of PC1, whereas, the samples from Punjab region exhibit variation from negative to positive values in case of PCA based on FTIR spectra (Figure 4.18). It was observed that through PCA samples were not classified with significantly high accuracy using FTIR spectral data, though it was observed that some of the samples from Punjab region were lying in the area covered by the samples of Haryana. This may be due to the reason that these samples from the Punjab and Haryana region present similarity in the nutritional composition due to least difference in the environment condition of the collection sites. Furthermore, additional effort was also made to further classify these samples based on the proximate composition. As shown in Figure 4.20, it was observed that the samples from Punjab region lies on the top left whereas, the samples from Haryana region lies on bottom right. Thus, it was observed that the samples on the basis of properties, roughly able to classified using Principal component analysis (PCA) due to effect of environmental conditions of Haryana and Punjab on the nutritional parameters.

Therefore, as a result, the total PCs accounted as 96.1% of variance for NIR spectral data and 96.2% for FTIR as well as 57.2% for the chemical data. It states that the complete information of IR spectral data from various regions were covered in first two PCs. Similar results were also reported in literature when Vis-NIR spectroscopy along with PCA and other classification methods was employed to trace the geographical origins of millets with efficiency of 94%.[26]

PCA is a powerful technique employed as mathematical tool for analyzing, classifying and dimensionality reduction of numerical data sets in multivariate problems.[9,10,32–34] PCA based upon the decomposition of data matrix X into two matrices V and U and these matrices are orthogonal to each other. The eigenvectors of data matrix are known as PCs.

In mathematical terms, the variables PC_k is the linear function of combination of n response vectors X_{ij} for the analyte under study.

$PC_k = a_{1k}x_{1j} + a_{2k}x_{2j} + \ldots a_{nk}x_{nj}$, $(k = 1, 2, \ldots)$

and $PC_k = PC_1, PC_2, PC_3..$, represents the principal components. In this case, the first principal component PC_1 has greatest possible variance in the all possible linear functions and second principal component PC_2 account for next highest possible variance being mutually uncorrelated to each other.[35] The magnitude of each eigenvector indicated through its possessed eigenvalue that measure the variance which is related to the principal component and their number is equal to the number of dimensions of data.[10] The main feature of PCA are loading plot and score plot. Loading plot represents relationship between all dependent and independent variables and helps in providing correlation between them. While, the score plot showed the classification of data clusters.[36] Therefore, PCA conclude that it is feasible to attain the data dimensionally reduction to principal component and rejection of less important information without any loss of significant information.

Analytical techniques are necessary for the safety and quality control of natural compound-based food items and supplements, as well as to avoid any deceit. For authenticity and traceability, spectroscopy coupled with chemometric approaches is most generally employed. Near-infrared spectroscopy (NIRS) has gained popularity as a quick and nondestructive method that requires minimal sample preparation and easy to use.[37,38] Because of the vibration of C–H, O–H, and N–H bonds, the NIR spectrum runs from 700 to 2500 nm. In agriculture, near-infrared spectroscopy (NIRS) is commonly used as a "fingerprint technique" based on a substrate's chemical composition and physical characteristics, can be used to analyze solid materials in a non-destructive manner by collecting a spectrum. The high quantity of information provided in such a fingerprint may potentially allow species characterization using mathematical processing and classification methods.[39–41]

4.4 Summary

Pearl millet has gained significant attention from consumers and producers due to its high nutritional value and potent health benefits. It has been used as sixth cereal grain in various states of India. In this study, pearl millet samples collected from different locations of Punjab and Haryana states of India were analyzed for proximate composition by employing AOAC standard methods. It was observed that moisture content was measured at a minimum of 5.187% and a high of 4.833%. The ash content was within range of 1.480-1.880%, and the crude fat content was 3.640-6.560% respectively. Similarly, crude fiber was found to be in the range of 1.550-4.500%, while crude protein was found to be in the range of 5.556-11.946% and the carbohydrate content ranges from 66.529 to79.461% and energy in between 350.640-386.604 kcal. The highest values for moisture, ash, crude fiber, crude protein, and carbohydrate content were observed in the pearl millet samples collected from Haryana, whereas the samples from Punjab region presented lowest value for crude fat and crude protein with 3.640%, and 5.556%, respectively.

The correlation analysis of proximate composition revealed the negative correlations between carbohydrate and moisture, moisture and energy as well as carbohydrates and protein content and positive correlation in between crude fat energy and carbohydrates. These results were compared to PCA technique and an acceptable rate of variance was obtained. It was concluded that the PCs accounted for 96.1 % classification rate of the total variation in case of NIR spectral data and 96.2% for FTIR data and 57.2 % in case of chemical data. It appears that the almost complete spectral information of IR spectral data from various regions were covered in first two PCs, but pearl millet samples were hardly distinguished on the basis of geographical regions using principal component analysis. Therefore, there is a need to study the modified technique for classification of pearl millet samples on the basis of geographical origins.

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CHAPTER-5

DEVELOPMENT OF NON-DESTRUCTIVE METHOD FOR PEARL MILLET USING NIR/MIR SPECTROSCOPY

5 Development of non-destructive method for pearl millet using NIR/MIR spectroscopy

5.1 Introduction

Pearl millet is an important cereal crop because its nutritional compositions are superior to wheat and rice grains. It has great health benefits, including antidiabetic and anti-carcinogenic activity, since they are rich in minerals including magnesium, phosphorus, zinc, and protein.[1] In addition, they have a high fiber content compared to other cereal grains – wheat and rice grains, which results in a slower rate of glucose absorption into the blood and slows digestion.[2] Patients with diabetes can effectively use pearl millet to sustain a steady blood sugar level over an extended period of time. There is very less amount of gluten present in pearl millet grains.[3,4] Conventionally, wet chemistry laboratory procedures like soxhlet and kjeldahl are well recognized for determination of compositions due to their accuracy and dependability. However, they are typically time-consuming, labor-intensive, and need expert investigators. It is also more difficult to determine the nutrients parameters efficiently from food and feed materials because that required specialized facilities, costly equipment, specialized staff, and longer experiment times. Because of these disadvantages of wet chemistry there is need to replace conventional analysis with innovative, chemical-free, quick, and green technologies. Thus, Infrared spectroscopy is one of the most widely used methods offered to scientists and manufacturers since it is affordable, rapid, non-invasive, and requires minimal sample preparation.

In the 1800, Sir Frederick William Herschel (a professional musician and astronomer) discovered the first non-visible region in absorption spectrum that is known as NIR spectroscopy.[5,6] The NIR technique as compared to other analytical such as HPLC as well as conventional chemical methods, is rapid, chemical-free, cost-effective, easy to use (once calibration model have been developed) and non-destructive in manner. NIR as well as MIR spectroscopic methods are used as secondary methods (i.e. requiring the reference values for development of calibration model). The accuracy of NIR methods are highly

depends on how much precisian reference values of chemical data. As a result, in spectroscopy, the MIR region contain the fundamental molecular vibration peaks within 4000 to 400 cm^{-1} while NIR region originating from overtones and combinations of fundamental vibration bands within 750-2500 nm.[5,7] The statistical calibration models are developed by using the NIR techniques as well as it have been widely used in food and non-food industries. The important applications of NIR spectroscopy in food industry such as cereal, milk, fish, meat and its products, fruits and vegetables, beer, wine and also mostly used in food and agriculture products. The non-food application of NIR spectroscopy named as wood, soil, medical, pharmaceutical, polymer and plastics processing and in environmental investigations.[8]

Therefore, the quantitative analysis using MIR spectroscopy is still in development and has not been enhanced. Hence there have been several studies to understand MIR spectra, but most of them concentrate on the qualitative analysis of molecular structure in particular samples.[9–11] In the previous studies, NIR/MIR vibrational spectroscopy and Chemometrics used to predict the quality factors of wheat grains[12], rice and its flour.[13] The use of vibrational spectroscopy to developed the PLS models for prediction of the physicochemical parameters of Manihot esculenta flour[14], wheat flour[15], potato flesh[16] and determine the adulteration in monofloral honey samples using FTIR vibrational spectra.[17] Another study reported the determination of adulteration of wheat flour in unripe banana flour using VIS-NIR spectroscopy.[18]

However, few research has been done on the development of statistical PLS models in food and cereals by using IR spectroscopy and very few literature has been found for comparison of NIR and FTIR/MIR vibrational spectroscopy for proximate compositions of millets. Therefore, this chapter aimed to reveal the NIR vibrational spectroscopy to predict the chemical compositions in pearl millet non-destructively with comparison of MIR spectroscopy. The work flow carried out in this chapter is shown in Figure 5.1.

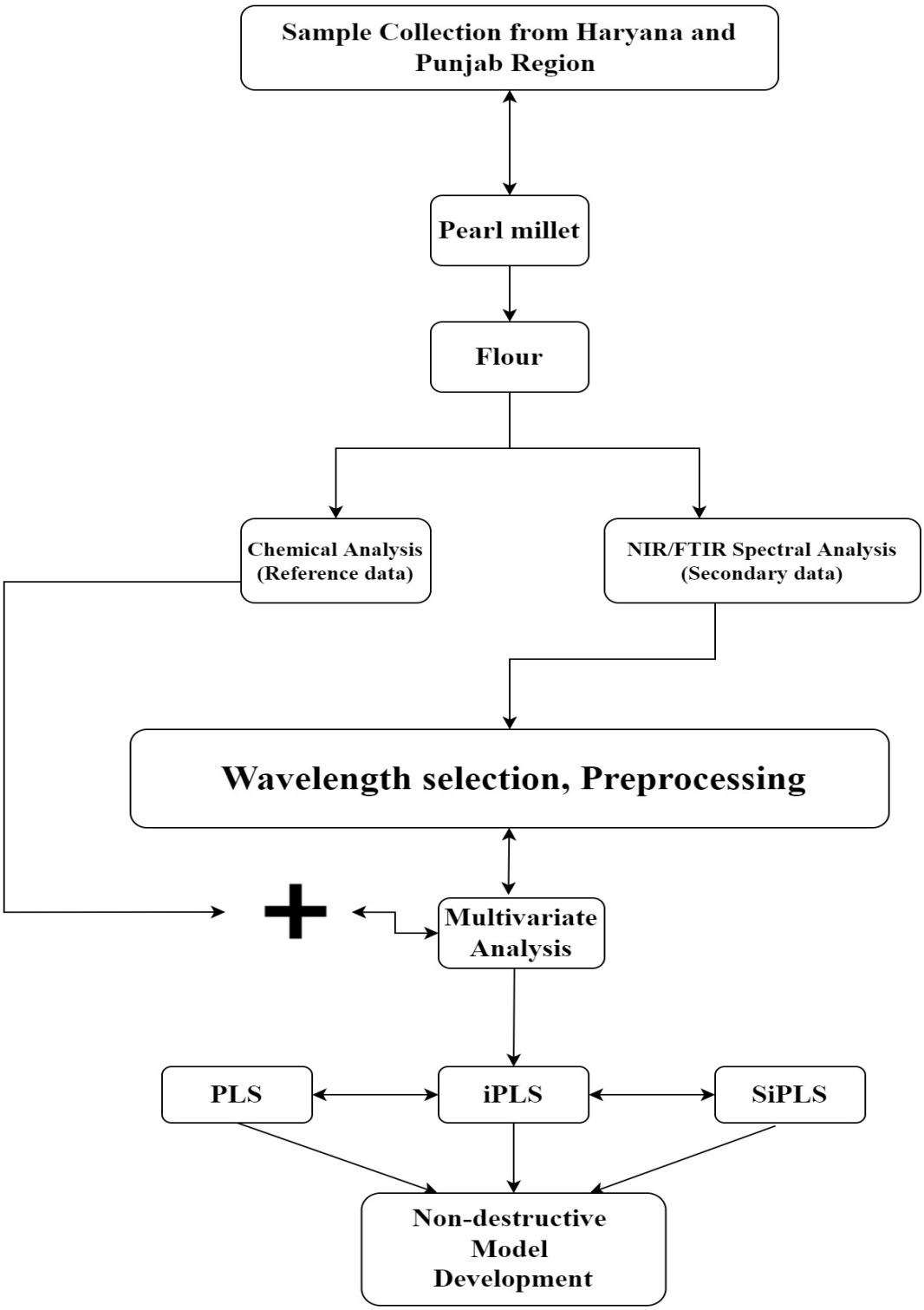

Figure 5.1 The work flow of nondestructive model development

5.2 Materials and Methods

5.2.1 Collection of pearl millet samples

In the year 2021, fifty samples of pearl millet were collected from local farmers of Haryana and Punjab state regions of India. The samples were thoroughly cleaned to remove foreign particles and broken grains and ground with AERO Mixer Grinder (500W) and sieved through a 40-mesh sieve to obtain a fine powder. Prior to further testing, the cleaned samples were kept in airtight polythene bags and refrigerated at 4°C. Samples of pearl millet were brought to room temperature for the further chemical and spectral analysis to ensure a consistent temperature.

5.2.2 Physico-chemical parameters

The chemicals required, sulfuric acid (98%), sodium hydroxide solution (45%), boric acid, and petroleum ether (60-80°C) for the reference analysis. The parameters named as, carbohydrates, crude protein, crude fiber, moisture, crude fat and ash, were evaluated by AOAC method brief discussed in previous chapter. All the data were collected in triplicate.

5.2.3 Acquisition of Spectral data

The NIR spectra of pearl millet samples of flour were collected from FOSS NIR DS-2500 spectrometer with VISION software and a circular sample holder were used in reflection mode from 400-2500 nm (@CSIR-CSIO Chandigarh, India). NIR spectrum was obtained at an interval of 0.5 nm in triplicate. Lambert Beer's law inbuilt in the VISION software was used to determine the absorbance of the spectra from the average number of scans.

The FTIR spectra collected by Shimazu 8400S spectrophotometer with diamond ATR and IRSOLUTION software (@LPU, Jalandhar, India). The spectra were collected in transmittance mode from 4000 cm^{-1} to 500 cm^{-1} at 100 cm^{-1} intervals for each sample. Spectra was recorded at room temperature.

5.2.4 Statistical analysis

The complexity in the spectral data was due to the fundamental, overtones and combinations of vibration bonds present in the pearl millet samples. To reduce this complexity in data the multivariate statistics techniques were used on the spectral data. Prior to that, the chemical data for all samples were maximum normalized and used as a primary data whereas, IR data were employed as secondary data. The multivariate analysis was carried on using chemometric software (The Unscrambler X version 10.5.1; CAMO Software AS, Oslo, Norway). The various types of the preprocessing and their combinations were used in the analysis. The various number of preprocessing and there combinations namely, SGS, SNV, MSC, first, second and fourth-order derivatives, DT, MN was used in the analysis. Data set was divided into calibration and prediction sets in the ratio of 4:1. To develop the NIR and MIR regression models, the chemometrics approach named as PLS regression method was used. According to the different parameters, the developed models were analyzed on the basis of R^2_C, R^2_V and RMSEC, RMSEV.

5.3 Results and Discussion

5.3.1 Chemical Analysis and statistics

Pearl millet is cherished for its high nutritional compositions. In the present study, the physicochemical compositions for all 50 samples were evaluated by using AOAC standard methods and these data were used as a reference method and in compositions moisture, ash, crude fat, crude protein, crude fiber and carbohydrates content were determined. The moisture content was lying in between 5.19 to 14.83%, ash content was in between 1.48 to 1.88%, crude fat in 3.64 to 6.56%, crude protein was in 5.56 to 11.95%, crude fiber was in between 1.55 to 4.50% and carbohydrate content was lying in between 66.53 to 79.46%. The descriptive statistics represented as mean, standard error, standard deviation, minimum and maximum values for all samples were employed in calibration and validation set represented in Table 5.1. The crude fat, crude protein, crude fiber and carbohydrates were

observed highest i.e. 6.56, 4.50, 11.95 and 79.64% respectively. Results were according to study on flours of pearl millet carried out to find the physiochemical and antioxidant activity.[19,20] The carbohydrates content shows the highest standard deviation 2.34 and lowest deviation were seen in ash content of 0.10. The broad range of moisture, crude protein and carbohydrates were helpful to obtain the precise results from calibration model and evaluation of performance of the PLS model built on the basis of various spectroscopic techniques.

Table 5.1 Descriptive statistics summary of chemical compositions of 50 (pearl millet) samples extracted by standard methods

Chemical Composition (%)	Mean	Standard Error	Standard Deviation	Minimum	Maximum	No. of Samples
Moisture	9.85	0.25	1.76	5.19	14.83	50.00
Ash	1.71	0.01	0.10	1.48	1.88	50.00
Fat	5.23	0.08	0.57	3.64	6.56	50.00
Crude Fiber	2.48	0.09	0.66	1.55	4.50	50.00
Crude protein	7.83	0.20	1.40	5.56	11.95	50.00
Carbohydrates	72.90	0.33	2.34	66.53	79.46	50.00

5.3.2 Spectral characteristics

In the present study, the physicochemical properties were used as a reference data and with the help of chemometric PLS regression, the statistical model for pearl millet were

developed with spectroscopic approach for prediction of different parameters. The mid infrared spectrum contained the fundamental bands with four regions named as fingerprint region that lying in between 1500-600 cm^{-1}, double bond region present in 2000-1500 cm^{-1}, triple bond in 2500-2000 cm^{-1} and X-H bonding region presented in between 4000-2500 cm^{-1}.[21,22] Whereas, interaction in between NIR radiation and pearl millet flour sample give rise the overtones and combination bands of X-H group due to presence molecules in organic compounds, which cause absorption bands at specific wavelengths. The FTIR and NIR vibrational spectroscopy contained the fundamental as well as overtones and combination bands present in the organic samples to determine the molecular structure of compounds.

FTIR spectra consist of fundamental bands at 3269 cm^{-1}, 2923 cm^{-1}, 1645 cm^{-1}, 1542 cm^{-1} 1412 cm^{-1} and 1000 cm^{-1} with bond vibration of O−H stretching (broad band), CH_2 group (alkanes), N-H stretching (Amides), N−H stretching (Amides), CH_3 bending{COOH(carboxyl) group}, C−O stretching (Alkene).[23,24] When the NIR radiation interact with the pearl millet sample, the overtones and combinational bonds shown that present in the pearl millet at the particular wavelengths. Absorption bands were observed at the wavelength 1225 nm, 1450 nm, 1725 nm, 1900 nm, 2100 nm, 2310 nm, 2380 nm and 2488 nm. These wavelength corresponds to C−H second overtone, O−H stretch first overtone, C−H stretch first overtone, C=O stretch second overtone, O−H bend/C−O stretch combination, C−H bend second overtone and C−H stretch/C−C stretch combination.[23] The NIR spectrum is difficult to visualize interpretation due to overlapping of peaks, overtones and combinational vibrational bands present in pearl millet. Therefore, chemometric study is necessary to understand the complicated nature of the NIR spectra and identify their relationship with the quality attributes.

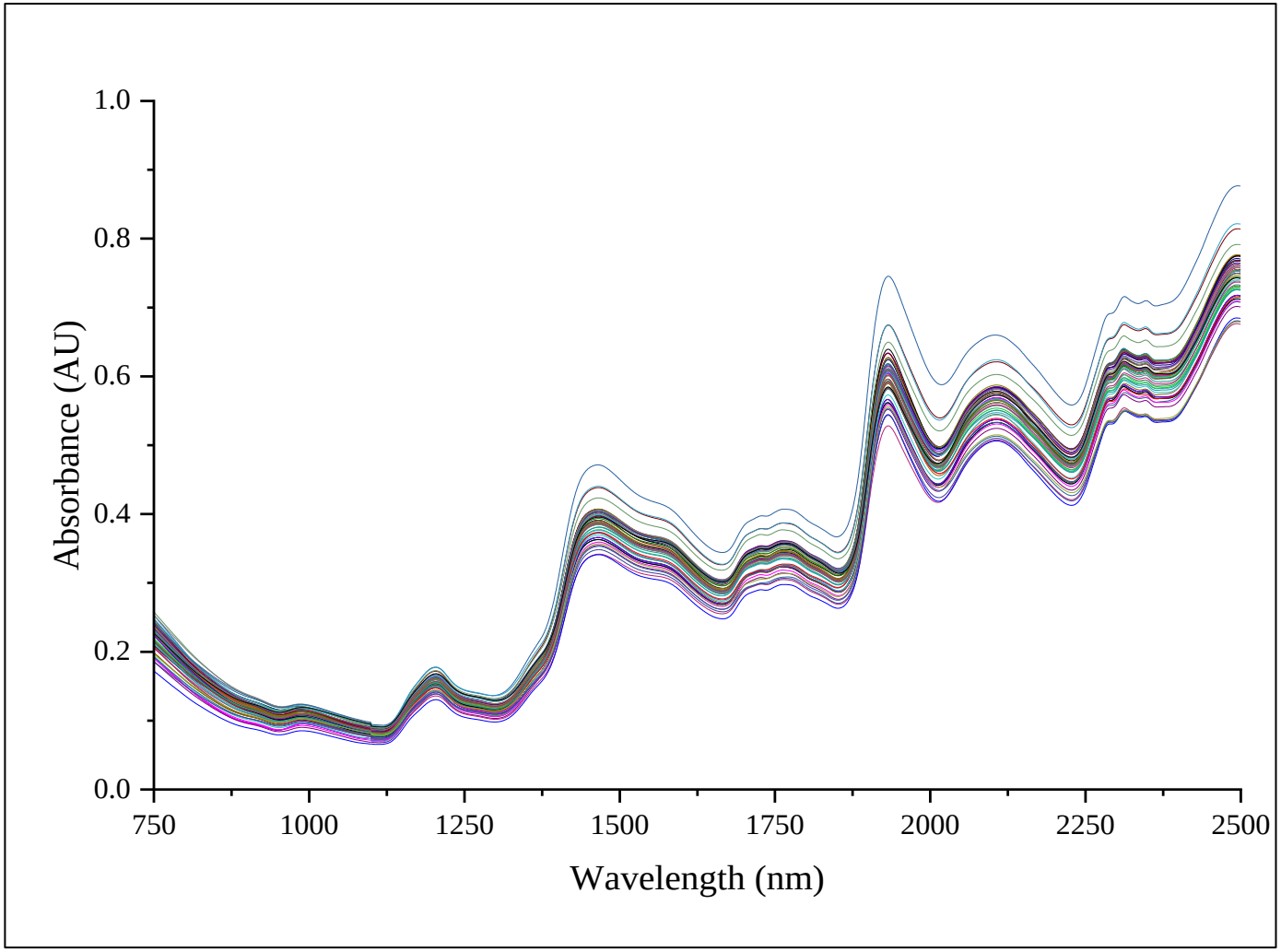

Figure 5.2 The raw average near infrared spectra of all pearl millet samples

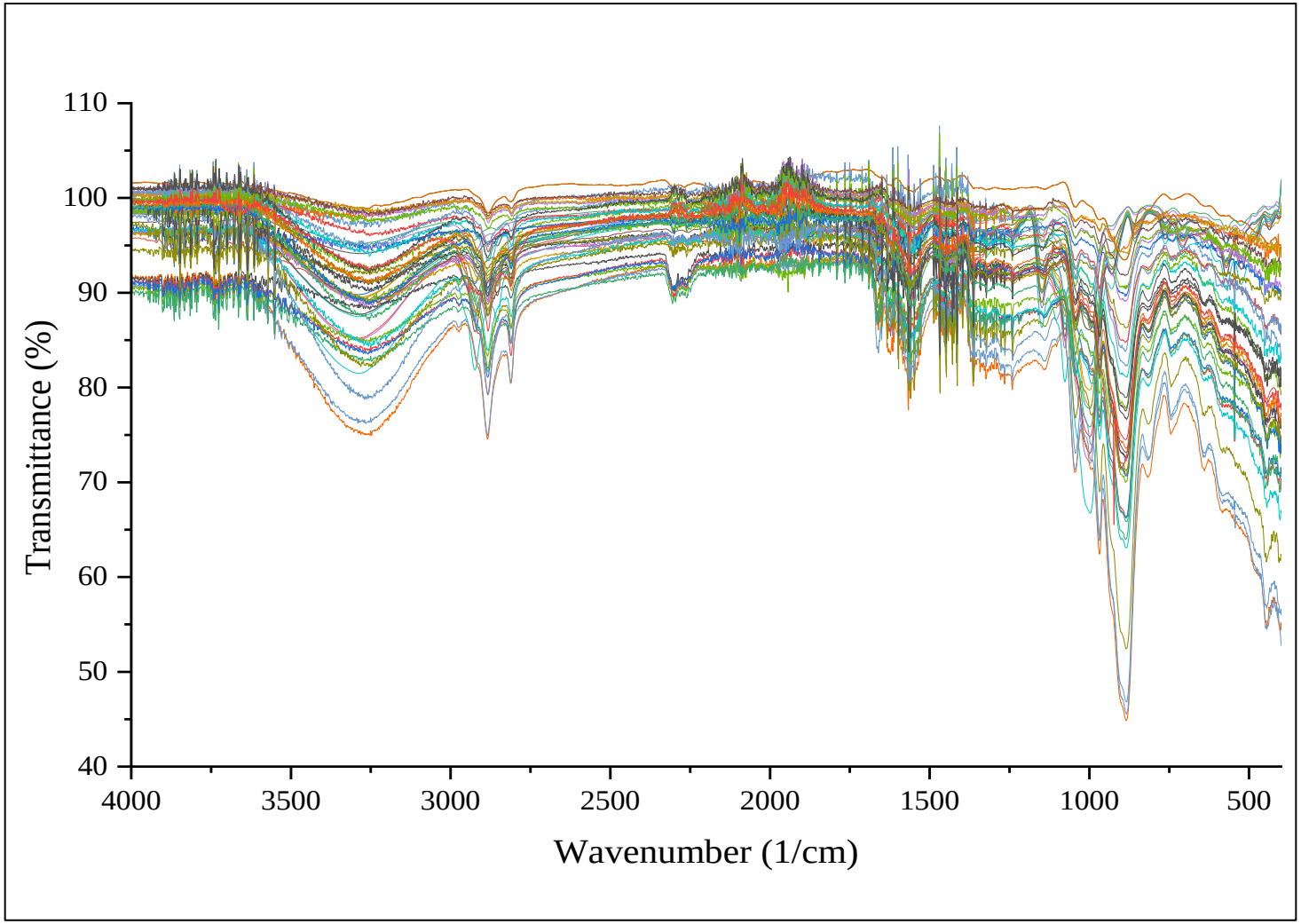

Figure 5.3 A typical Fourier transform infrared spectra of pearl millet

The raw NIR spectra of all samples is represented in Figure 5.2 and the average FTIR spectra were obtained in transmittance mode for all samples is shown in Figure 5.3. Visual interpretation of the NIR spectrum is challenging due to its extreme complexity. However, chemometric approaches could be used to obtain the molecular information from NIR spectra.

5.3.3 Chemometrics

Preprocessing

During the spectra acquisition, the unwanted background information and noise could be introducing. To avoid these unwanted background information and noise signals, the pretreatment or pre-processing on the spectral data is required also it enhances the chemical information signals.[5] In the present study, the popular pre-processing and combinations include smoothing, first derivate, second derivative (Savitzky–Golay derivative), normalized, SNV, MSC, DT and derivatives were used on spectral data before development of calibration model suggested by previous studies.[25,26]

The various pretreatment and their combinations were used to reduce the various types of chemical interference as well as physical variations.[7,27] In the preprocessing, DT aims to eliminate nonlinear patterns from spectroscopic data. Combining DT with SNV reduces multicollinearity, baseline shift, and curvature.

MSC preprocessing was first introduced by Martens et al. in 1983 and further elaborated on by Geladi et al. in 1985. MSC is preprocessing technique which is most widely used for NIR spectrum to remove the undesirable scattered effect from the data matrix. In order to decrease light scattering and eliminate baseline offsets, MSC and SNV have been widely employed[5,7,27,28] as shown in Figure 5.4 and 5.5. The preprocessing methods are mostly used in NIR spectral data to enhance the accuracy of calibration and classification model and force to obey the lambert –Beer's law. The best spectral pre-processing and regression algorithms are often chosen by trial and error.

The 2nd and 4th order derivatives shown in Figure 5.6 and Figure 5.7 were used to correct the baseline shifts in spectra for aimed to eliminate nonchemical effects and build reliable calibration models as well as differentiate overlapping peaks which can enhance the understanding of spectral data.

It was observed that the samples are being divided region wise in four groups this shown in Figure 5.8. No classification could be observed in preprocessed NIR spectra, but same was visible in 4th derivative preprocessed NIR data. This was observed because the raw spectral absorbance differences for the samples from different regions was very small to classify the same, but application of derivative helped in highlighting the variances among them. The upper left group was defined for Haryana and Punjab region samples and upper right group represent the Punjab region samples whereas, various district of Haryana region also divided in two groups lower left and right shown in Figure 5.8. These samples were divided into groups because they were collected from different locations of Haryana. Therefore, after applying the preprocessing, it was observed that classification of pearl millet samples can be possible according to their region.

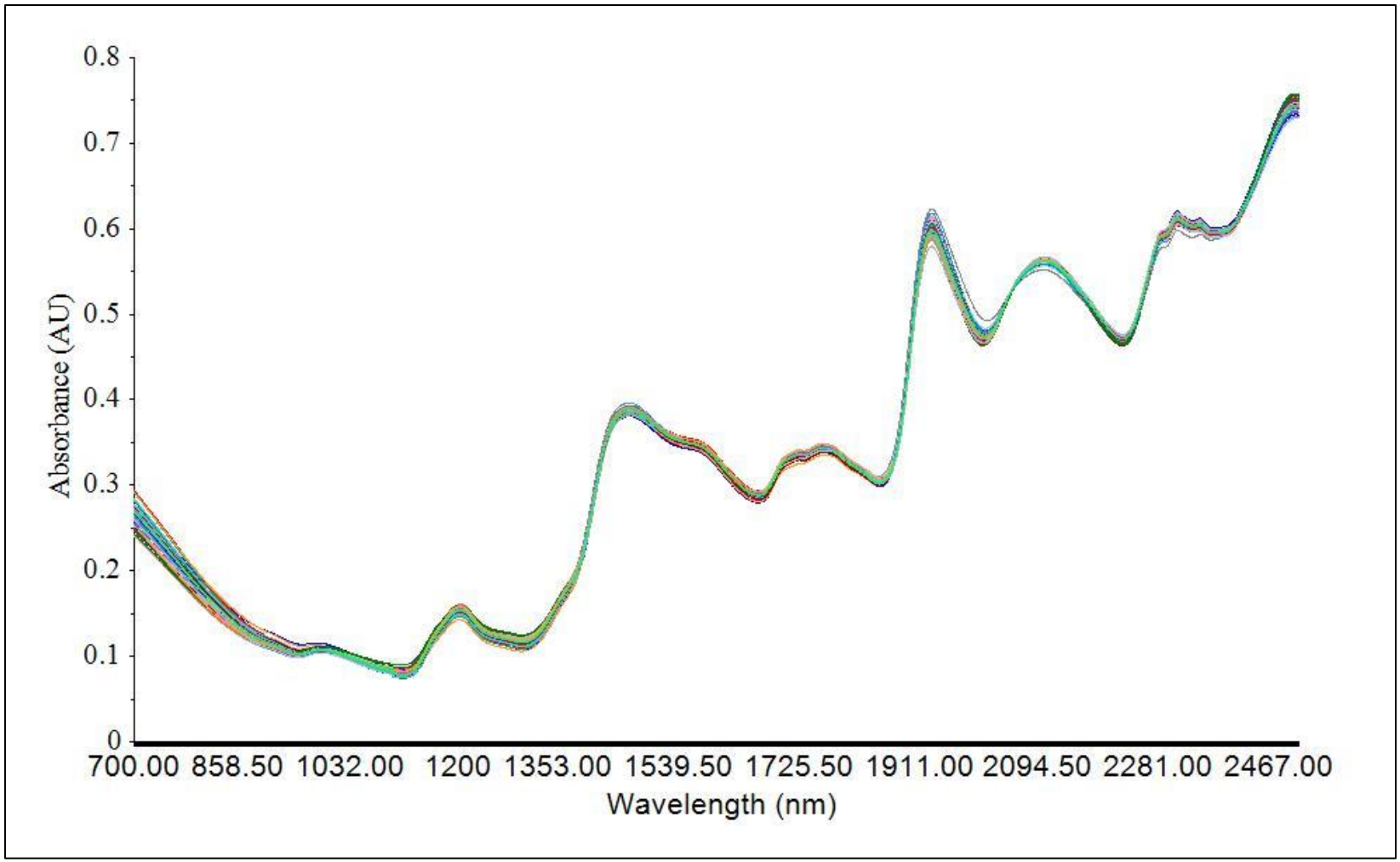

Figure 5.4 The pearl millet spectra treated by MSC

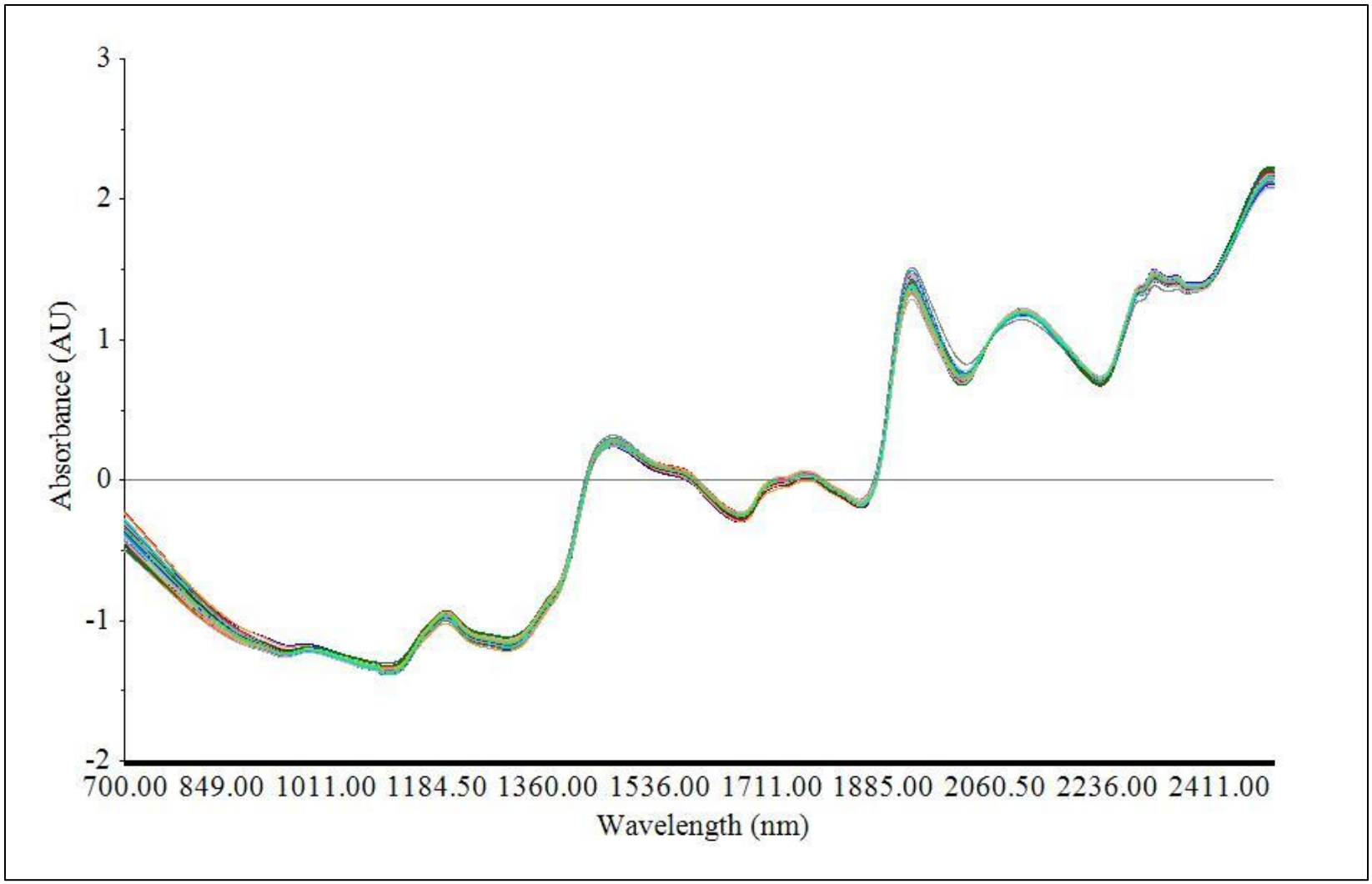

Figure 5.5 The pearl millet spectra treated by SNV

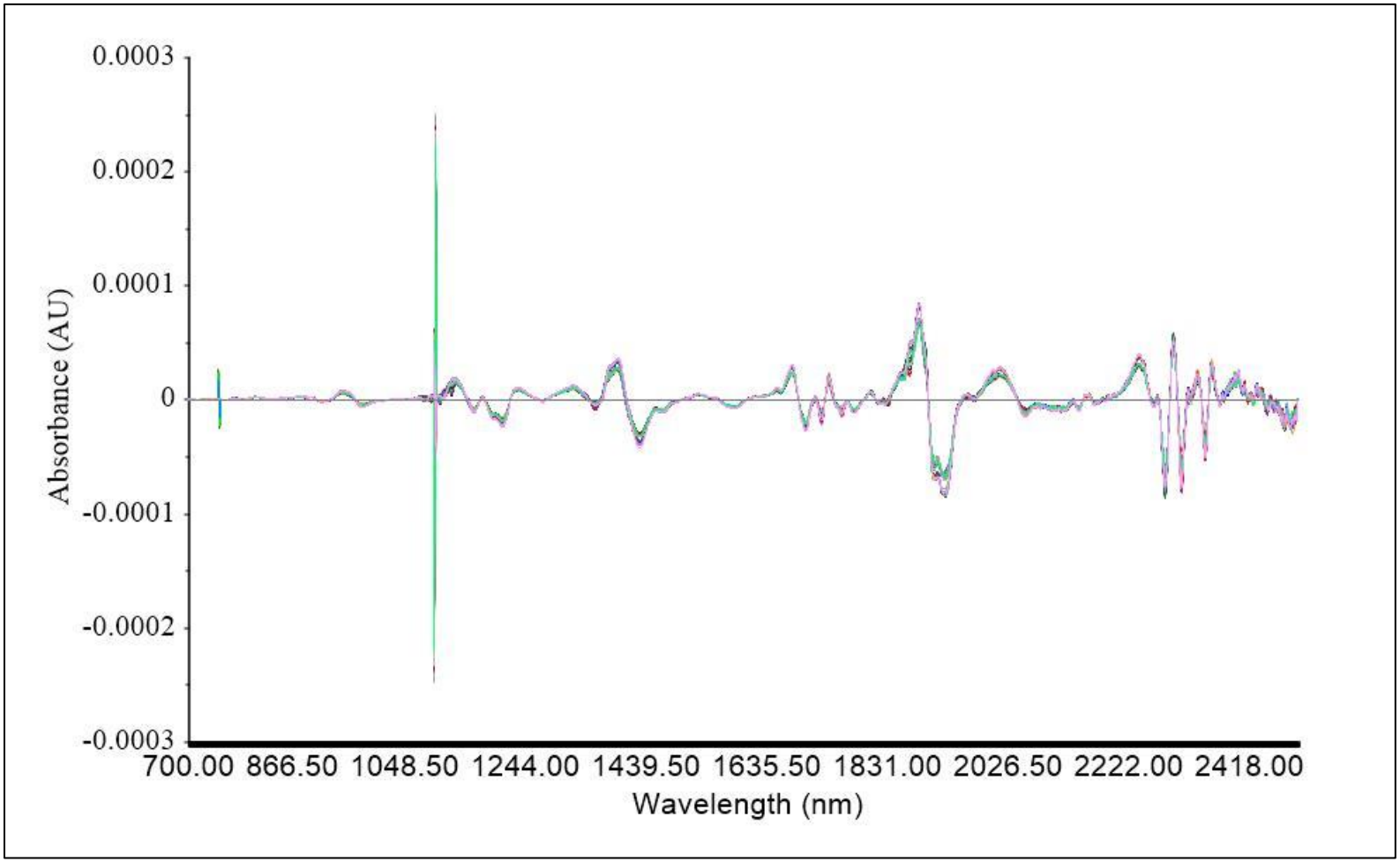

Figure 5.6 The pearl millet NIR spectra treated by second order derivatives (Savitzky–Golay derivative)

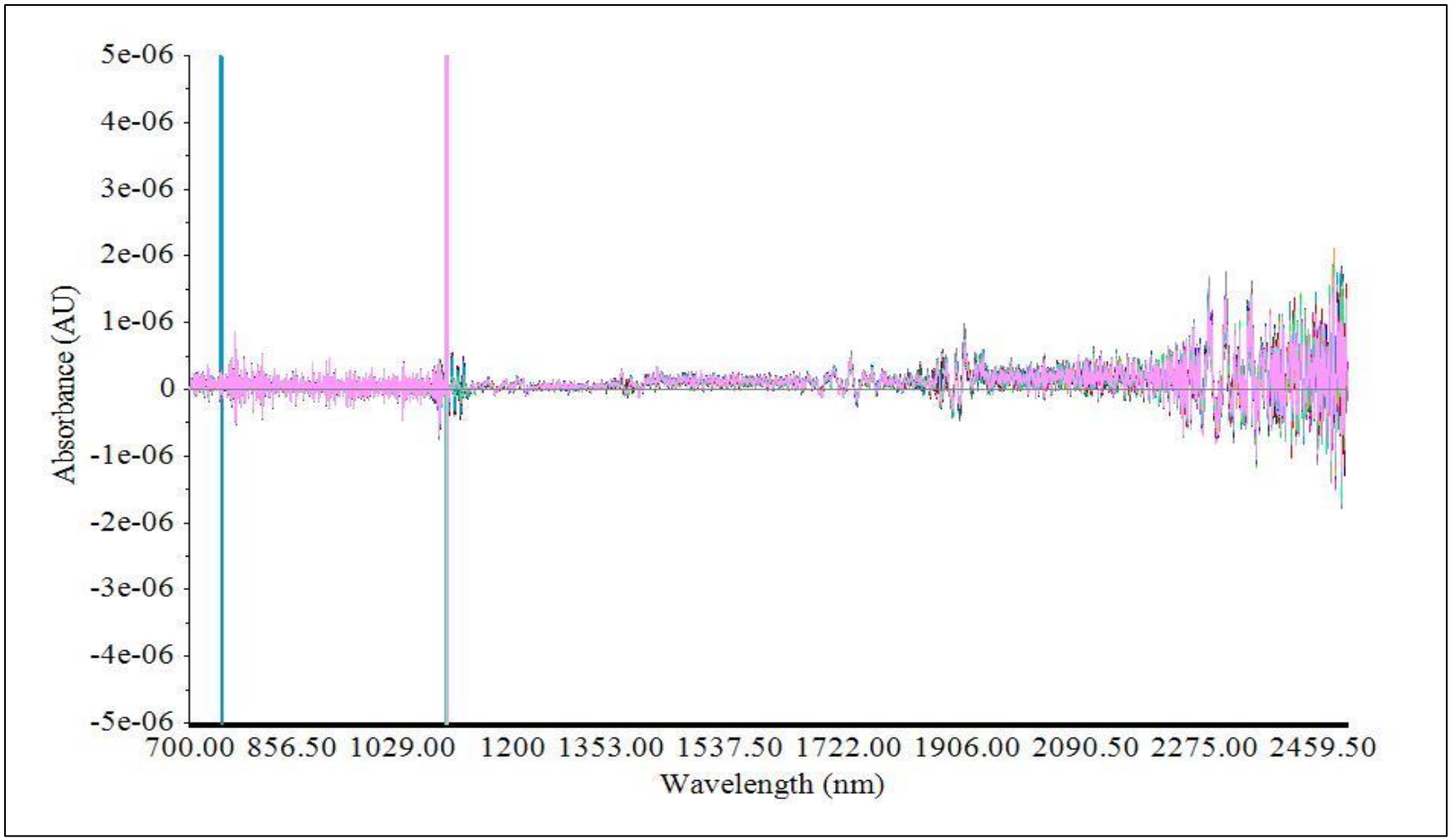

Figure 5.7 The pearl millet NIR spectra treated by fourth order derivatives (Savitzky–Golay derivative)

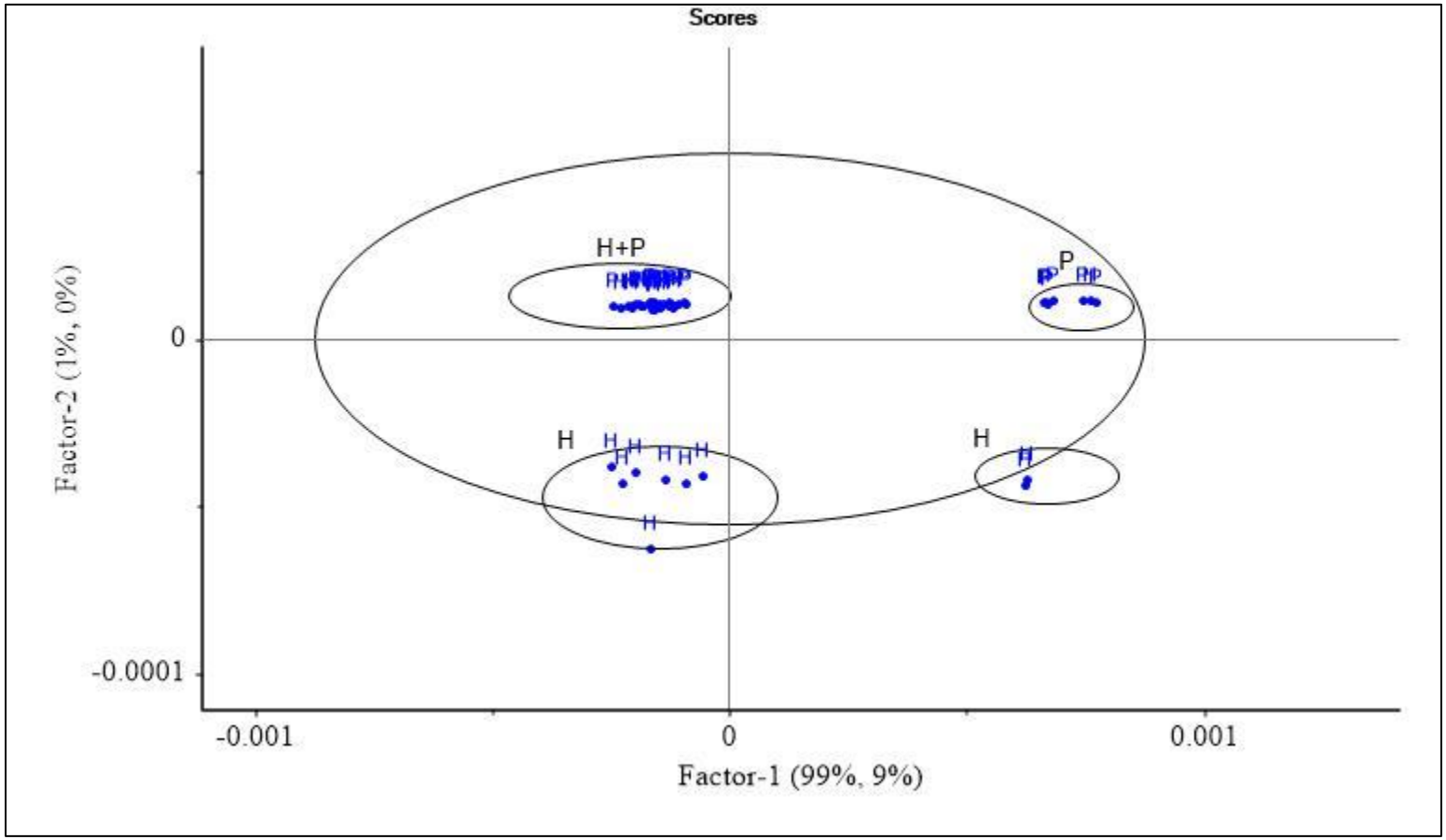

Figure 5.8 Principal component analysis (PCA) classification of pearl millet samples using preprocessed (fourth order derivatives) NIR spectra

Model development

The spectral data and the properties of selected samples are both correlated. This correlation was determined by using multivariate calibration techniques.[28] In present study, Partial least square regression (PLS), interval (iPLS) and synergy (SiPLS) were employed with the various combinations of preprocessing on the NIR as well as FTIR spectral data. PLS consist of full range of spectral data, whereas iPLS exhibits the spectra divided into several group with equal intervals. The combination of two or more intervals with low error were used in the synergy iPLS.[29–31]

Partial Least Square (PLS)

In the multivariate statistical regression methods, PLS regression is very popular technique to determine the correlation in between dependent and independent variables [32–34]. The calibration model mainly identified by R^2_C that evaluate the quality to fit and RMSEC. It was observed from the literature, for the excellent model the coefficient of determination should be greater than 0.91 whereas, good model obtained when R^2_C values in between 0.66-0.91 and models with R^2_C values between 0.50-0.65 are capable of performing a discriminating analysis between high and low values.

Spectral data were processed by various preprocessing techniques and the combinations of preprocessing applied on NIR raw pearl millet spectra shown in Table 5.2 and Table 5.3. The PLS regression model were developed for the physicochemical properties of pearl millet and its performance was assessed by the coefficient of determination (R^2) and root-mean square error (RMSE) for calibration and validation sets. The R^2 and RMSE values present in the all compositions in pearl millet were reported for calibration and validation set using NIR and MIR spectra are reported in Table 5.2. In the PLS regression model the value of R^2_C for moisture in range from 0.68 to 0.93, crude fat 0.41 to 0.93, crude protein 0.27 to 0.91 crude fiber 0.24 to 0.94 and carbohydrates 0.60 to 0.94 for NIR PLS model and R^2_C for moisture lies in between of 0.67 to 0.99, crude fat 0.41 to 0.90, crude protein 0.53 to 0.96, crude fiber 0.53 to 0.97 and carbohydrates 0.45 to 0.99 for FTIR regression

model. The PLS NIR regression model developed with maximum value of R^2_C for moisture, crude fat, crude protein, crude fiber and carbohydrates was 0.93, 0.93, 0.91, 0.88, 0.94 respectively also predicted and reference values are represented in Figure 5.9 – Figure 5.13.

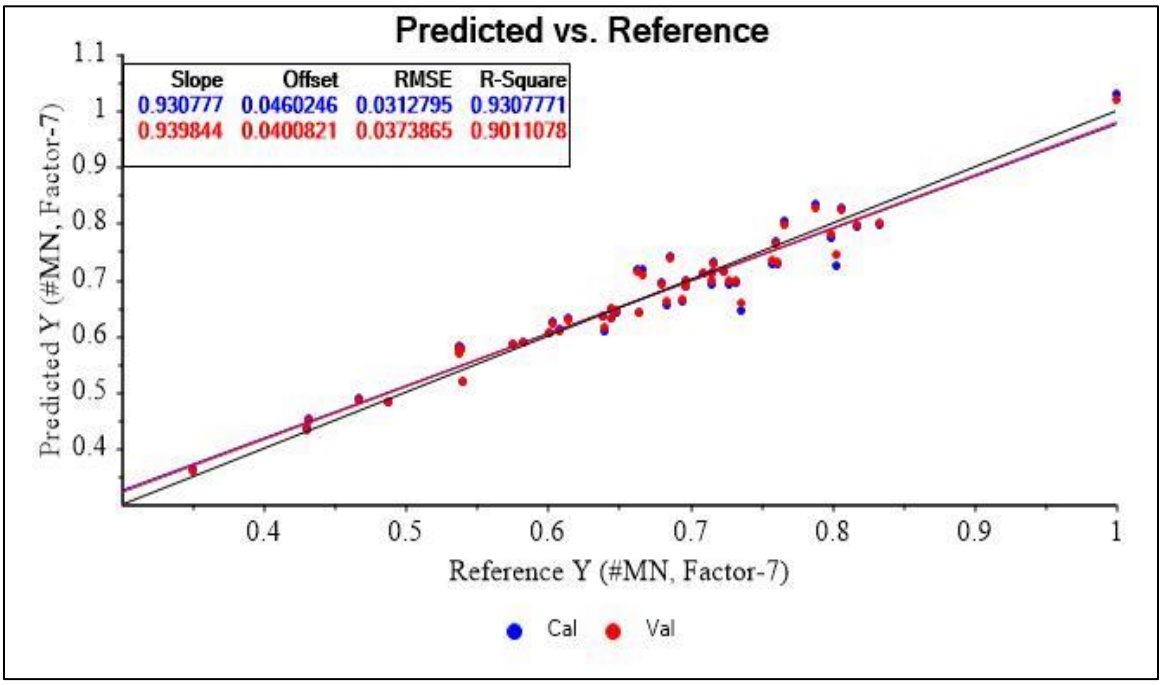

Figure 5.9 Reference and Predicted NIR values for moisture using PLS regression.

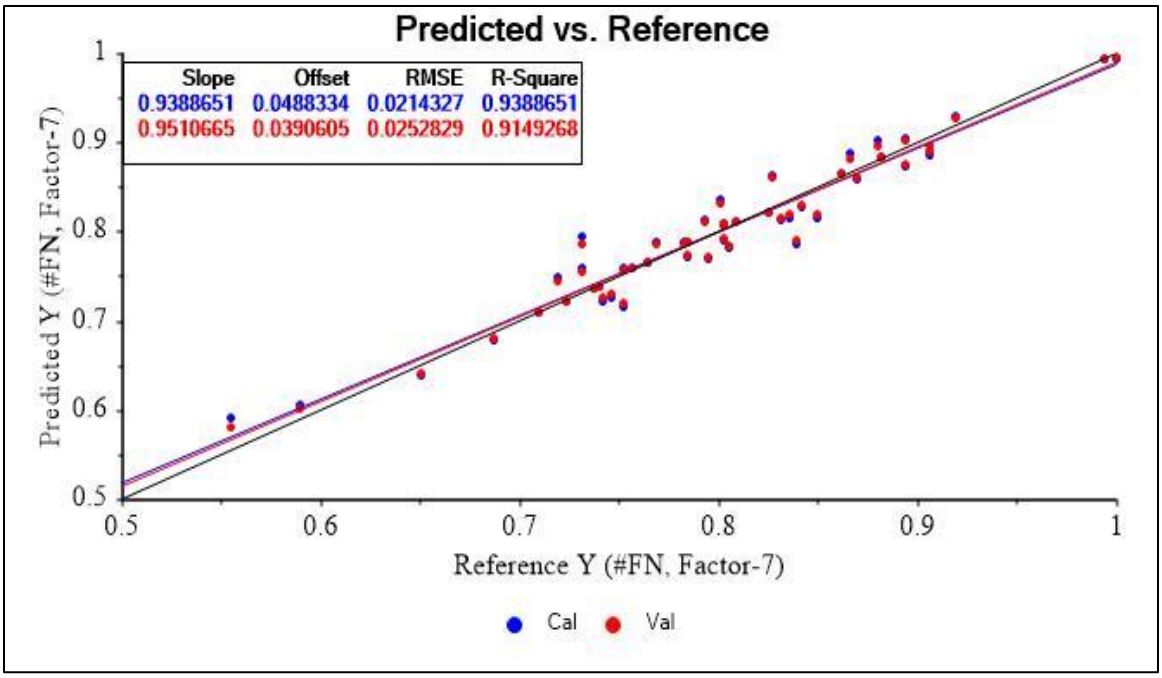

Figure 5.10 Reference and Predicted NIR values for crude fat using PLS regression.

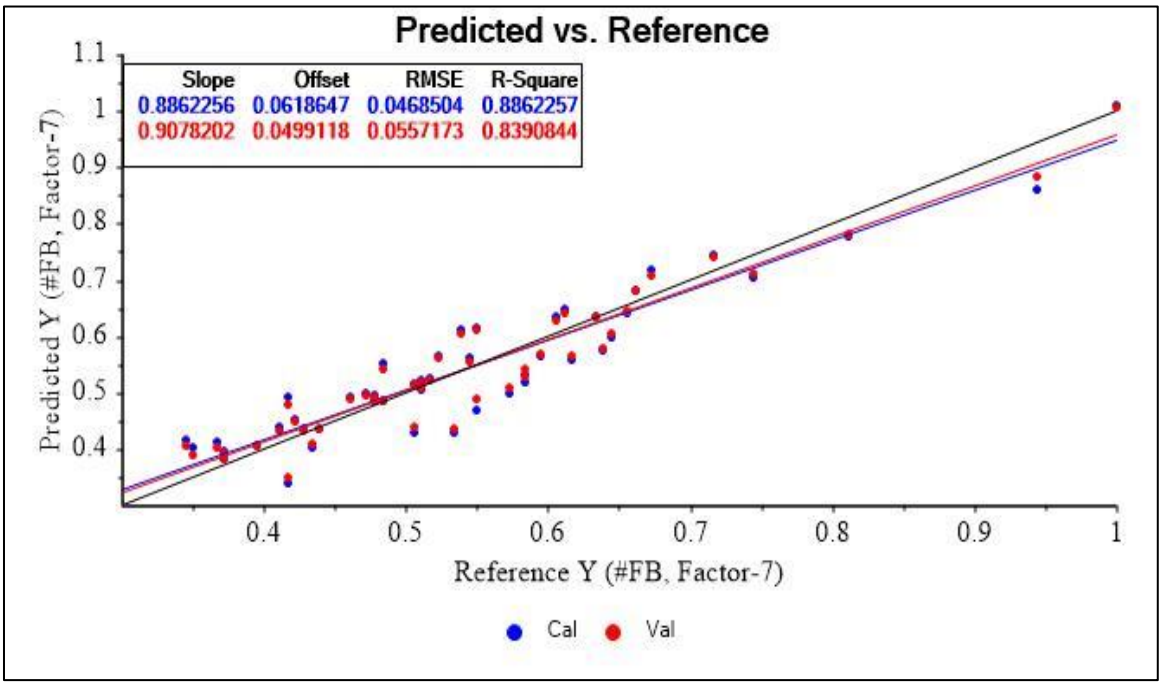

Figure 5.11 Reference and Predicted NIR values for crude fiber using PLS regression.

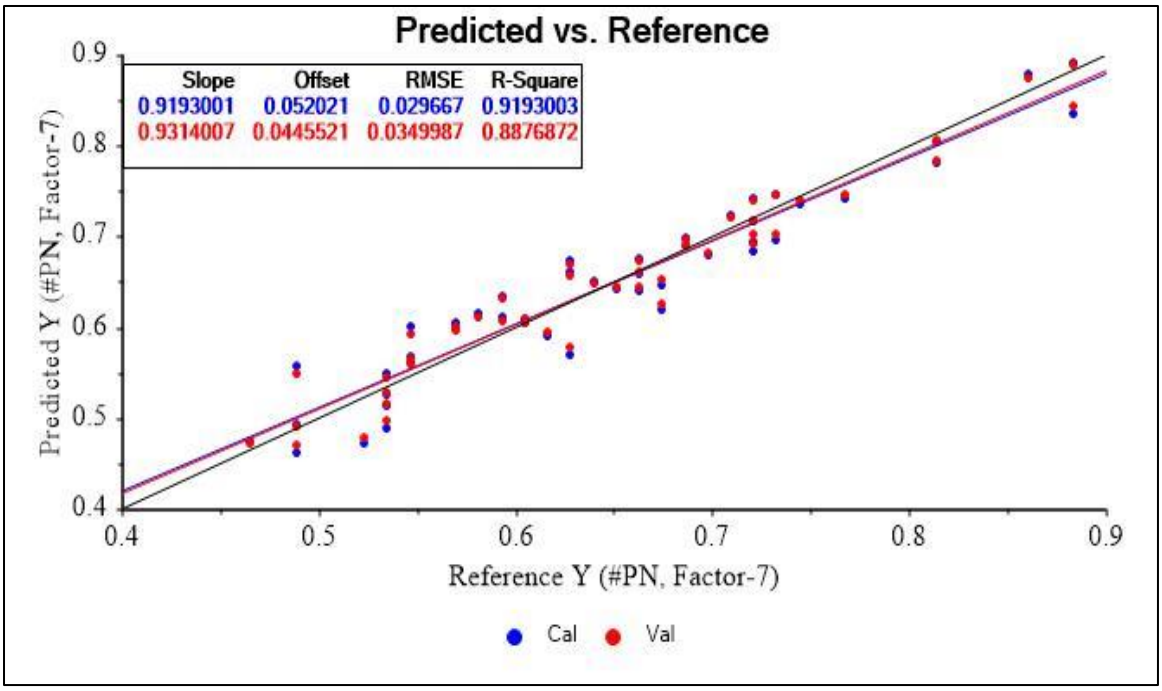

Figure 5.12 Reference and Predicted NIR values for crude protein using PLS regression.

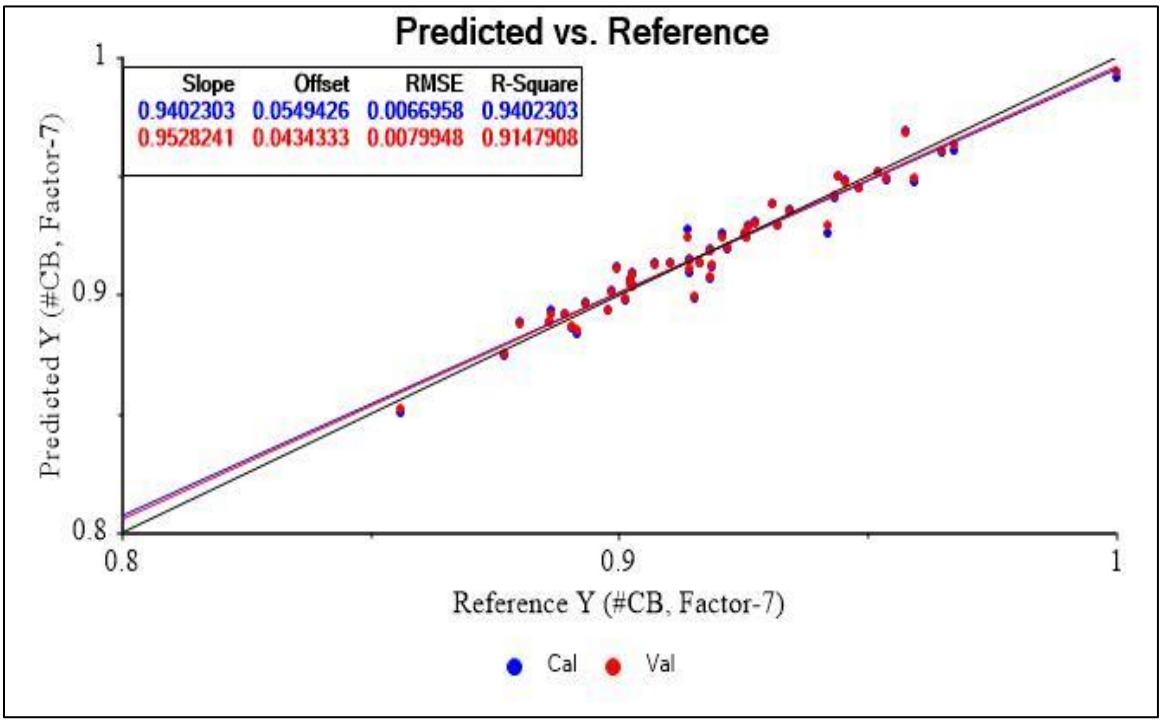

Figure 5.13 Reference and Predicted NIR values for carbohydrates using PLS regression.

Table 5.2 Statistical analysis of PLS regression model in all range of wavelength using NIR and FTIR spectroscopy for evaluation of proximate compositions in pearl millet

Pre-processing		Calibration										Validation									
		Moisture		Crude fat		Crude Protein		Crude Fiber		Carbohydrates		Moisture		Crude fat		Crude protein		Crude fiber		Carbohydras	
		R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE
Smoothing +1DV	NIR	0.77	0.05	0.54	0.05	0.51	0.07	0.33	0.11	0.68	0.015	0.68	0.06	0.34	0.07	0.30	0.08	NA	0.13	0.56	0.018
	FTIR	0.98	0.012	0.90	0.02	0.95	0.02	0.97	0.02	0.99	0.002	0.98	0.014	0.87	0.03	0.95	0.02	0.96	0.02	0.98	0.003
SNV	NIR	0.68	0.06	0.41	0.06	0.29	0 .08	0.24	0.12	0.60	0.017	0.61	0.07	0.15	0.07	NA	0.10	NA	0.15	0.43	0.020
	FTIR	0.67	0.06	0.41	0.06	0.53	0.07	0.53	0.09	0.49	0.019	0.44	0.08	0.20	0.07	0.31	0.08	0.29	0.11	0.35	0.021
1DV+SNV	NIR	0.83	0.04	0.56	0.05	0.52	0.07	0.39	0.10	0.75	0.013	0.76	0.05	0.39	0.06	0.30	0.08	0.04	0.13	0.65	0.016
	FTIR	0.97	0.01	0.87	0.03	0.95	0.02	0.95	0.02	0.98	0.003	0.95	0.02	0.81	0.03	0.92	0.02	0.93	0.03	0.96	0.005
De-Trending	NIR	0.69	0.06	0.41	0.06	0.27	0.08	0.24	0.12	0.62	0.016	0.60	0.07	0.12	0.08	NA	0.10	NA	0.15	0.46	0.019
	FTIR	0.82	0.05	0.64	0.05	0.72	0.05	0.78	0.06	0.83	0.011	0.74	0.06	0.48	0.06	0.58	0.06	0.68	0.07	0.76	0.013
1DV+DT	NIR	0.78	0.05	0.52	0.05	0.46	0.07	0.34	0.11	0.70	0.014	0.70	0.06	0.30	0.07	0.24	0.09	0.04	0.13	0.59	0.017
	FTIR	0.90	0.01	0.90	0.02	0.95	0.02	0.97	0.02	0.99	0.002	0.98	0.01	0.87	0.03	0.95	0.02	0.96	0.02	0.98	0.003
S+NM+1DV	NIR	0.90	0.03	0.77	0.04	0.85	0.04	0.72	0.07	0.89	0.008	0.85	0.04	0.67	0.04	0.79	0.04	0.59	0.08	0.86	0.010
	FTIR	0.99	0.01	0.9	0.02	0.96	0.02	0.96	0.02	0.99	0.002	0.98	0.013	0.88	0.02	0.95	0.02	0.95	0.02	0.98	0.002
S+NM+1DV+ SNV	NIR	0.83	0.04	0.56	0.05	0.59	0.06	0.35	0.11	0.73	0.014	0.77	0.05	0.38	0.06	0.39	0.08	NA	0.14	0.62	0.016
	FTIR	0.97	0.01	0.87	0.03	0.95	0.02	0.95	0.02	0.98	0.003	0.95	0.02	0.81	0.03	0.92	0.02	0.93	0.03	0.96	0.005
2DV	NIR	0.90	0.03	0.80	0.03	0.74	0.05	0.74	0.07	0.90	0.008	0.87	0.04	0.72	0.04	0.64	0.06	0.62	0.08	0.87	0.009
	FTIR	0.98	0.01	0.89	0.02	0.96	0.01	0.97	0.02	0.99	0.002	0.98	0.01	0.87	0.03	0.95	0.02	0.96	0.02	0.98	0.003
1DV+MSC	NIR	0.80	0.05	0.55	0.05	0.46	0.07	0.35	0.11	0.71	0.014	0.73	0.06	0.37	0.06	0.21	0.09	0.01	0.13	0.61	0.017
	FTIR	0.67	0.06	0.44	0.06	0.55	0.06	0.63	0.08	0.69	0.015	NA	0.18	NA	0.18	NA	0.16	0.24	0.12	NA	0.043
4DV	NIR	0.93	0.03	0.93	0.02	0.91	0.02	0.88	0.04	0.94	0.006	0.90	0.03	0.91	0.02	0.88	0.03	0.83	0.05	0.91	0.007
	FTIR	0.98	0.01	0.89	0.02	0.96	0.01	0.96	0.02	0.99	0.002	0.98	0.15	0.86	0.03	0.95	0.02	0.95	0.02	0.98	0.003

Wavelength Selection

In the PLS regression, all range of wavelengths may contain unwanted chemical information which may reduce the efficiency of the calibration model. Therefore, specific wavelengths must be used in order to obtain precise results of model. The enhancement of model stability depends on the wavelength selection method by reducing the error. The equation of model for the parameters of pearl millet were developed by help of wavelength selection method.

$$Y = b_0 + \sum_{k=1}^{n} b_k X_k$$

Y represents for dependent variable (reference value), b_0 shows the intercept terms, b_k known for regression coefficient and X_k denotes for the independent variable (absorbance of spectral data).

The iPLS regression were used on equal intervals, by diving complete spectra in three groups with 600 nm gap, six groups with a gap of 300 nm, nine groups within gap 200 nm and eighteen groups with gap 100 nm each to collect the precise results. In the gap of 600 nm wavelength (Figure 5.14), the highest coefficient of determination for moisture, crude fat, crude fiber, crude protein and carbohydrate content was 0.95, 0.84, 0.84, 0.88 and 0.95 lied within 1900-2500 nm range respectively. In gap of 300 nm (Figure 5.15), the coefficient of determination lying in between 700-1000, 1000-1300, 1300-1600, 1600-1900, 1900-2200 and 2200-2500 for moisture, was 0.81, 0.73, 0.83, 0.77, 0.90, 0.93 for crude fat, 0.52, 0.42, 0.60, 0.45, 0.76, 0.83, for crude fiber, 0.81, 0.0006, 0.63, 0.36, 0.73, 0.76, for crude protein 0.41, 0.36, 0.74, 0.45, 0.82 and 0.86 and for carbohydrates 0.70, 0.66, 0.78, 0.68, 0.88 and 0.94 respectively. It was observed that the best coefficient of determination for moisture was lies in the range of 1300-1600 and 1900-2500 nm was chosen for NIR regression model development. Similarly, the best coefficient of determination for crude fat was lying in the range of 1900-2500 nm, crude protein was lying in between 1300-1600, 1900-2500 nm. Hence the best wavelengths for regression model for crude fiber and carbohydrates was 2200-2500 nm. Whereas in the gap of 200

and 100 nm, the intervals are 700-900, 900-1100, 1100-1300, 1300-1500, 1500-1700, 1700-1900, 1900-2100, 2100-2300 and 2300-2500 nm, and 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 1900-2000, 2000-2100, 2100-2200, 2200-2300, 2300-2400 and 2400-2500 nm respectively and coefficient of determination are shown in Figure 5.16- Figure 5.17. Similarly, the wavenumbers were selected at the gap of 500 and 1000 cm^{-1} within the intervals of 4500-4000, 4000-3500, 3500-3000, 3000-2500, 2500-2000, 2000-1500, 1500-1000, 1000-500 cm^{-1} and 4500-3500, 3500-2500, 2500-1500, 1500-500 cm^{-1} respectively. The coefficient of determination for moisture, crude fat, crude protein, crude fiber, carbohydrate content was in range of 0.93 to 0.98, 0.81 to 0.90, 0.92 to 0.97, 0.94 to 0.97, 0.89 to 0.99 respectively within gap of 1000 cm^{-1} for FTIR regression model are shown in Figure 5.18. It was observed that the coefficient of determination for moisture content lies in between 0.85-0.98, crude fat was lying in between 0.79 to 0.90, crude protein in between 0.87 to 0.97, crude fiber in between 0.85 to 0.97 and for carbohydrates present in between 0.88 to 0.99 within the gap of 500 cm^{-1} wavenumbers. The maximum value of R^2_C for moisture, crude fat, crude protein and carbohydrates was 0.98, 0.90, 0.97 and 0.99 within 2500-3500 cm^{-1} respectively and maximum R^2_C for crude fiber was 0.97 in range of 3500-4000 cm^{-1} as shown in Figure 5.19.

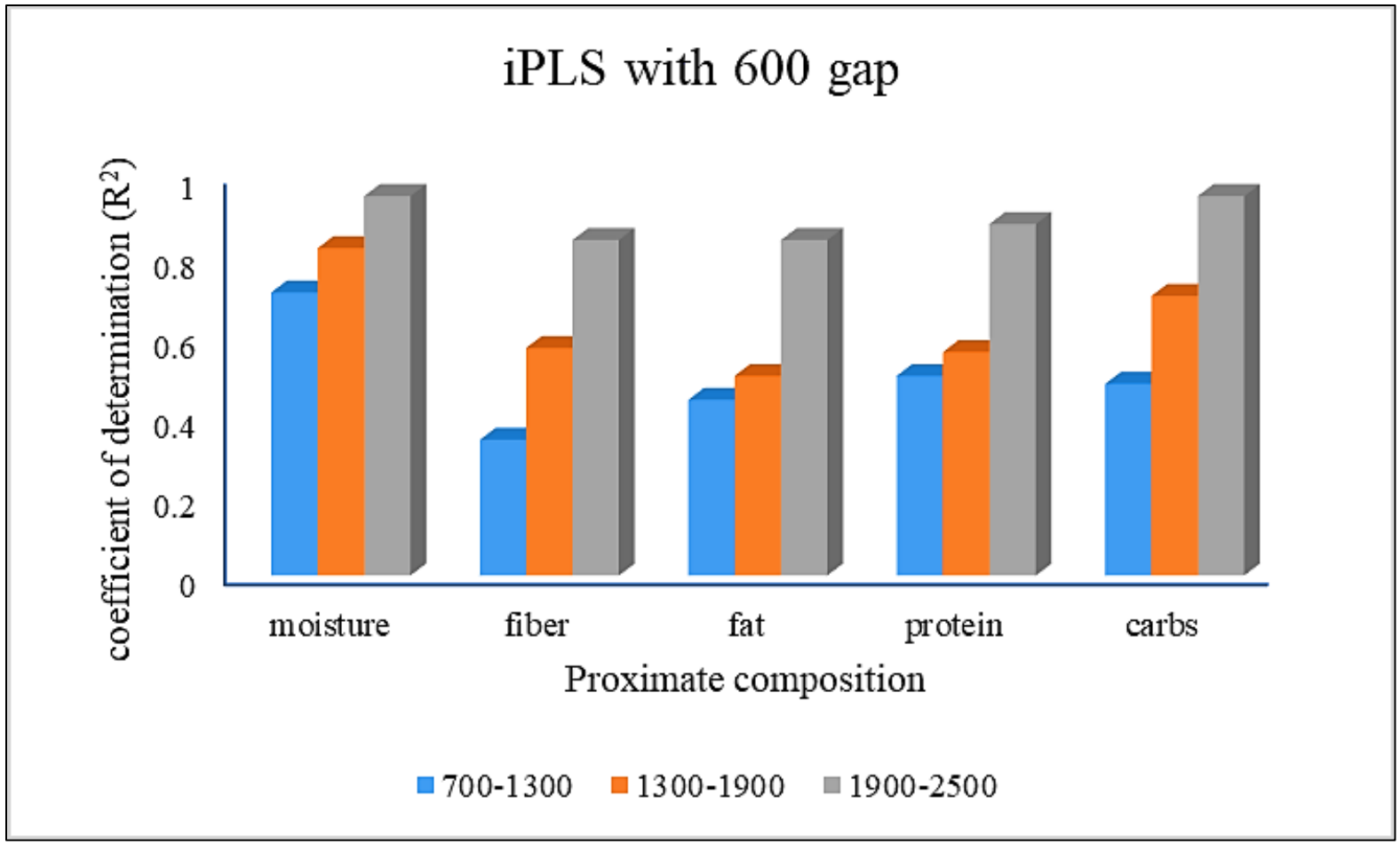

Figure 5.14 Bar graph plot using iPLS regression method within 600 gap for proximate composition present in pearl millet

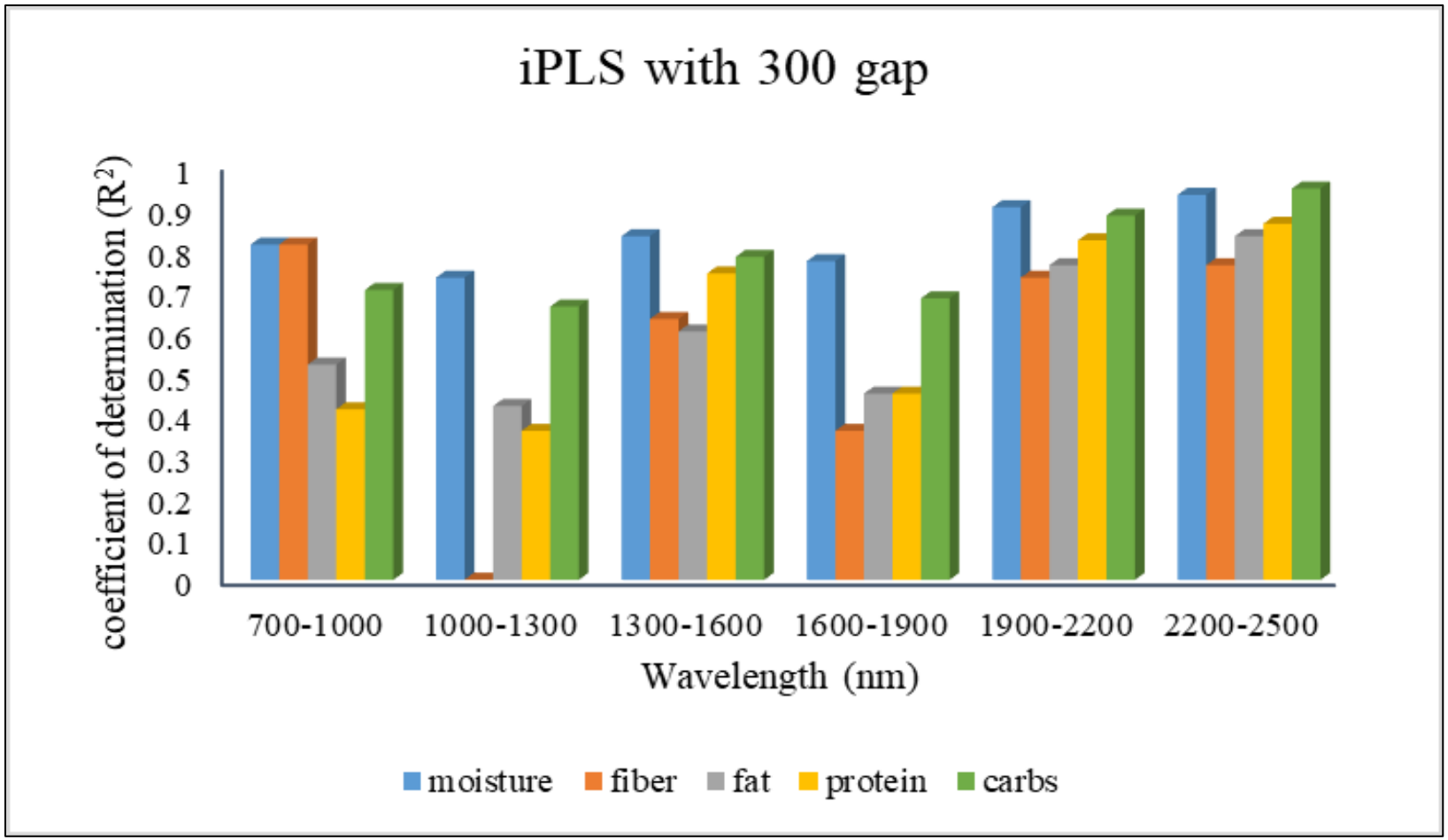

Figure 5.15 Bar graph using iPLS regression method within 300 gap for proximate composition present in pearl millet

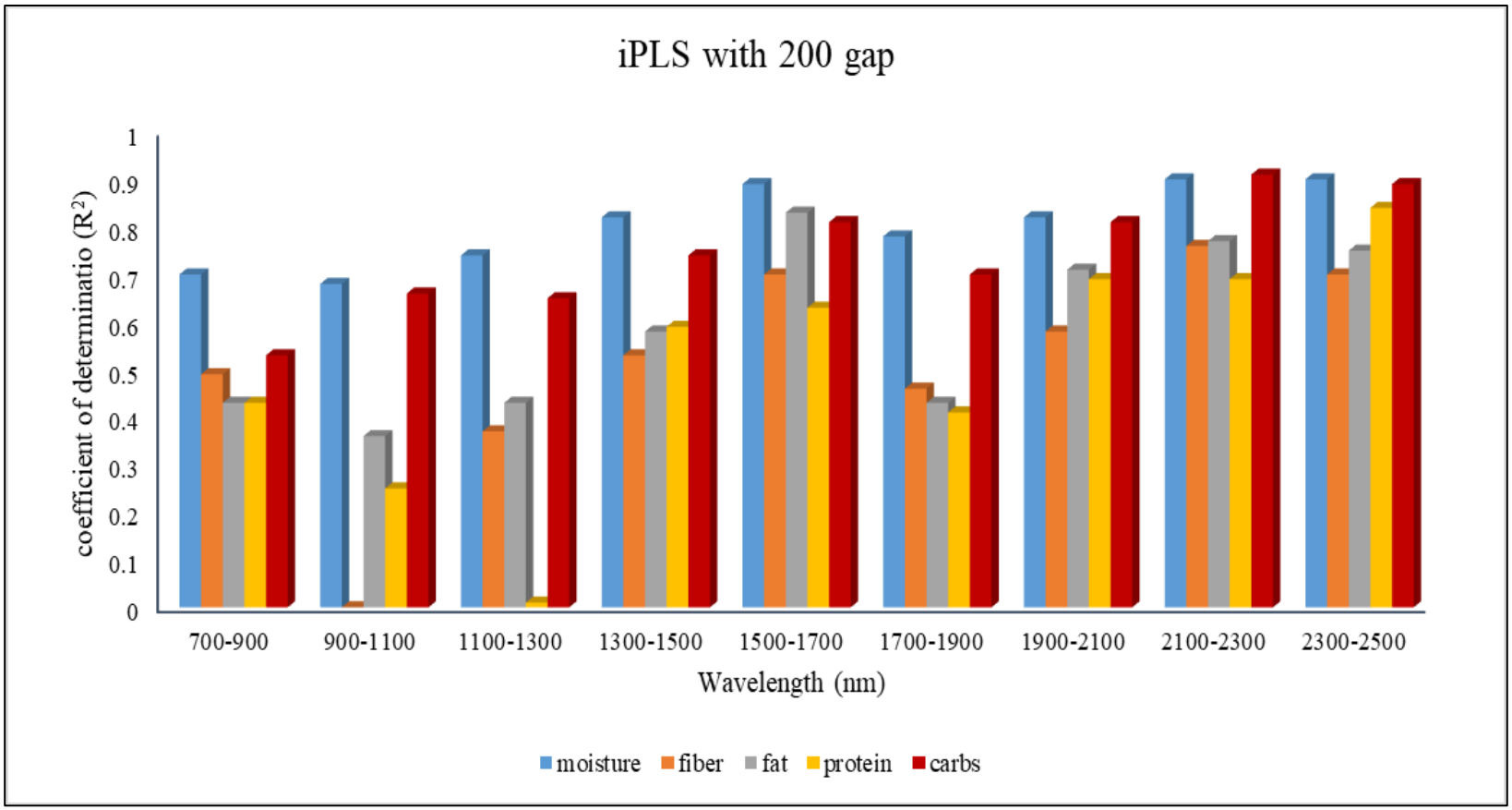

Figure 5.16 Bar graph using iPLS regression method within 200 gap for proximate composition present in pearl millet

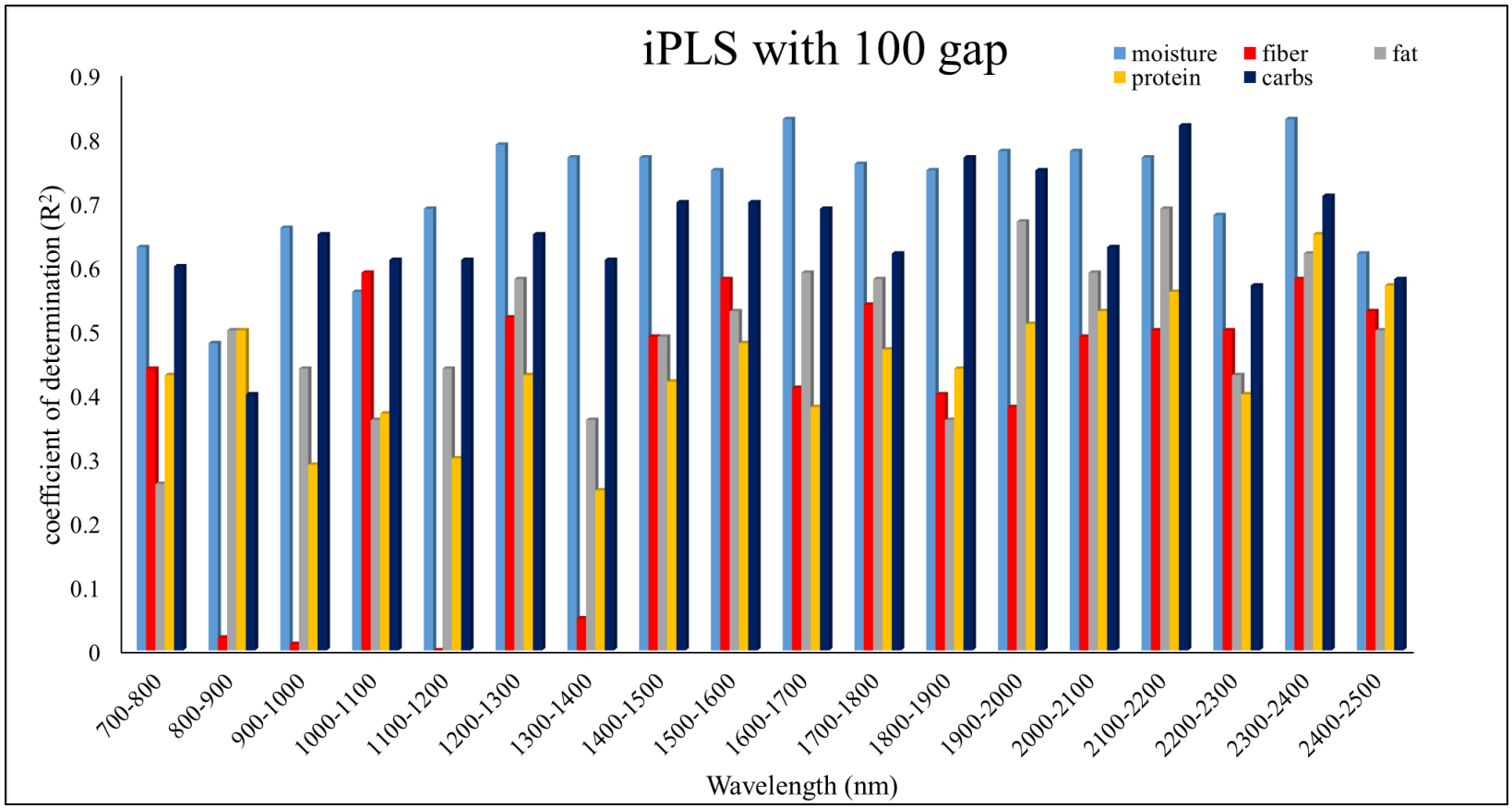

Figure 5.17 Bar graph using iPLS regression method within 100 gap for proximate composition present in pearl millet

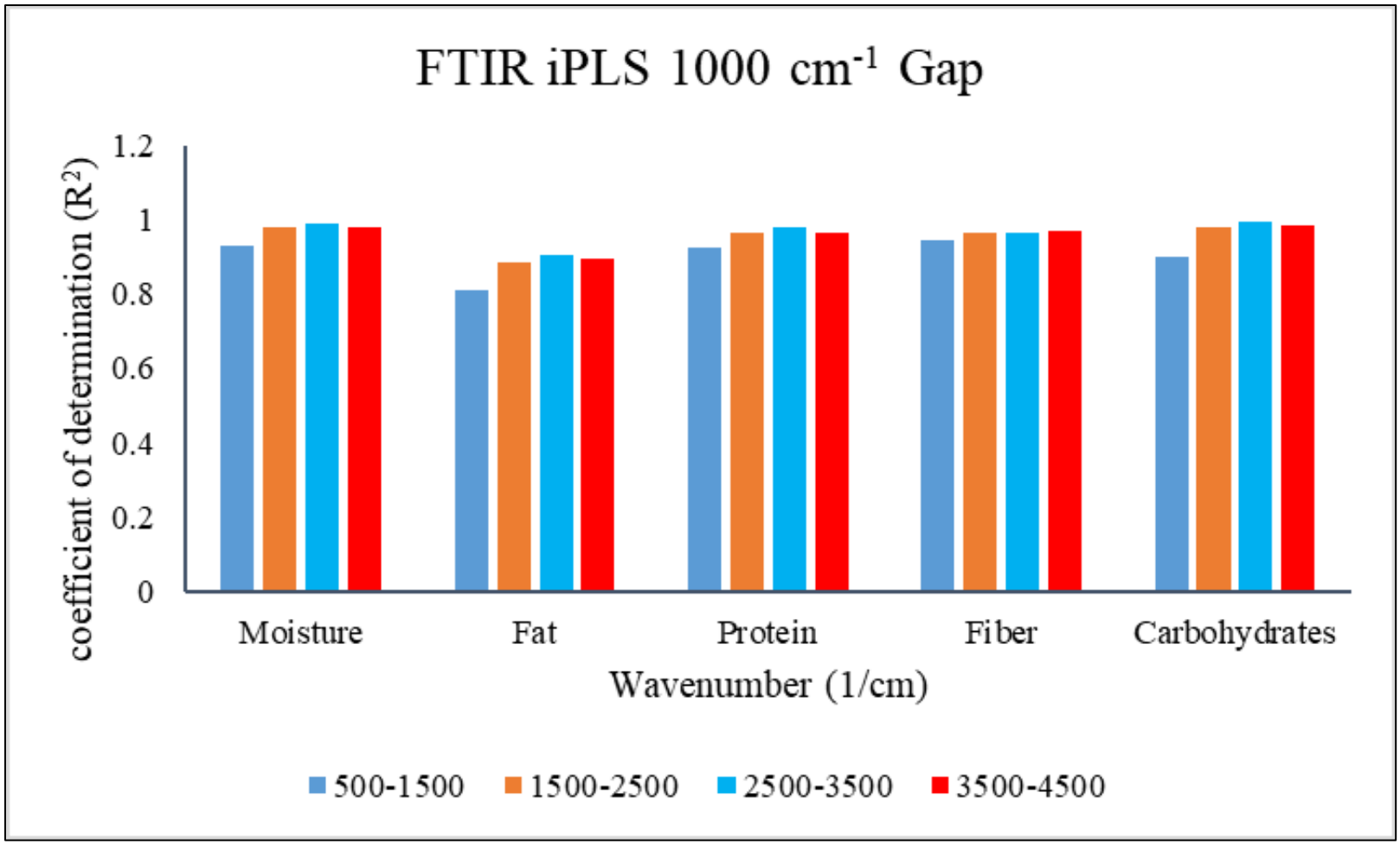

Figure 5.18 Bar graph of FTIR using iPLS regression method within 1000 cm^{-1} gap for proximate composition

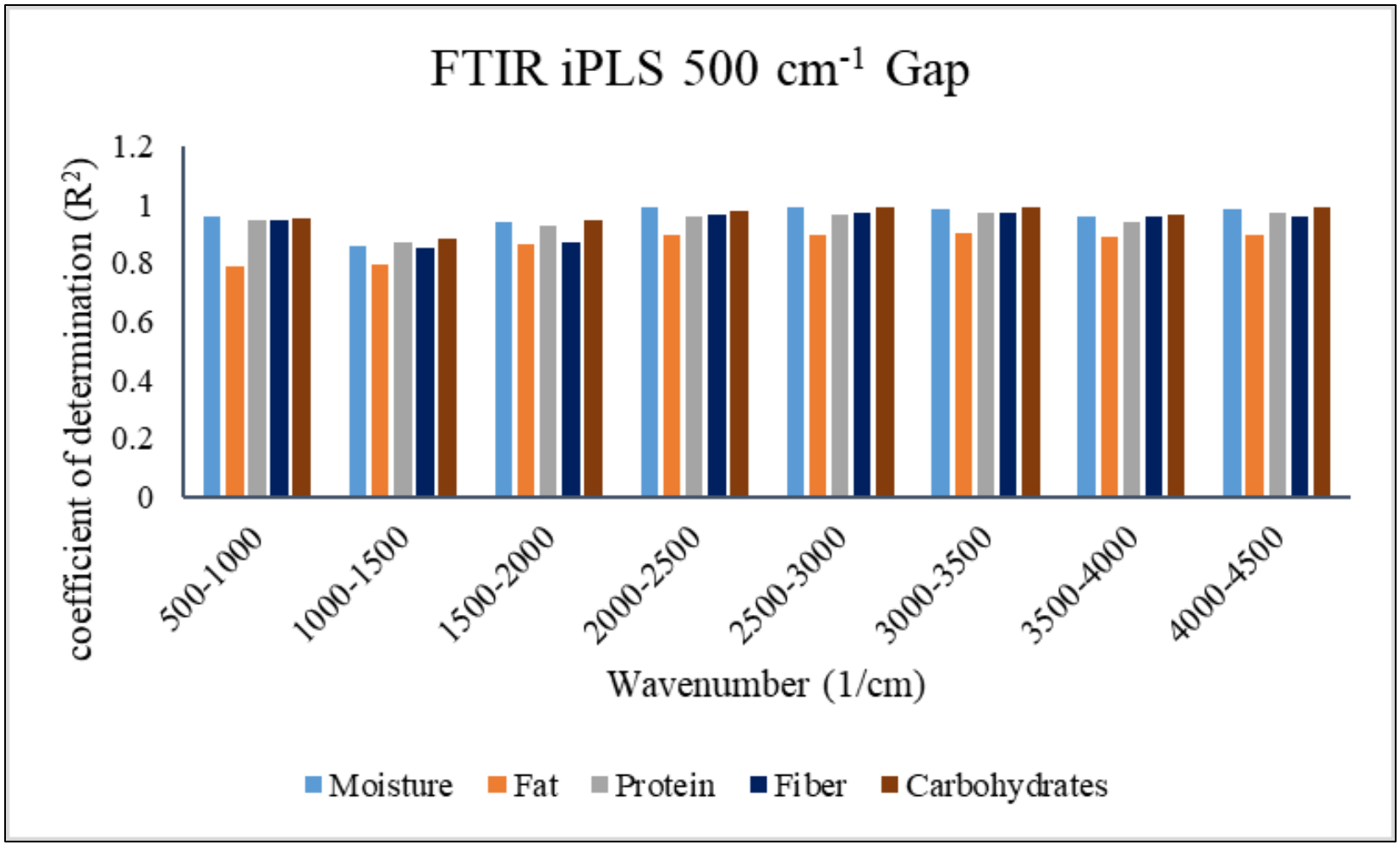

Figure 5.19 Bar graph of FTIR using iPLS regression method within 1000 cm^{-1} gap for proximate composition

Synergy regression (SiPLS) model

For the moisture content the excellent model was obtained within the range of 1350-1600, 1900-2500 nm of MSC+4DV (smoothing point 7, L=3, R=3) with preprocessed NIR spectral data, which shows the good prediction (R^2_C = 0.995, RMSEC= 0.008) in pearl millet sample. The best NIR model were developed for crude fat within the range of 1900-2500 nm with parameters R^2_C = 0.994, RMSEC = 0.006; NIR model were developed for crude protein within spectral range of 1300-1600, 1900-2300 and 2320-2500 nm with R^2_C = 0.989, RMSEC= 0.010; the NIR model were built for crude fiber within range of 2000-2100, 2200-2500 nm with R^2_C = 0.963, RMSEC= 0.026; the best model were generated by NIR technique with same combination of preprocessing for carbohydrates content within spectral range of 1500-1700, 2100-2500 nm with good prediction of R^2_C = 0.992, RMSEC= 0.002 shown in Table 5.4. The important wavelengths for regression model were observed from regression coefficient plots for NIR data shown in Figure 5.30- Figure 5.34. Wavelengths with high values of regression coefficient were considered as important wavelengths.

MIR model was also developed with the combination of smoothing, normalized and first derivative preprocessing. The best MIR model were developed for moisture within 4500-4000, 3500-2000 cm^{-1} with good prediction of (R^2_C= 0.986, RMSEC= 0.013); MIR model were developed for crude fat within spectral range of 4500-4000, 3500-2000 cm^{-1}, with R^2_C = 0.902, RMSEC= 0.027; the MIR model were built for crude protein within range of 4500-4000, 3500-2000 and 1000-500 cm^{-1} with R^2_C= 0.968, RMSEC= 0.018; the MIR model were developed for crude fiber within range of 3500-2500 cm^{-1} with R^2_C= 0.960, RMSEC= 0.027 and the excellent model were developed by MIR for carbohydrates content within spectral range of 4500-4000, 3500-2000 and 1000-500 cm^{-1} with R^2= 0.990, RMSEC= 0.002 shown in Table 5.4. The variation in predicted and references values in proximate compositions for excellence performance of NIR and MIR are shown in Figure 5.20 - Figure 5.24 and Figure 5.25- Figure 5.29.

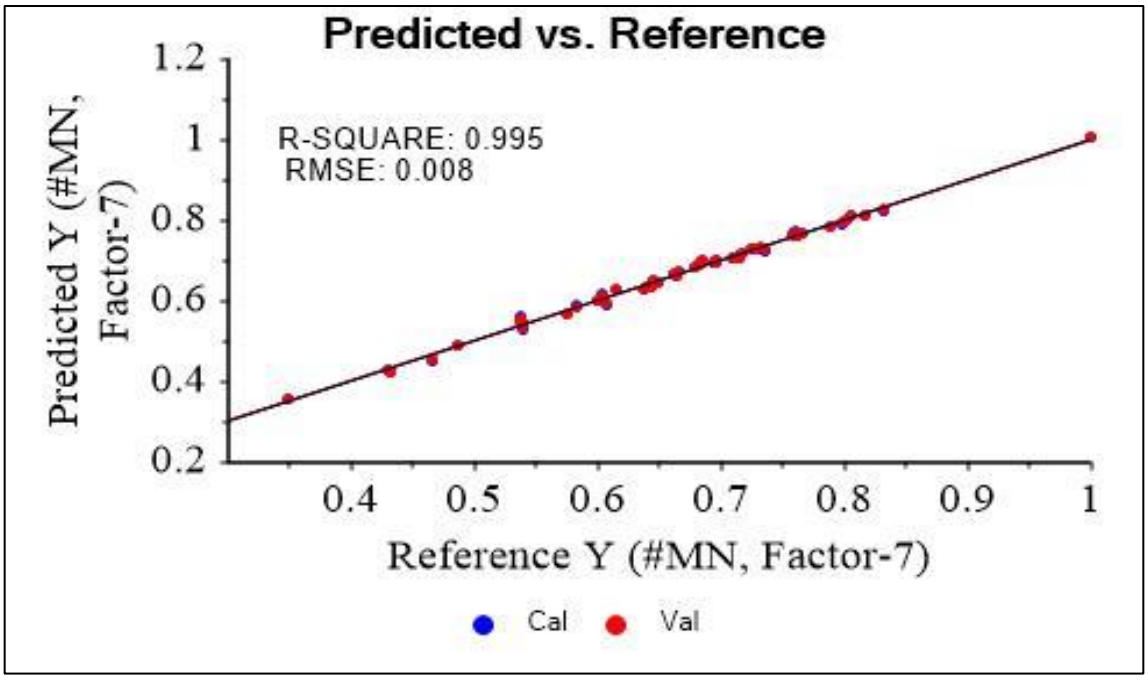

Figure 5.20 Reference and predicted NIR values for moisture calibration model with R-Square and RMSE using synergy intervals partial least square (SiPLS) regression

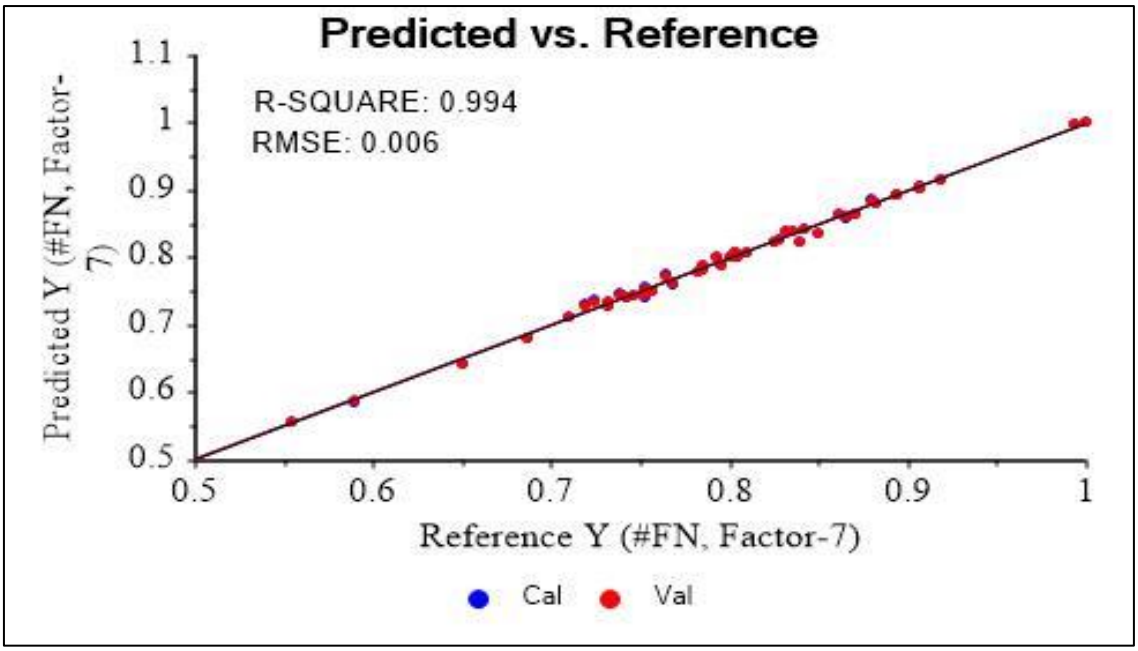

Figure 5.21 Reference and predicted NIR values for crude fat calibration model with R-Square and RMSE using synergy intervals partial least square (SiPLS) regression

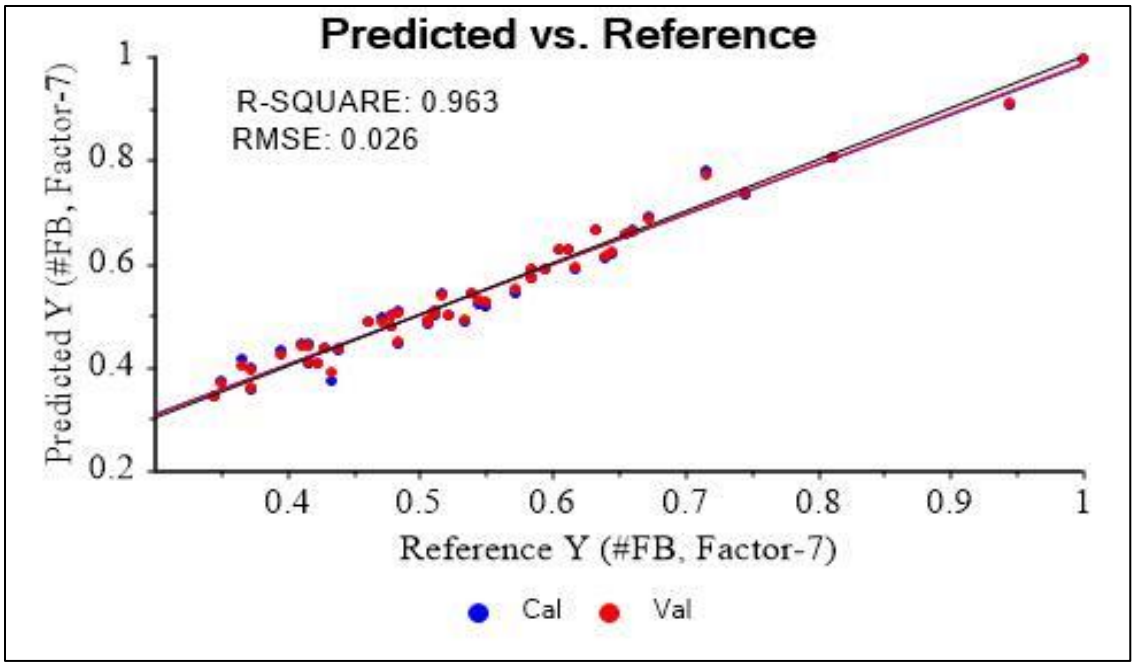

Figure 5.22 Reference and predicted NIR values for crude fiber calibration model with R-Square and RMSE using synergy intervals partial least square (SiPLS) regression

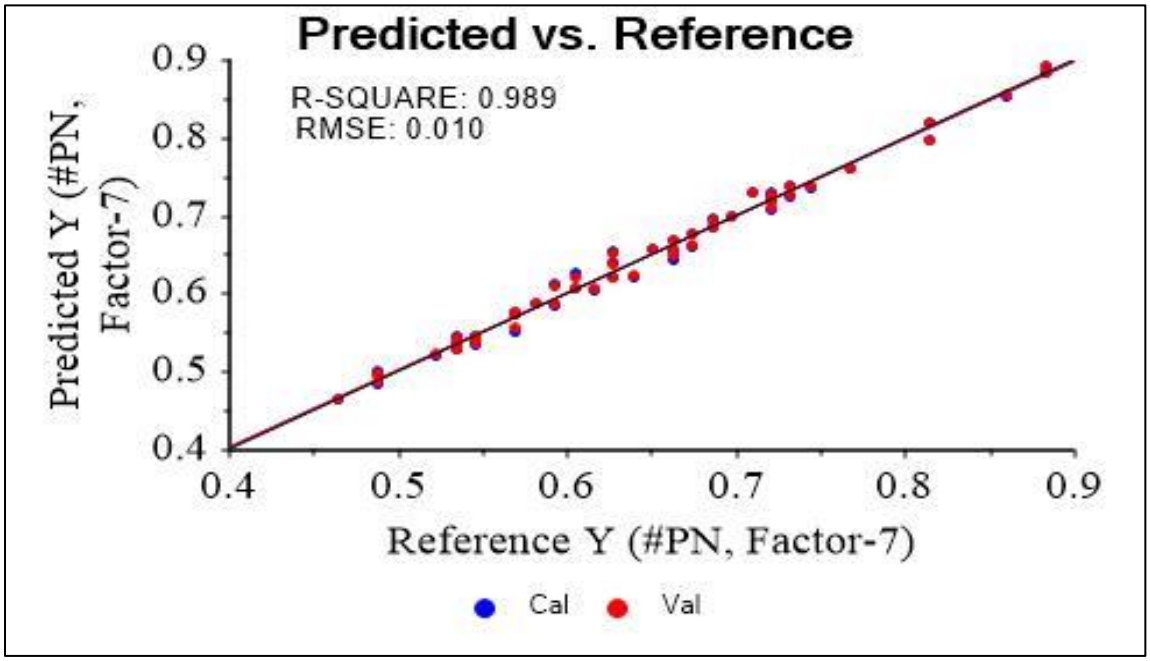

Figure 5.23 Reference and predicted NIR values for crude protein calibration model with R-Square and RMSE using synergy intervals partial least square (SiPLS) regression

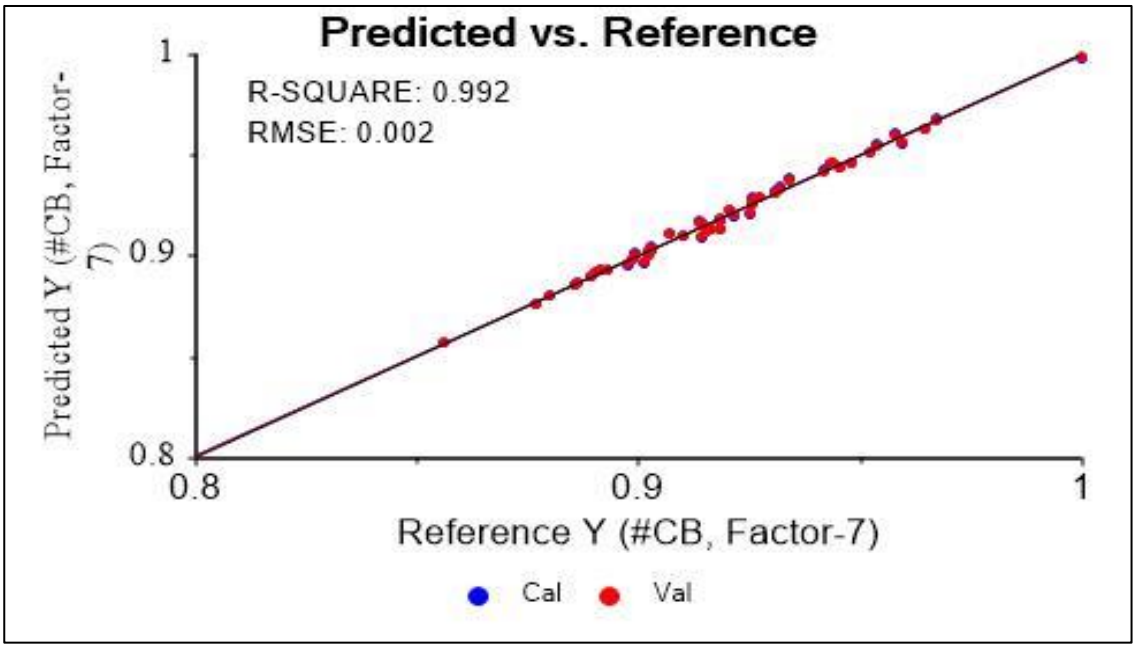

Figure 5.24 Reference and predicted NIR values for carbohydrates calibration model with R-Square and RMSE using synergy intervals partial least square (SiPLS) regression

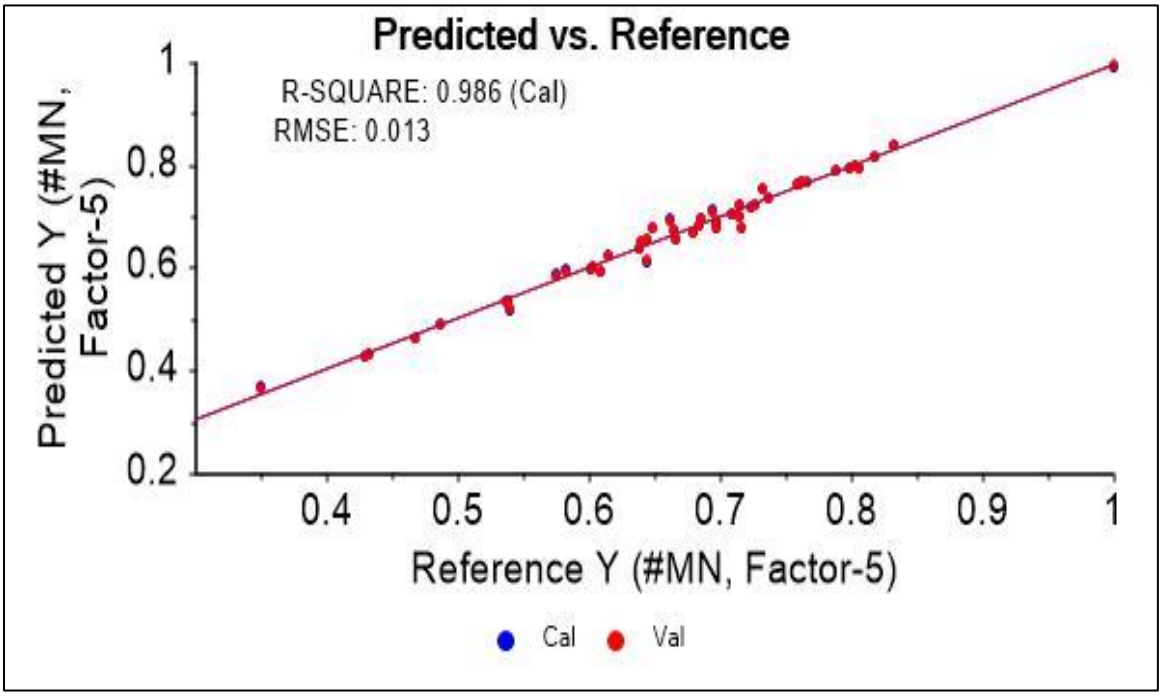

Figure 5.25 Reference and predicted FTIR values for moisture using SiPLS regression

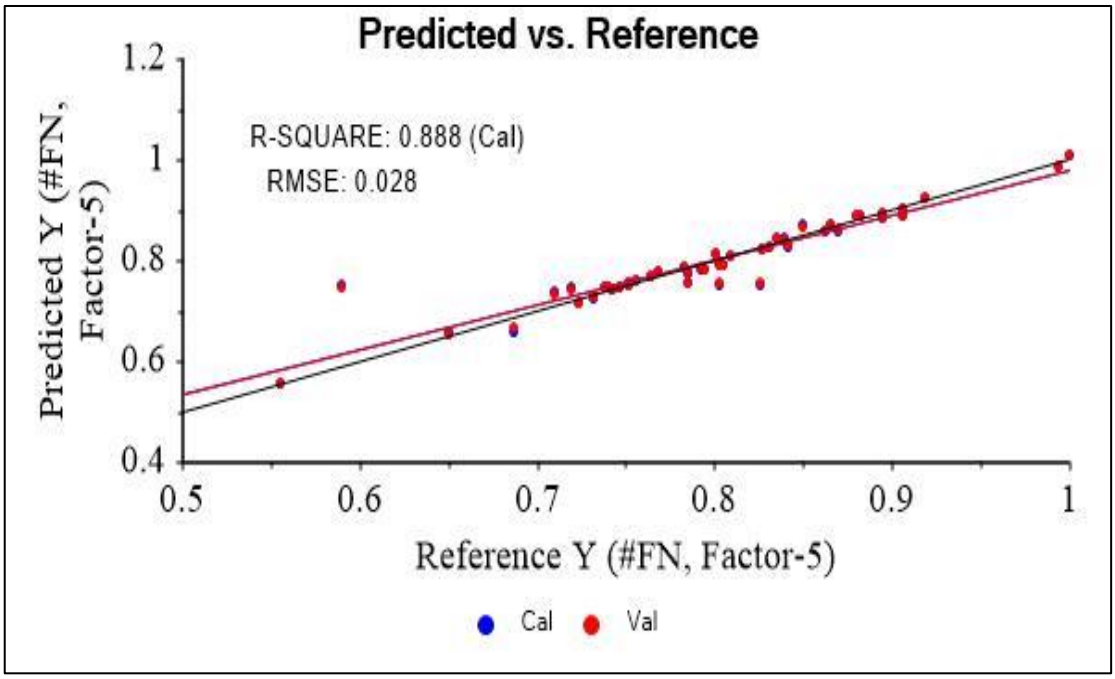

Figure 5.26 Reference and predicted FTIR values for crude fat using SiPLS regression

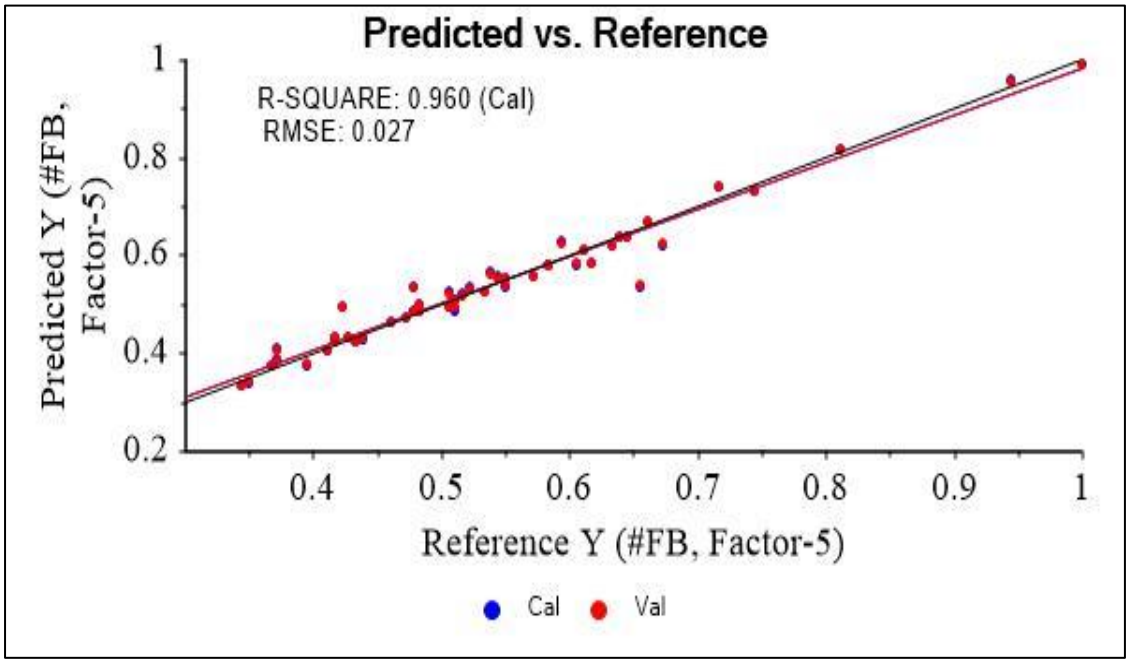

Figure 5.27 Reference and predicted FTIR values for crude fiber using SiPLS regression

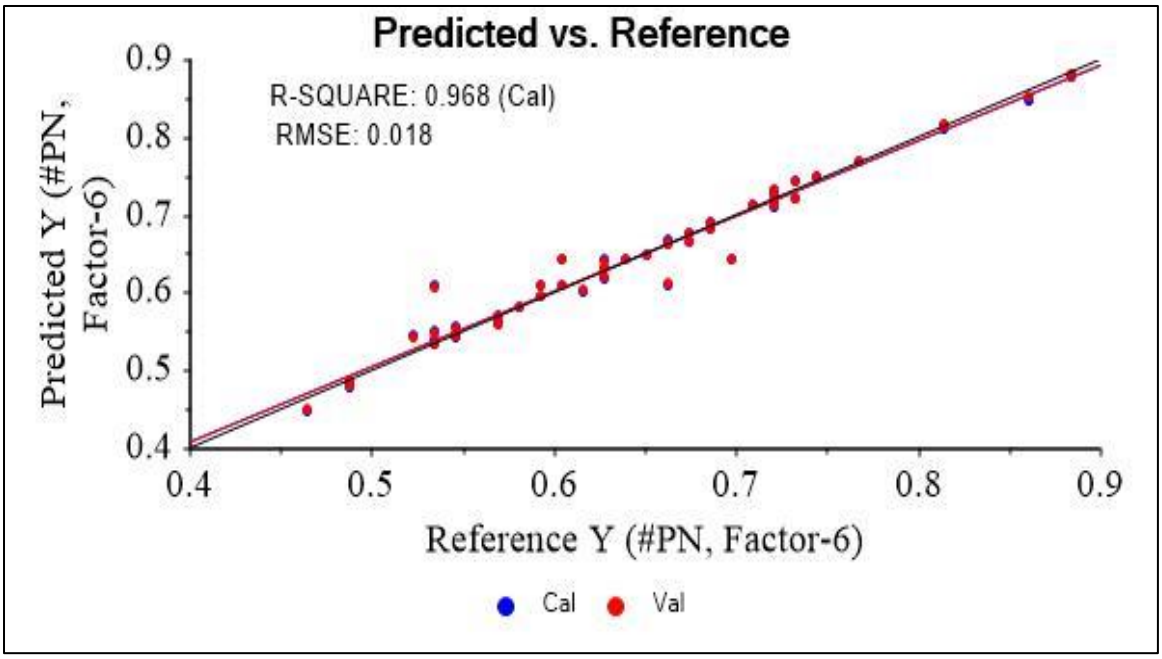

Figure 5.28 Reference and predicted FTIR values for crude protein using SiPLS regression

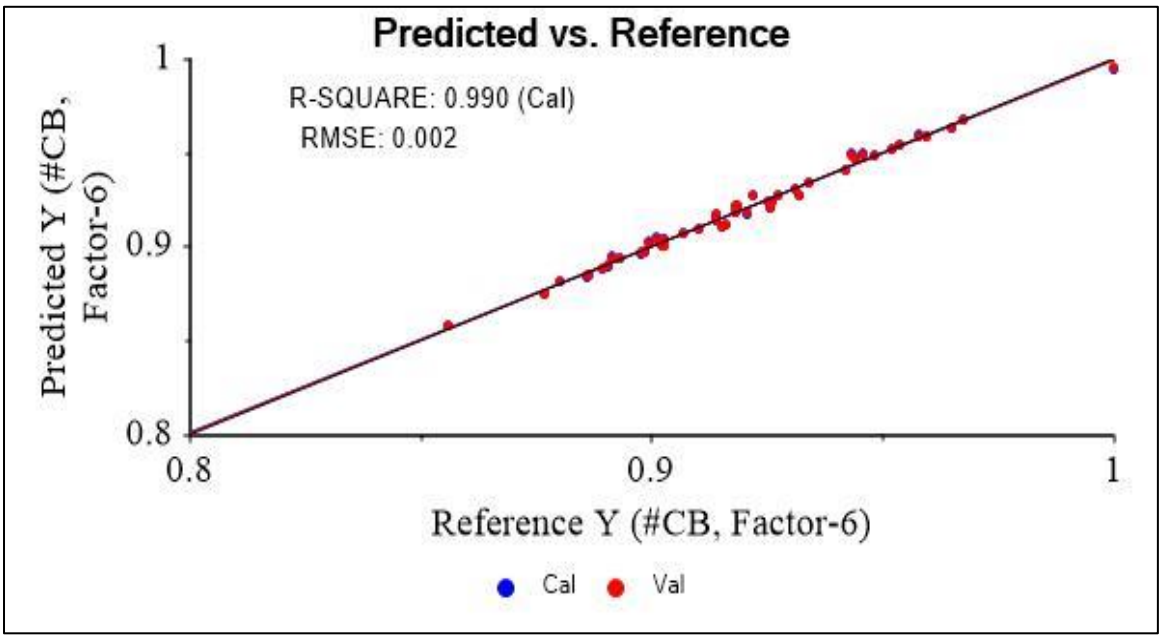

Figure 5.29 Reference and predicted FTIR values for carbohydrates using SiPLS regression

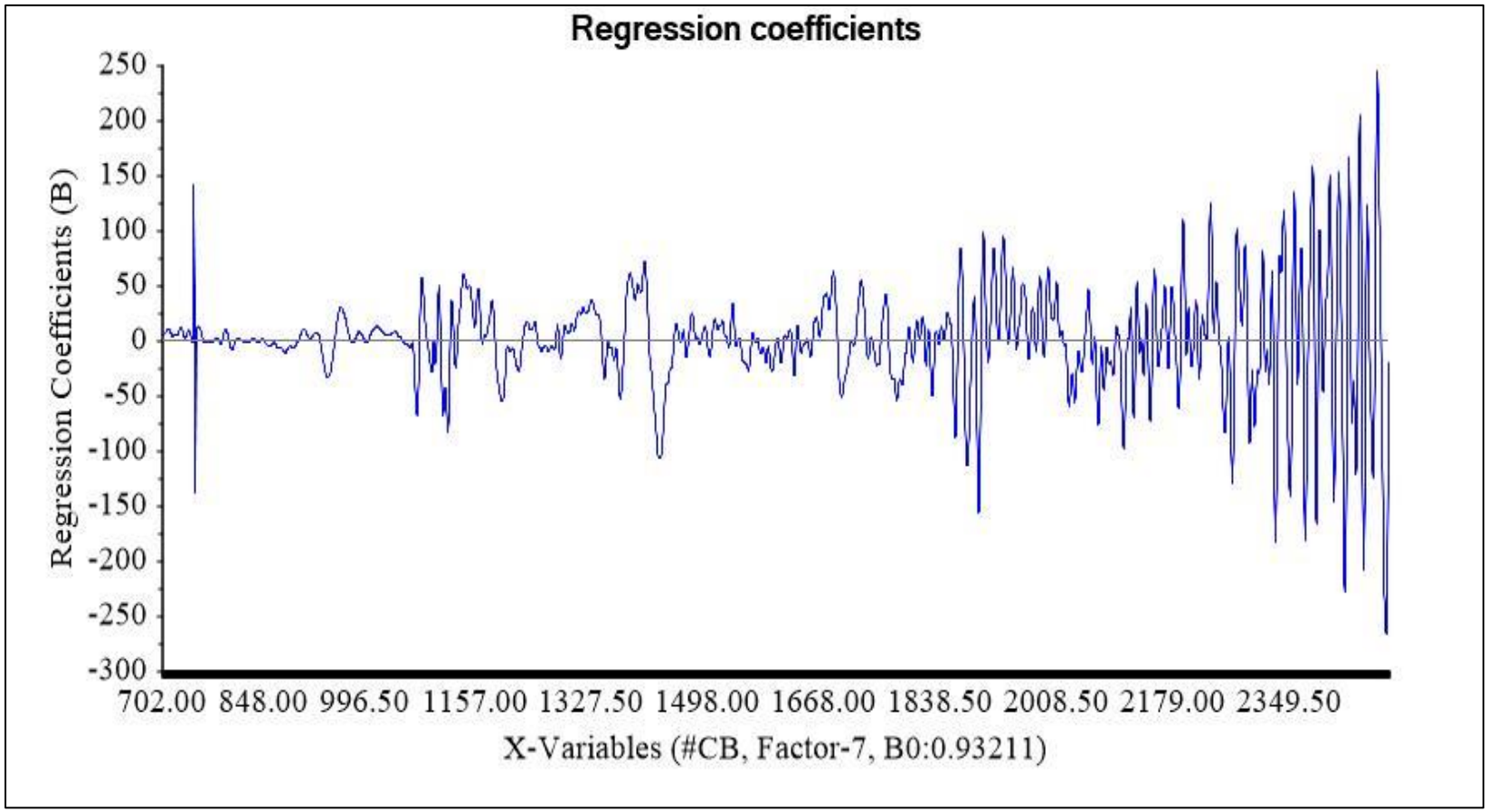

Figure 5.30 Regression coefficients of carbohydrate content for PLS model using NIR spectroscopy

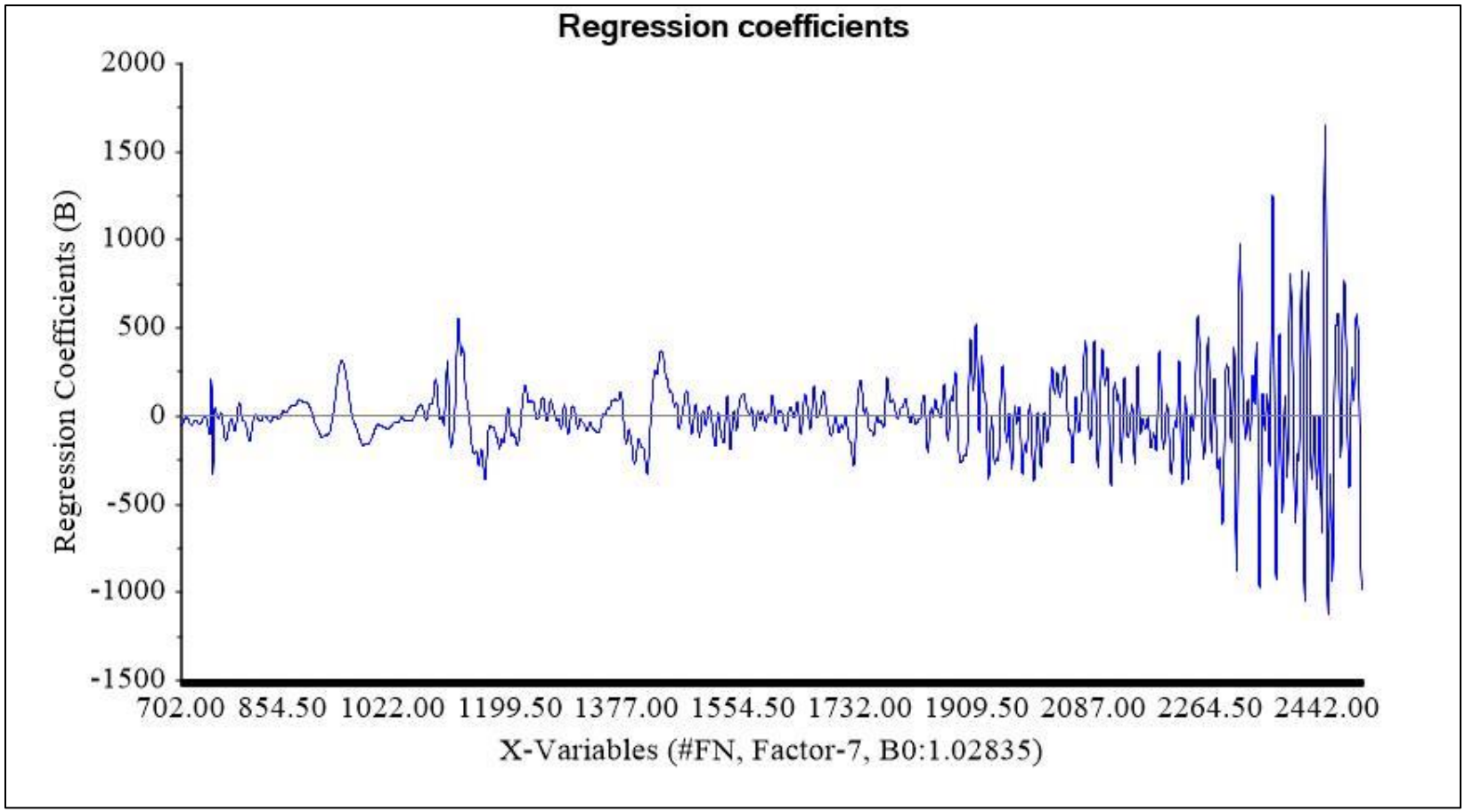

Figure 5.31 Regression coefficients for crude fat content for PLS model using NIR spectroscopy

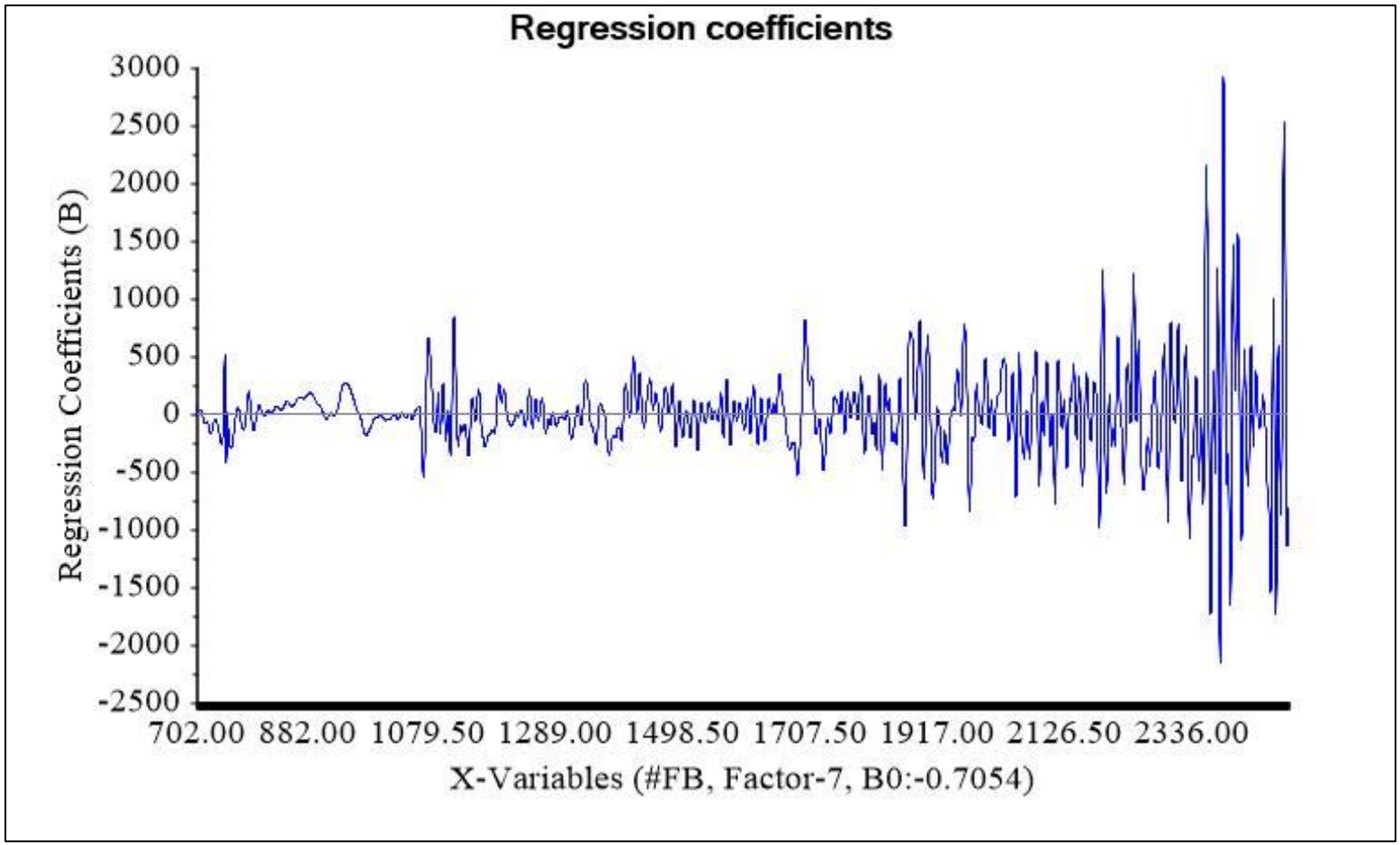

Figure 5.32 Regression coefficients for crude fiber content for PLS model using NIR spectroscopy

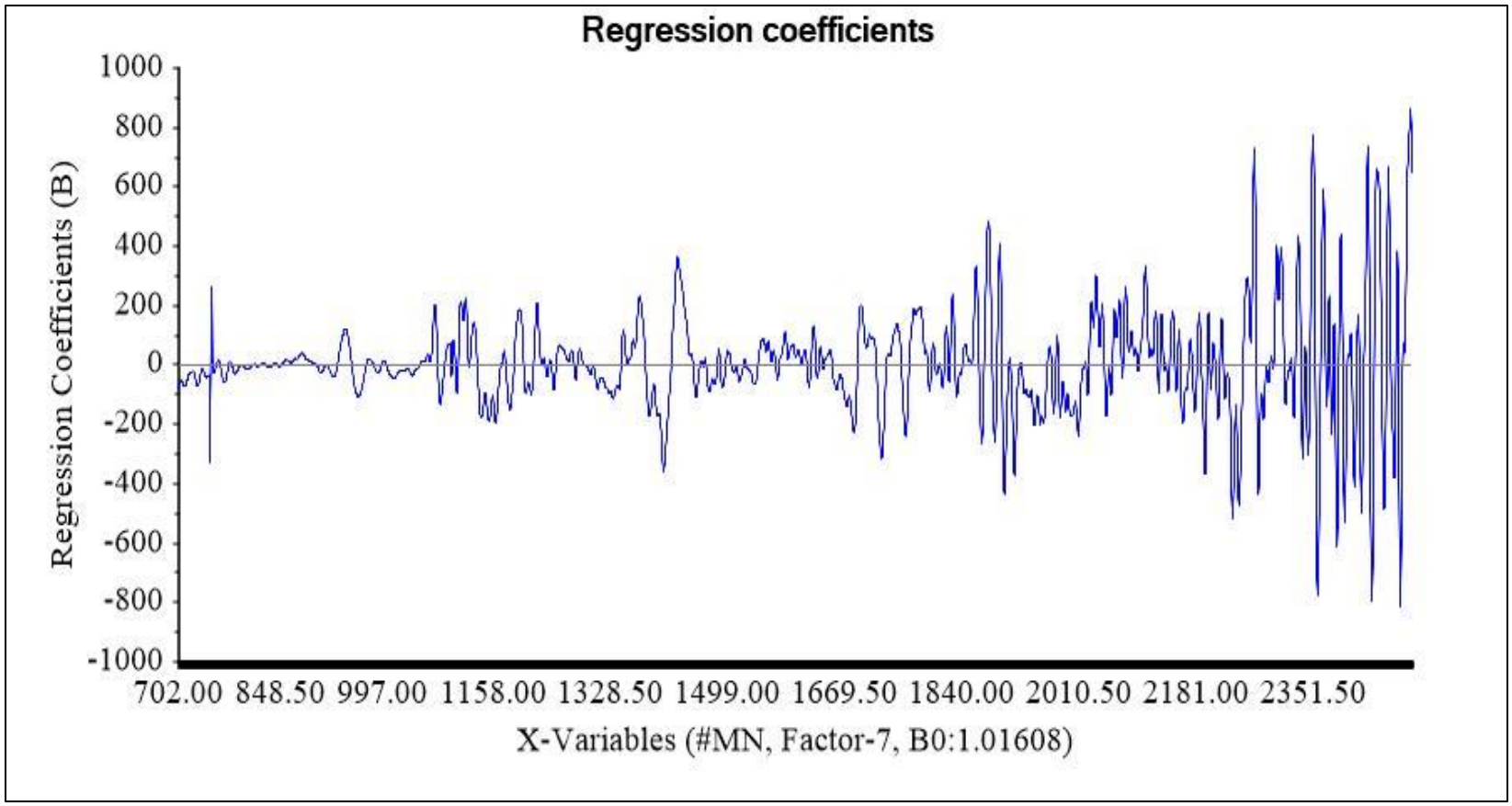

Figure 5.33 Regression coefficients for moisture content for PLS model using NIR spectroscopy

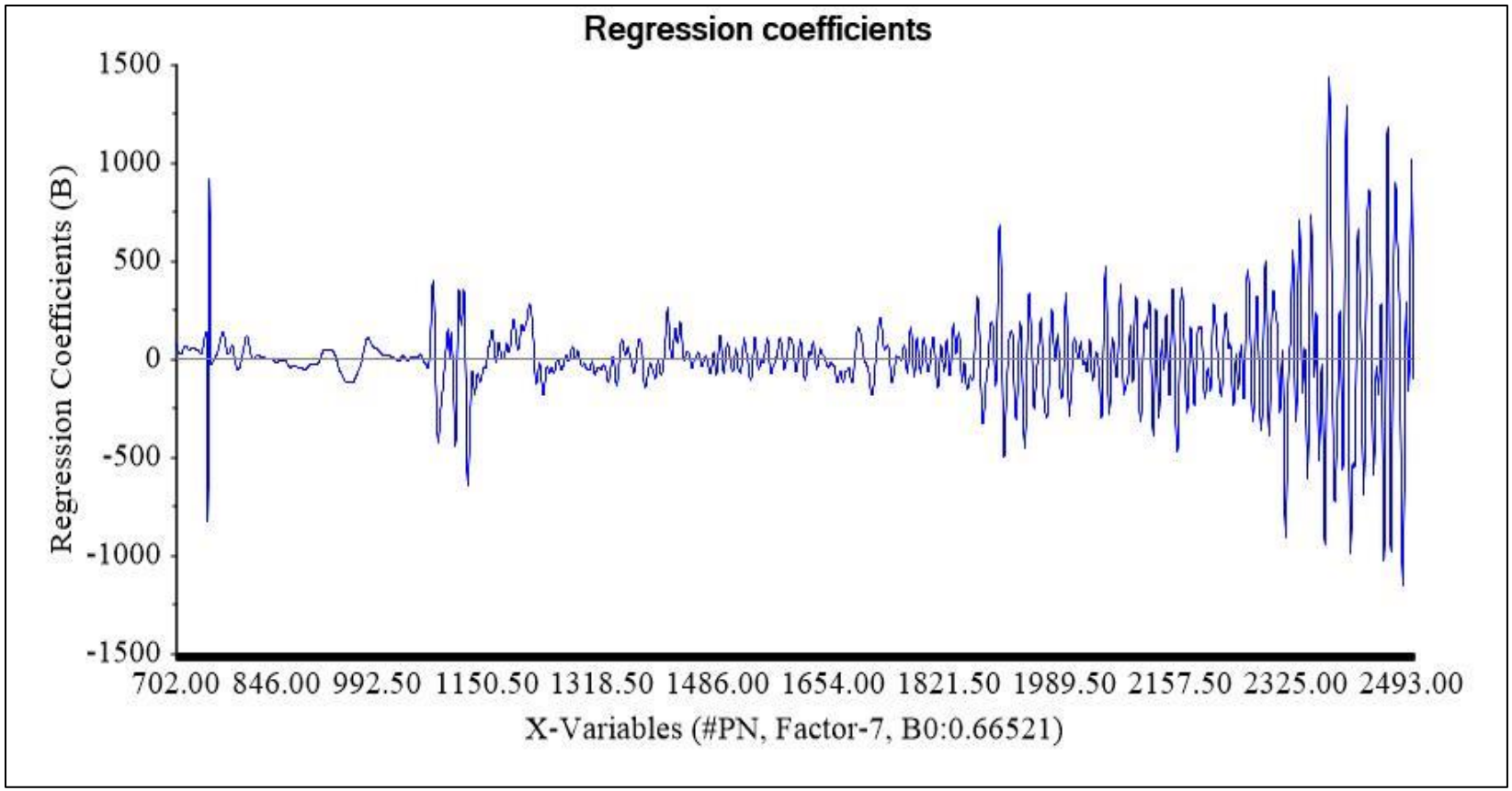

Figure 5.34 Regression coefficients for crude protein content for PLS model using NIR spectroscopy

Table 5.3 Statistical analysis of SiPLS regression model from NIR technique to determine the compositions present in pearl millet

Pre-processing	Calibration										Validation									
	Moisture		Crude fat		Crude Protein		Crude Fiber		Carbohydrates		Moisture		Crude fat		Crude protein		Crude fiber		Carbohydrates	
	R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_C	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE	R^2_V	RMSE
1DV	0.87	0.04	0.60	0.05	0.61	0.06	0.60	0.05	0.87	0.009	0.82	0.049	0.39	0.06	0.45	0.07	0.42	0.10	0.82	0.011
1DV+SM	0.87	0.04	0.60	0.05	0.61	0.06	0.60	0.08	0.87	0.009	0.82	0.05	0.39	0.06	0.45	0.07	0.42	0.10	0.82	0.011
1DV+SNV	0.88	0.03	0.66	0.05	0.64	0.06	0.65	0.08	0.88	0.009	0.84	0.04	0.50	0.06	0.49	0.07	0.50	0.09	0.83	0.011
1DV+MSC	0.88	0.03	0.66	0.05	0.64	0.06	0.65	0.08	0.88	0.009	0.84	0.04	0.50	0.06	0.49	0.07	0.50	0.09	0.83	0.011
SNV	0.73	0.06	0.50	0.06	0.22	0.09	0.40	0.10	0.63	0.016	0.63	0.07	0.30	0.07	NA	0.11	0.17	0.12	0.46	0.019
MSC	0.72	0.06	0.53	0.05	0.22	0.09	0.38	0.10	0.62	0.016	0.61	0.07	0.33	0.07	NA	0.11	0.14	0.12	0.46	0.020
MSC+1DV	0.87	0.04	0.66	0.05	0.64	0.06	0.67	0.07	0.88	0.009	0.81	0.05	0.49	0.06	0.49	0.07	0.54	0.09	0.84	0.010
2DV	0.93	0.02	0.83	0.03	0.85	0.03	0.84	0.05	0.96	0.005	0.91	0.03	0.76	0.04	0.77	0.04	0.76	0.06	0.94	0.006
2DV+SNV	0.93	0.02	0.86	0.03	0.85	0.03	0.83	0.05	0.95	0.005	0.91	0.03	0.80	0.03	0.79	0.04	0.74	0.07	0.93	0.006
2DV+MSC	0.93	0.03	0.84	0.03	0.84	0.04	0.82	0.05	0.95	0.005	0.90	0.03	0.79	0.03	0.78	0.04	0.72	0.07	0.93	0.006
MSC+2DV	0.95	0.02	0.88	0.02	0.89	0.03	0.83	0.05	0.96	0.005	0.93	0.03	0.83	0.03	0.84	0.04	0.73	0.07	0.94	0.006
2DV+MSC+SNV	0.93	0.02	0.86	0.03	0.85	0.03	0.83	0.05	0.95	0.005	0.91	0.03	0.80	0.03	0.79	0.04	0.74	0.07	0.93	0.006

Table 5.4 Best model parameters at various variables to determine proximate compositions of pearl millet using SiPLS

Properties	Technique	Variables (nm, cm^{-1})	Factor	Calibration		Validation	
				R^2_C	RMSEC	R^2_V	RMSEV
Moisture	NIR	1350-1600, 1900-2500	7	0.995	0.008	0.992	0.010
	FTIR	3500-2000, 4500-4000	5	0.986	0.013	0.984	0.014
Crude Fat	NIR	1900-2500	7	0.994	0.006	0.992	0.007
	FTIR	3500-2000, 4500-4000	5	0.888	0.028	0.876	0.030
Crude Protein	NIR	1300-1600,1900-2300,2320-2500	7	0.989	0.010	0.985	0.012
	FTIR	1000-500, 3500-2000, 4500-4000	6	0.968	0.018	0.964	0.019
Crude Fiber	NIR	2000-2100, 2200-2500	7	0.963	0.026	0.946	0.032
	FTIR	3500-2500	6	0.960	0.027	0.953	0.029
Carbohydrates	NIR	1500-1700, 2100-2500	7	0.992	0.002	0.989	0.002
	FTIR	1000-500, 3500-2500, 4500-4000	5	0.990	0.002	0.986	0.003

5.3.4 Evaluation of Performance of model

The development of PLS regression model plays the important role to check the performance of the model for further use on unknown samples. It was observed that the best calibration model was developed with the coefficient of determination 0.995, 0.994, 0.989, 0.963 and 0.992 for moisture, crude fat, crude protein, crude fiber and carbohydrates respectively by employing NIR spectral preprocessed data for pearl millet samples of Haryana and Punjab region. After development of regression model, the performance of model was checked by using external prediction method. In prediction method, there were two unknown samples used to predict the proximate composition of pearl millet. It was observed that the very less deviation has noticed in the predicted and referenced values of moisture, crude fat and carbohydrate content for both samples. The predicted values for moisture, crude fat, crude protein, crude fiber and carbohydrates were 9.93, 5.24, 7.88, 2.25 and 71.514 and references values were 8.64, 4.88, 9.72, 2.45 and 72.59 respectively for unknown sample. Similarly, Table 5.5-5.9 represents the predicted and reference values with maximum normalization as well as deviation.

Table 5.5 References and external predicted values for moisture content of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.6782324	0.009894663	0.5824719
52-Mix	0.6762902	0.008129253	0.694382

Table 5.6 References and external predicted values for crude protein of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.6686113	0.0124274	0.8139532
52-Mix	0.6086107	0.01009981	0.9999997

Table 5.7 References and external predicted values for carbohydrates of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.9061718	0.002741987	0.9134843
52-Mix	0.9040529	0.002259191	0.8372531

Table 5.8 References and external predicted values for crude fat of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.8004881	0.007066495	0.7439024
52-Mix	0.8144918	0.006060158	0.8028455

Table 5.9 References and external predicted values for crude fiber of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.5074058	0.02974316	0.5444444
52-Mix	0.6713815	0.02596594	0.9166667

Similarly, the MIR calibration model used for prediction and predicted values for moisture, crude fat, crude protein, crude fiber and carbohydrates was 7.11, 5.51, 7.54, 2.71 and 74.85 respectively. It was observed that the deviation in between predicted and reference using MIR model was higher as compare to NIR model. The lower deviation reveal the accuracy of calibration model and it can be further used as in non-destructive manner for pearl millet samples. Table 5.10- Table 5.14 shows the performance of MIR calibration model using unknown samples.

Table 5.10 References and external prediction using MIR calibration model for moisture content of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.4807658	0.01108209	0.5824719
52-Mix	0.4535993	0.01295031	0.694382

Table 5.11 References and external prediction using MIR calibration model for crude fat of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.8425798	0.02227696	0.7439024
52-Mix	0.8313027	0.02628518	0.8028455

Table 5.12 References and external prediction using MIR calibration model for crude protein of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.6325506	0.01351357	0.8139532
52-Mix	0.6115707	0.01552315	0.9999997

Table 5.13 References and external prediction using MIR calibration model for crude fiber of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.6030948	0.02241023	0.5444444
52-Mix	0.5513877	0.03117543	0.9166667

Table 5.14 References and external prediction using MIR calibration model for carbohydrates of unknown pearl millet samples

Pearl millet samples	Predicted	Deviation	Reference
51-HR(2)	0.942933	0.002115351	0.9134843
52-Mix	0.9338192	0.002488468	0.8372531

In order to evaluate various organic samples (such as various feeds/cereals, fruits, grains combinations) using infrared methods, it is important to construct the specific statistical models in accordance with the spectral and chemical characteristics of target samples.[35,36] Therefore, this study shows the chemical compositions of pearl millet can be evaluate by the small subsets of NIR as well as MIR spectra.

5.4 Summary

The NIR, FTIR spectra and laboratory chemical data were used to evaluate the proximate compositions in pearl millet. The various preprocessing and combination were employed on spectral data to smoothen and remove the noise in data. The best combination for NIR data was MSC and 4DV and the best combination for FTIR spectra was smoothing with maximum normalize and 1DV gave the best results. The effective range of wavelengths for the moisture was 1350 – 1600 nm, 1900 – 2500 nm, for crude Fat 1900 – 2500 nm, crude protein lies in 1300 – 1600 nm, 1900 – 2300 nm, 2320 – 2500 nm, crude fiber present in between 2000 – 2100 nm and 2200 – 2500 nm and carbohydrates found in the range of 1500 – 1700 nm and 2100 – 2500 nm. Similarly, spectra the various wavenumbers were represents the chemical compositions in FTIR spectra. It was observed that the model built from NIR spectra shows the better performance as compared to models develop from MIR spectra, which was due to presence of fundamental bands present in the FTIR spectra. The outcomes of this study also reveal that IR spectroscopy has the great ability to predict the compositions and nutrient digestibility. The result can be enhanced with large sample size in terms of verities as well as locations. The developed statistical model may be employed to determine the chemical compositions contained in pearl millet in a quick, efficient, eco-friendly and non-destructive manner.

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CHAPTER-6

CONCLUSION & FUTURE SCOPE

6 Conclusion and Future scope

6.1 Conclusion

The objectives, Qualitative and quantitative determination of physical & chemical composition and rheological properties of pearl millets. To fulfil this objective the research was carried out to determine the physical as well as chemical composition of pearl millet. The pearl millet samples were collected from region of Haryana and Punjab states India. In the physical parameters the moisture and ash content were determined by employing AOAC standard methods. The determination of moisture content was important to check the shelf life and storage of the sample. The ash contents consist of numerous mineral contents like as magnesium, phosphorus, zinc and proteins that are responsible for the potent health benefits of pearl millets such as antidiabetic and anti-carcinogenic activity. In addition, the effect of moisture content on the pearl millet grain gains the more attention. The sample of two varieties were chosen for analysis. In this thesis, the effect of moisture content on physical parameters of grains at various moisture levels (11-18%) were measured by Vernier calliper with 0.001 mm least count. Farmers of Sirsa, Haryana, India provided the Bajra-1827 and Pioneer 84M88 grains samples. Foreign particles were removed from the grains, and immature and broken grains were manually removed. The physical parameters of pearl millet were investigated in the moisture range of 11.55 to 18.44%. Firstly, the grains were divided into the four equal portions and the calculated amount of water sprayed on them. By adding the estimated amount of water, the required moisture content was attained using hot air oven method. Grains with varying moisture levels were packed in polythene zip-lock bags and stored in a refrigerator (4°C) for 15 days to achieve consistent moisture content. To ensure moisture and constant temperature, samples were equilibrated for 2 hours at room temperature before to each experiment. After this time period, grains were ready for determination of physical properties at different moisture levels. In the physical properties, the parameters named as size, geometric mean diameter, volume, surface area, sphericity, and bulk density of pearl millet grains are determined using established methods.

The length of the pearl millet grains within moisture content of 11.55, 12.95, 16.35, and 17.29% is shown to be 3.672, 3.754, 3.920, 3.826 mm for the Bajra-1827 variety, and length within moisture content of 11.85, 13.96, 16.18, and 18.44% is shown to be 3.171, 3.337, 3.505, and 3.671mm respectively for the Pioneer 84M88 variety. The width of the Bajra-1827 variation was 2.087 to 2.672 mm and the Pioneer84M88 variety was 2.504 to 2.838 mm. Similarly, the thickness of the Bajra-1827 and Pioneer84M88 varieties ranged from 2.004 to 2.838 mm and 2.002 to 2.171 mm, respectively. All observations were taken into account in terms of desirable moisture contents. The pearl millet's length, width, and thickness appear to be increasing. Sphericity ranged from 0.713 to 0.762 for the bajra-1827 variety and 0.770 to 0.814 for the Pioneer 84M88 type of pearl millet. Whereas, the bulk density within range of 0.877 to 0.806 for both varieties. Thus, the sphericity and bulk density decreased as per increase of moisture content. It was found that the grain of variety Bajra-1827 had a greater influence on size than Pioneer 84M88 for the same moisture content. These findings may be used by engineers to build apparatus for cleaning, grading, processing, and storing pearl millet grains with moisture effects.

Simultaneously, the pearl millet prominent crop in the term of its nutrient parameters. Pearl millet is rich in protein, fiber content as well as contains less amount of gluten which help to reduce the heart attacks and also prevent from diabetic problems. Thus, the various parameters named as crude fat, crude protein, crude fiber and carbohydrates contents were determined by AOAC standards. The moisture content was within range of 5.19 to 14.83% with standard deviation 1.76, ash content was in between 1.48 to 1.88% with 0.10 standard deviation, fat content was lying in between 3.64 to 6.56% with 0.57 standard deviation. Whereas, the crude fiber was present in between 1.55 to 4.50%, crude protein in 5.56 to 11.95% and carbohydrates was in 66.53 to 79.46% and with standard deviation of 0.66, 1.40 and 2.34 respectively. In comparison of another studies, it was observed that the pearl millet of Haryana region contains 7.44% moisture, 2.11% ash, 5.53% crude fat, 9.81% crude protein, 3.41% crude fiber and 71.70% carbohydrates. Therefore, it can be seen that the pearl millet high in protein and fiber content as compare to other cereals.

In food consumption, production, and in handling the rheological has several uses. The study of rheology is of based upon how food materials behave under the impact of applied forces and deformations. Rheology data is helpful not only for production control and equipment design, but it may also be used to understand the behavior of dough during processing and the quality of the items produced from it. The viscosity of the various varieties was therefore measured with a Labman 200 rotating viscometer. It was observed that the viscosity at 100 and 200 RPM were determined within range of 1366.66-1377.55 and 685.52-688.89 mPa-Sec.

The NIR and FTIR spectroscopy techniques were used to determination of qualitatively and quantitatively analysis of organic materials, as well as to determine the chemical structure of numerous inorganic chemicals. The spectral data results give quantitative and qualitative information about the organic substance pearl millet flour.

In the present work the near infrared and fourier infrared spectroscopy were used to classify the pearl millet samples according to their region by employing Principal Component Analysis (PCA). The raw spectral data were collected from NIR and FTIR spectroscopy and outliers from 50 samples were removed by using PCA. PCA was applied to all chemical and spectral data sets, which explains the region-based classification of pearl millet samples. After that it was observed that the total PCs accounted for 96.1% of the variation in NIR spectral data, 96.2% in FTIR data, and 57.2% in chemical data. The first two PCs appear to include nearly all of the spectrum information of IR spectral data from various regions, although pearl millet samples were hardly separated on the basis of geographical regions using principal component analysis.

In continuation of this work, the near infrared spectroscopy was used to predict the physicochemical compositions with the help of multivariate regression analysis such as important wavelength selection method, pre-processing, PLS, coefficient of determination, regression coefficients, iPLS, SiPLS. Firstly, the chemical compositions were determined by AOAC standards and these are the time consuming and hazardous methods. To overcome this time taken procedures, the rapid, green and non-destructive methods are frequently used for determination of chemical compositions by NIR spectroscopy. The data

set was compiled using reference analyses and near infrared (NIR) spectra data to estimate physicochemical properties of pearl millet in a non-destructive and eco-friendly manner. Multivariate regression analysis was performed on the compiled data using CAMO Unscrambler X 10.5.1 software. Firstly, NIR spectral data and reference data were used to develop the statistical calibration model by using PLS regression. The various types of pre-processing were employed on the spectral data and select only those which shows the excellent model at the full range of wavelength in PLS regression. The pre-processing named as Svitzky-Golay derivatives (2DV and 4DV), SNV, MSC with their combinations shows the excellent outcomes. The pre-processing mostly used to remove the noise and unwanted signal of spectral data and also it enhances the quality of model with high coefficient of determination (R^2) and root mean square error (RMSE) of model. The coefficient of determination (R^2) and root-mean square error (RMSE) values for all pearl millet compositions were reported employing NIR and MIR spectra for calibration and validation.

For the NIR PLS model, the coefficient of determination (R^2) for moisture was 0.68 to 0.93, crude fat was 0.41 to 0.93, crude protein was 0.27 to 0.91, crude fiber was 0.24 to 0.94, and carbohydrate was 0.60 to 0.94. For the FTIR regression model, the coefficient of determination (R^2) for moisture was in range from 0.67 to 0.99, crude fat in 0.41 to 0.90, crude protein in 0.53 to 0.96, crude fiber in 0.53 to 0.97, and carbohydrates in between 0.45 to 0.99. The greatest coefficient of determination (R^2) for moisture, crude fat, crude protein, crude fiber, and carbohydrate in the PLS NIR regression model was 0.93, 0.93, 0.91, 0.88, and 0.94, respectively. Whereas, after applying wavelength selection method, the accuracy of calibration model enhanced with combination of spectral and chemometric approach. The wavelength was selected by the interval partial least regression methods and regression coefficients with the gap of 600, 300, 200, and 100 nm intervals of wavelength. It was observed that the effective wavelength was seen in the gap of 300 nm gap of wavelengths with high coefficient of determination. Thus, the coefficient of determination in wavelength 700-1000, 1000-1300, 1300-1600, 1600-1900, 1900-2200 and 2200-2500 nm was in range of 0.73 to 0.90 for moisture, 0.42 to 0.83 for crude fat, 0.0006 to 0.81 for

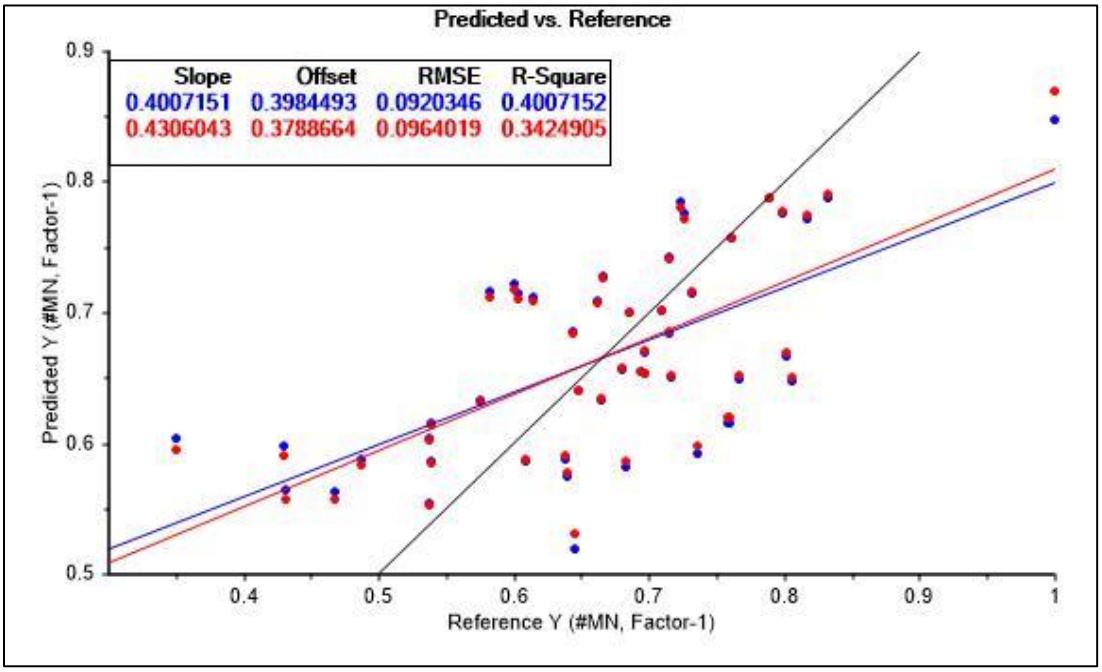
crude fiber, 0.36 to 0.86 for crude protein and 0.66 to 0.94 for carbohydrate content. The combination of various intervals gives the more accurate outcomes. These combinations of wavelengths for moisture content are 1300-1600 and 1900-2500 nm shows the excellent coefficient of determination (R^2) i.e., 0.995 and root mean square (RMSE) i.e., 0.008 of pearl millet using NIR spectroscopy. Whereas the crude fat (1900-2500 nm), crude protein (1300-1600, 1900-2300, 2320-2500 nm) crude fiber (2000-2100, 2200-2500 nm) and carbohydrates (1500-1700, 2100-2500 nm) contents having the good coefficient of determination0.994, 0.989, 0.963 and 0.992 respectively. The analysis of NIR spectroscopy were compared to the FTIR spectroscopy and NIR shows the excellent model performance as compare to FTIR analysis. After developing the calibration model, the accuracy of model was checked by the prediction using unknown samples. The predicted values for moisture, crude fat, crude protein, crude fiber and carbohydrates were 9.993, 5.24, 7.88, 2.25 and 71.514 respectively. These predicted results show the accuracy of calibration model. The results indicate that using wavelength selection approach improves the prediction accuracy of NIR-based regression models for the quality parameters of pearl millet samples when compared to full spectrum models.

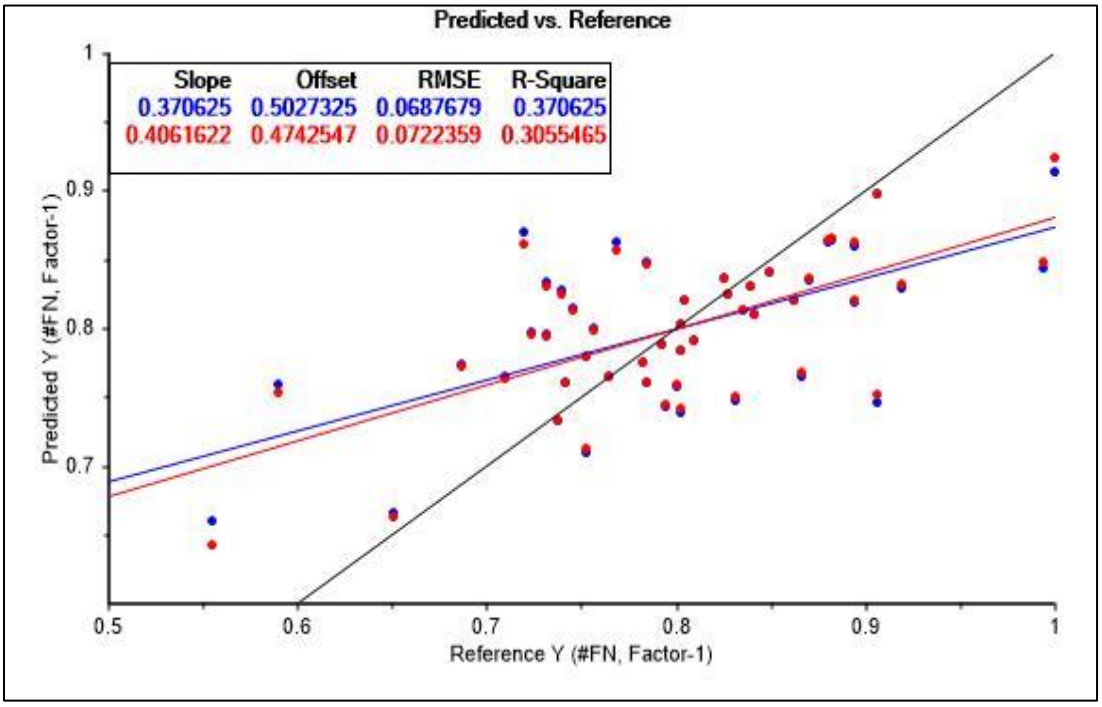
6.2 Future Scope

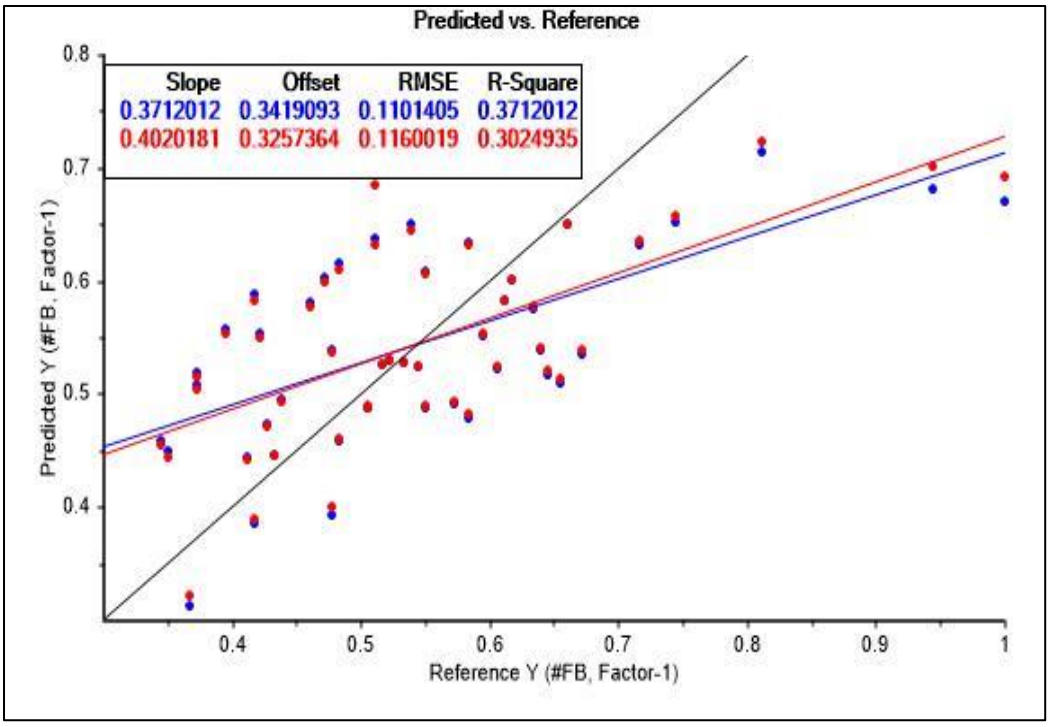
- NIR instruments may be designed for specific wavelengths utilizing LEDs of those wavelengths, minimizing the complexity and expense of the equipment.
- Other attributes of pearl millet such as moisture, ash content, crude fiber, crude protein, carbohydrates and biochemical parameters may be determined using the established approach and relevant wavelengths can be chosen from the prediction model. With a larger number of sample size, the usage of selective wavelength may be expanded to other cereals such as maize, barley, rice, and so on.
- In summary, it is feasible to construct an NIR device with low cost, rapid analysis time, and good prediction capacity using selective wavelength regression models.
- The method under consideration is an offline preliminary evaluation of the appropriateness of NIRS to assess the quality characteristics of pearl millet under laboratory conditions. The procedures, formulation, and other variable factors observed in commercial scale manufacturing can be investigated in the future.

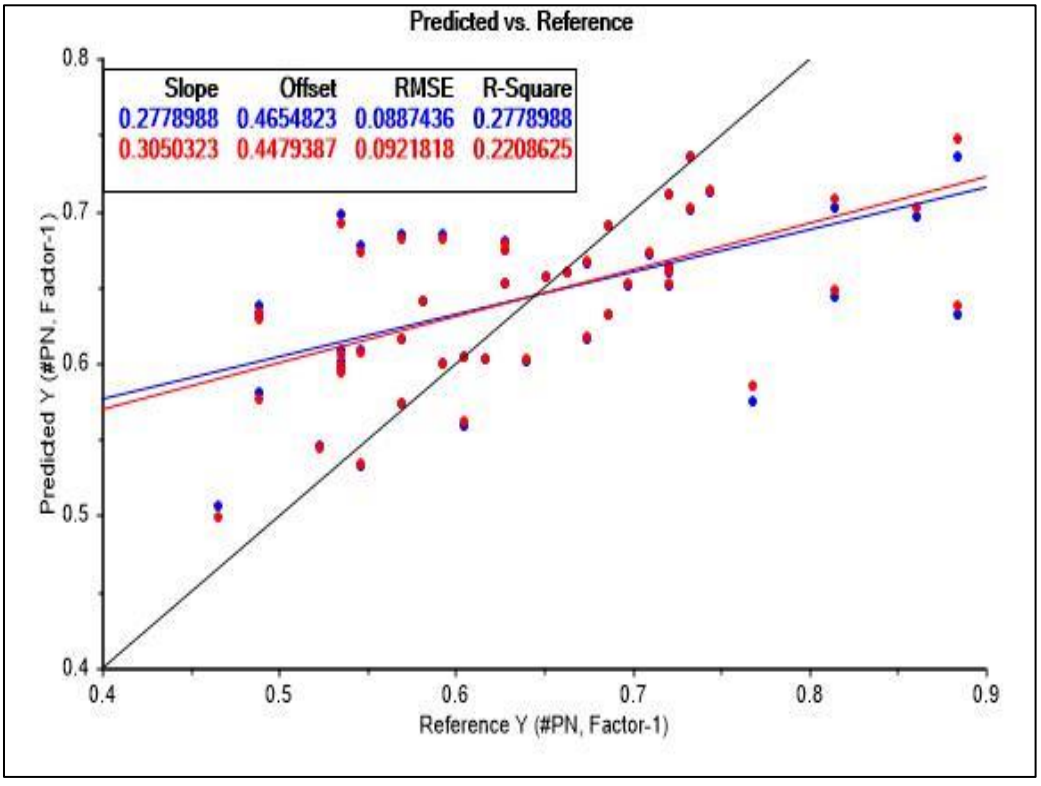
Appendices

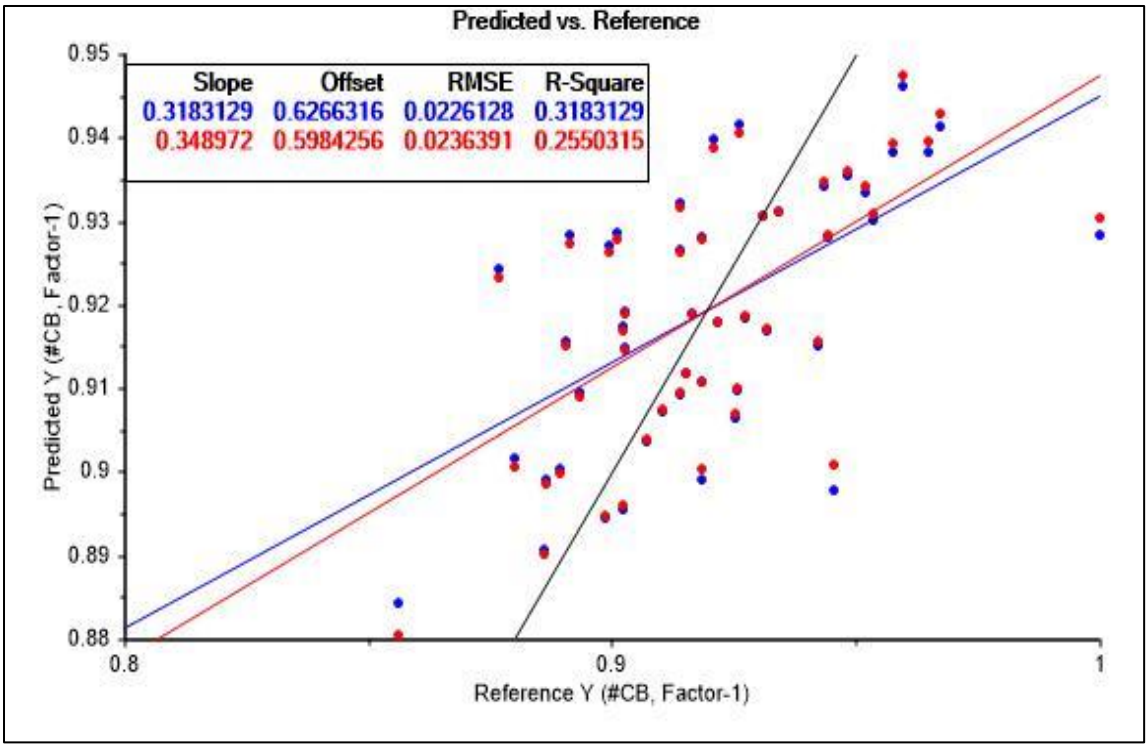
Appendix 1. Reference and predicted values of proximate compositions of pearl millet at Factor 1 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



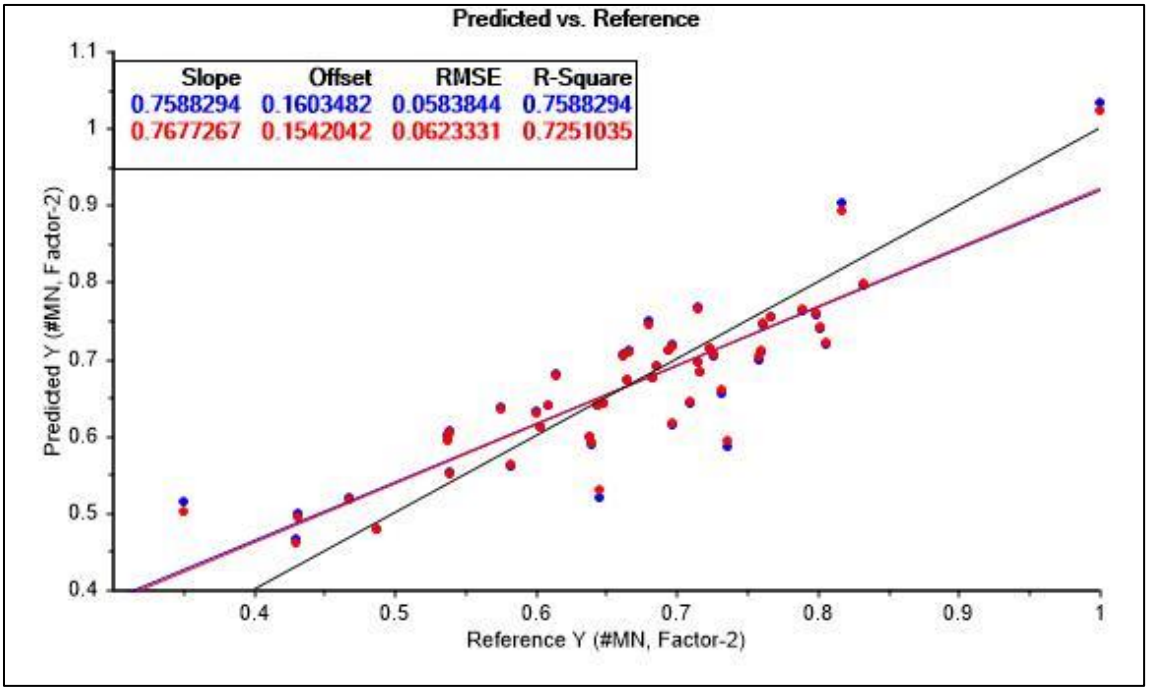


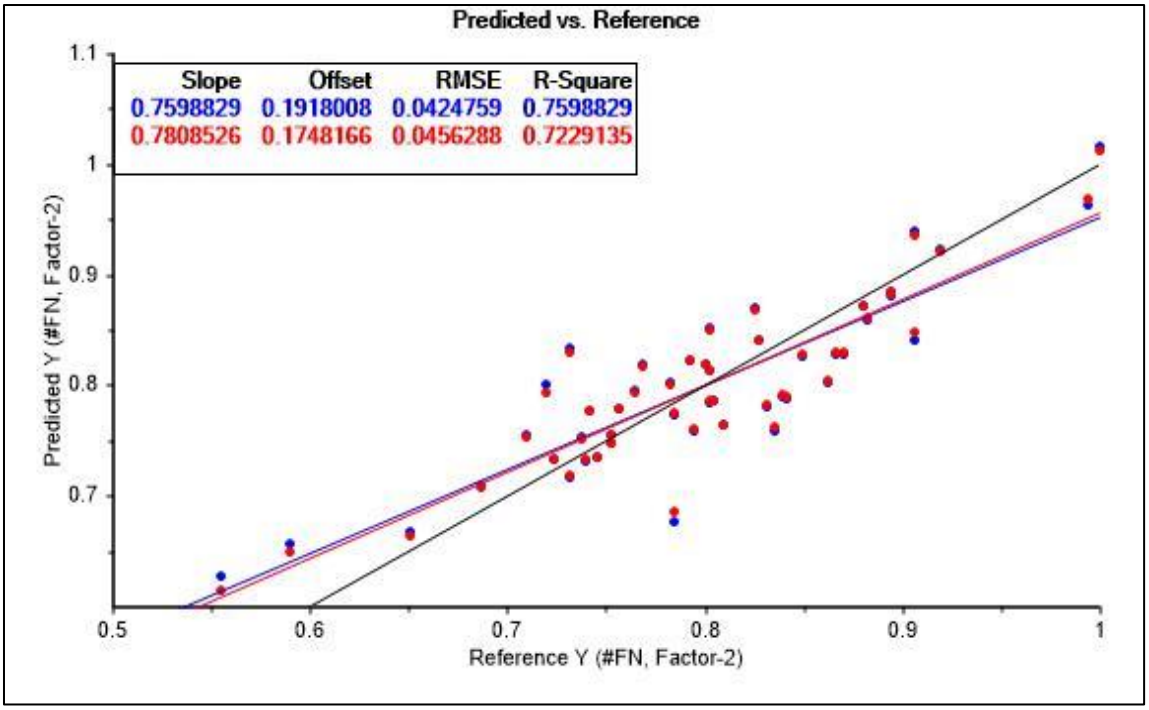

Predicted vs. Reference
Slope Offset RMSE R-Square
0.3712012 0.3419093 0.1101405 0.3712012
0.4020181 0.3257364 0.1160019 0.3024935
Predicted Y (#FB, Factor-1)
Reference Y (#FB, Factor-1)
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1

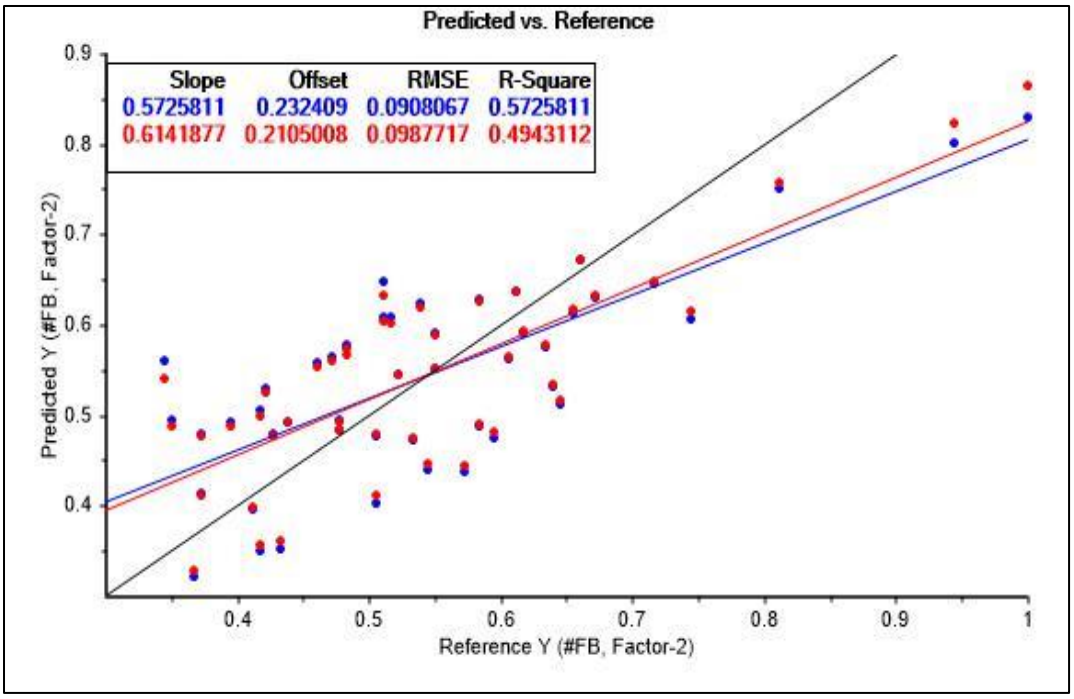

Predicted vs. Reference
Slope Offset RMSE R-Square
0.2778988 0.4654823 0.0887436 0.2778988
0.3050323 0.4479387 0.0921818 0.2208625
Predicted Y (#PN, Factor-1)
Reference Y (#PN, Factor-1)
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9

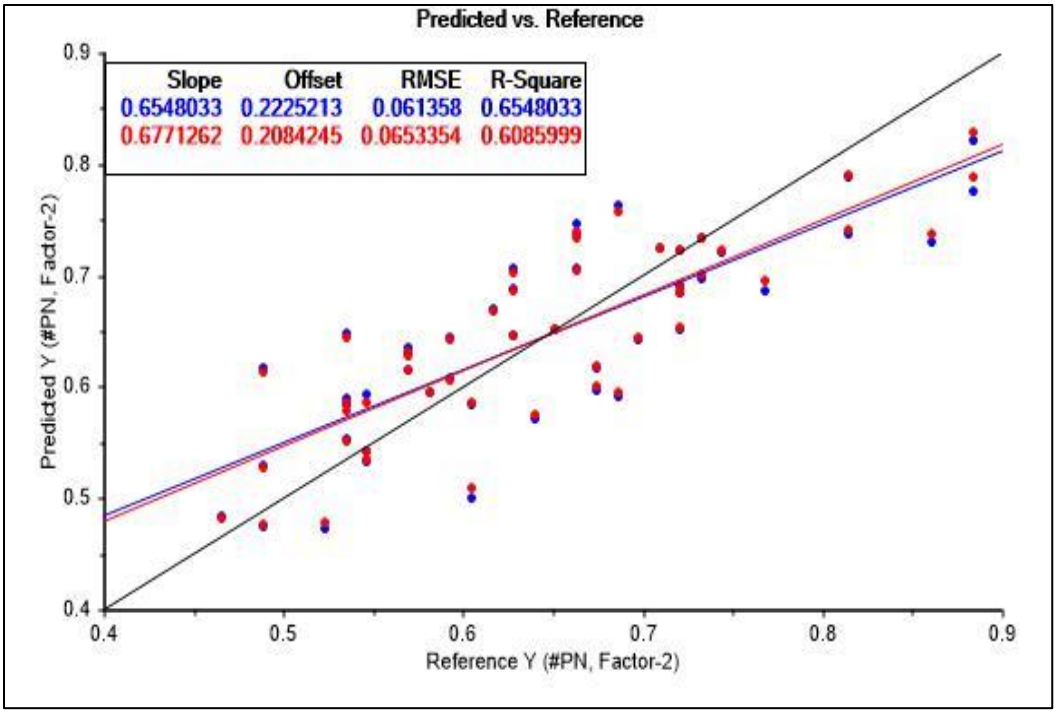

Predicted vs. Reference
Slope Offset RMSE R-Square
0.3183129 0.6266316 0.0226128 0.3183129
0.348972 0.5984256 0.0236391 0.2550315
Predicted Y (#CB, Factor-1)
Reference Y (#CB, Factor-1)
0.95
0.94
0.93
0.92
0.91
0.9
0.89
0.88
0.8
0.9
1

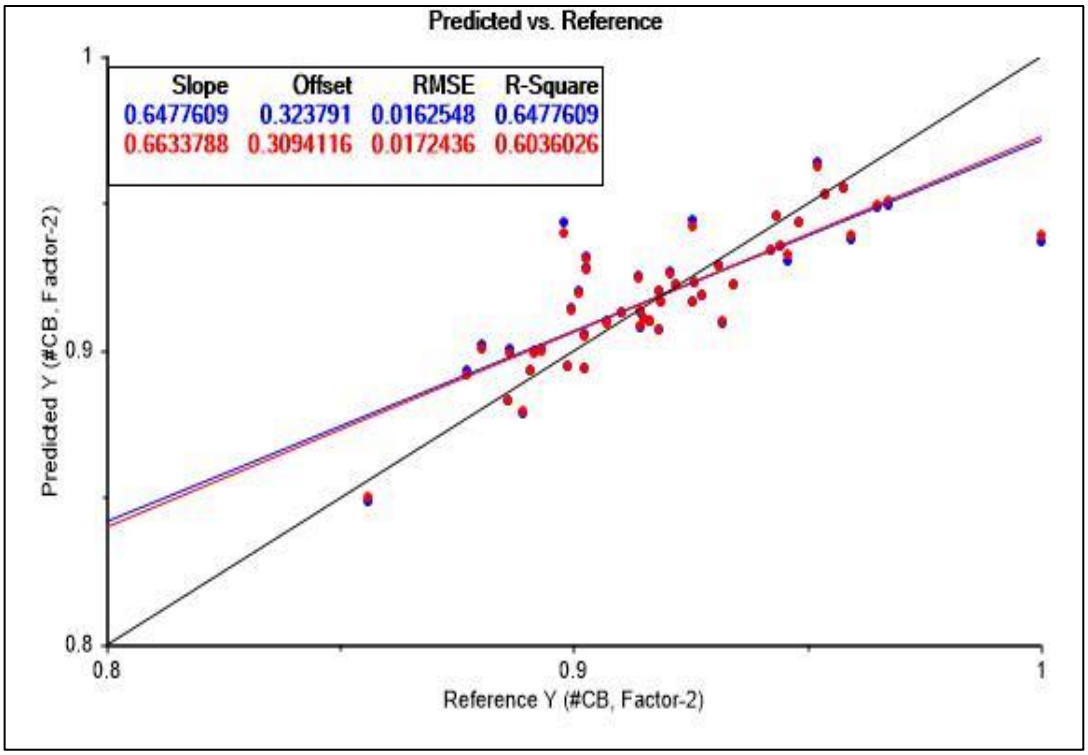
Appendix 2. Reference and predicted values of proximate compositions of pearl millet at Factor 2 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



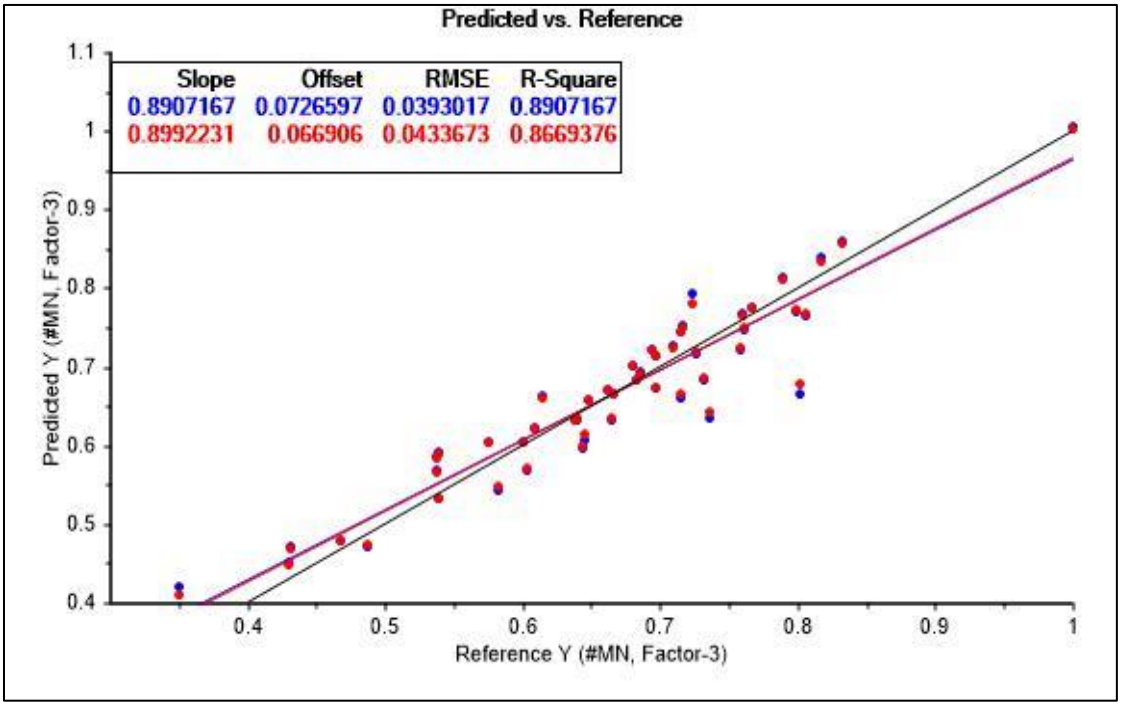


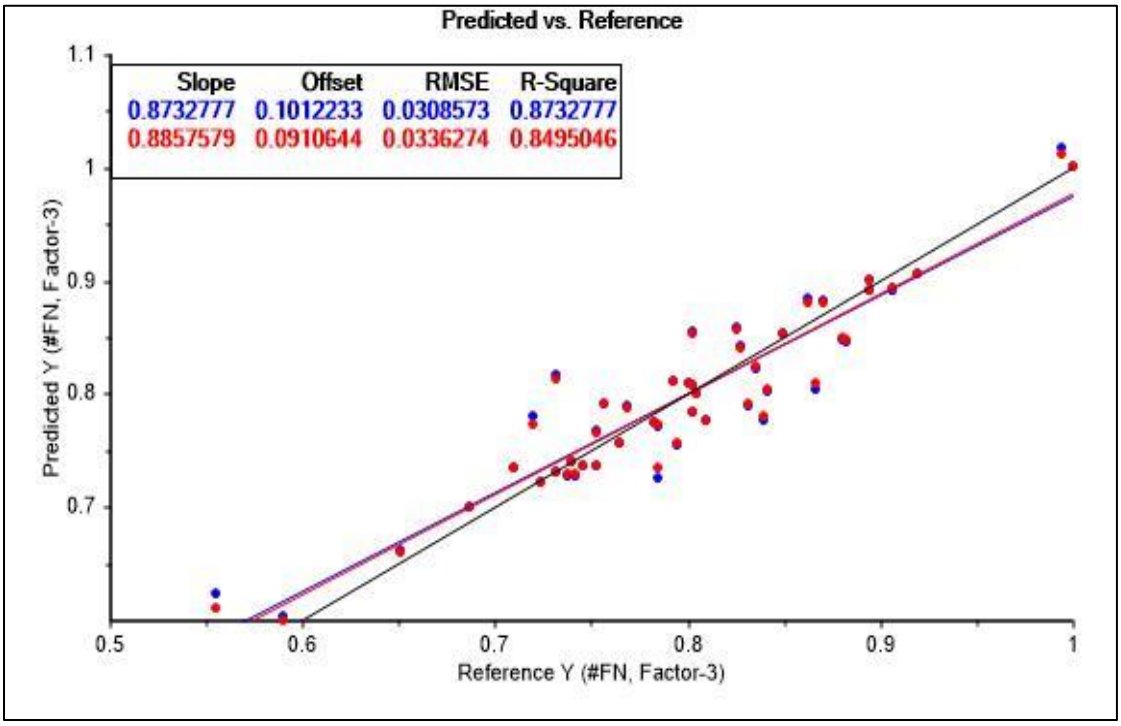

Predicted vs. Reference
Slope Offset RMSE R-Square
0.5725811 0.232409 0.0908067 0.5725811
0.6141877 0.2105008 0.0987717 0.4943112
Predicted Y (#FB, Factor-2)
Reference Y (#FB, Factor-2)
0.4
0.5
0.6
0.7
0.8
0.9
1

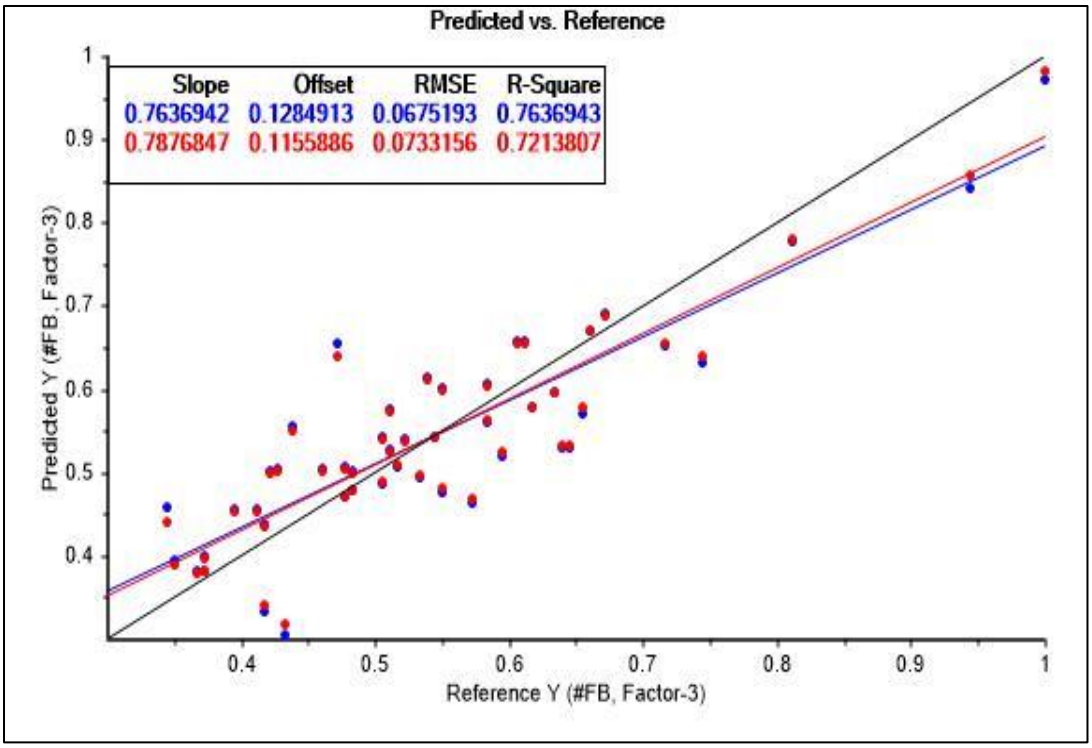

Predicted vs. Reference
Slope Offset RMSE R-Square
0.6548033 0.2225213 0.061358 0.6548033
0.6771262 0.2084245 0.0653354 0.6085999
Predicted Y (#PN, Factor-2)
Reference Y (#PN, Factor-2)
0.4
0.5
0.6
0.7
0.8
0.9

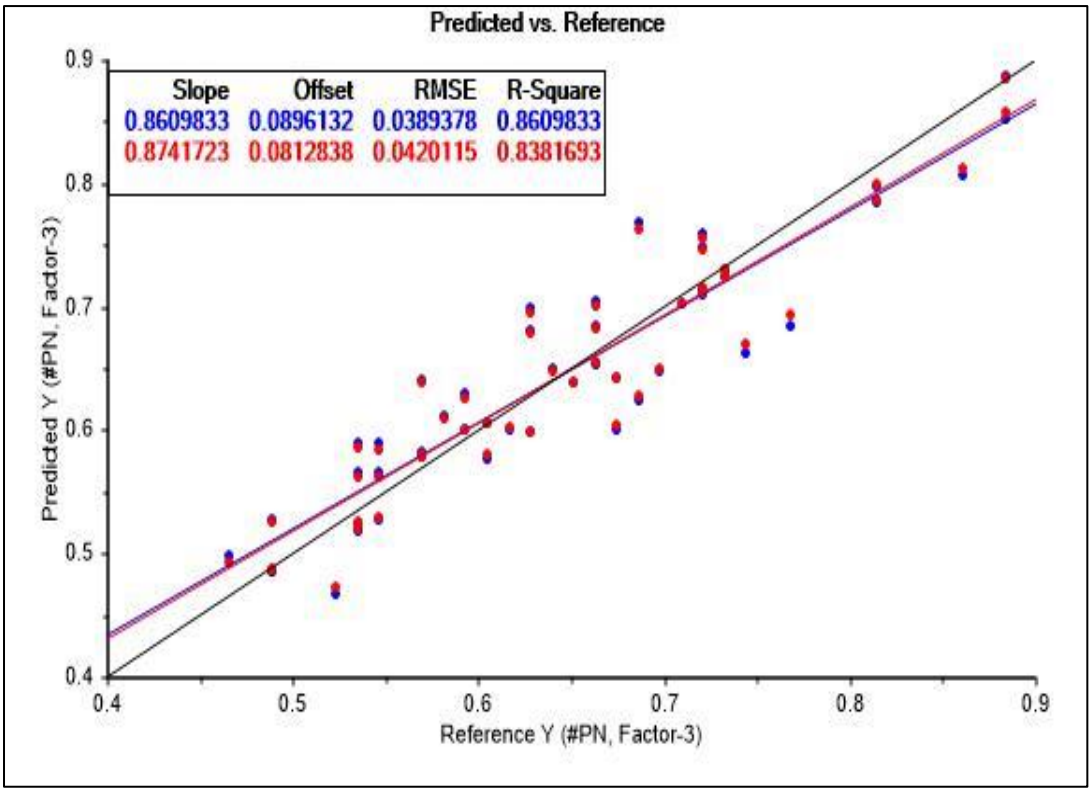

Predicted vs. Reference
Slope Offset RMSE R-Square
0.6477609 0.323791 0.0162548 0.6477609
0.6633788 0.3094116 0.0172436 0.6036026
Predicted Y (#CB, Factor-2)
Reference Y (#CB, Factor-2)
1
0.9
0.8
0.8
0.9
1

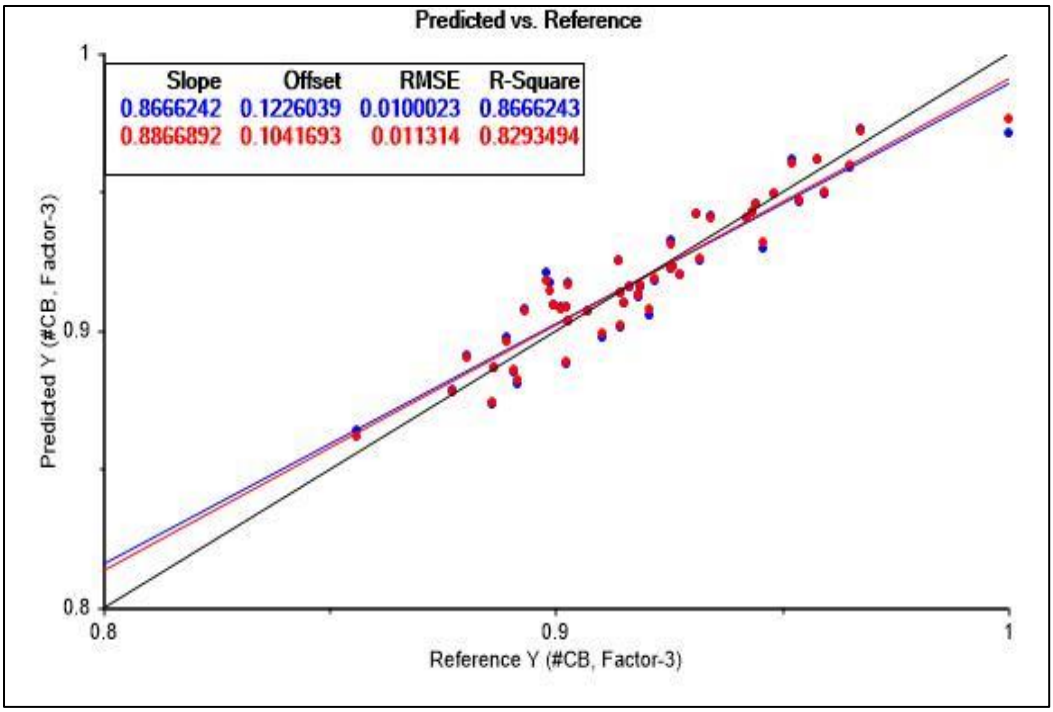
Appendix 3. Reference and predicted values of proximate compositions of pearl millet at Factor 3 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



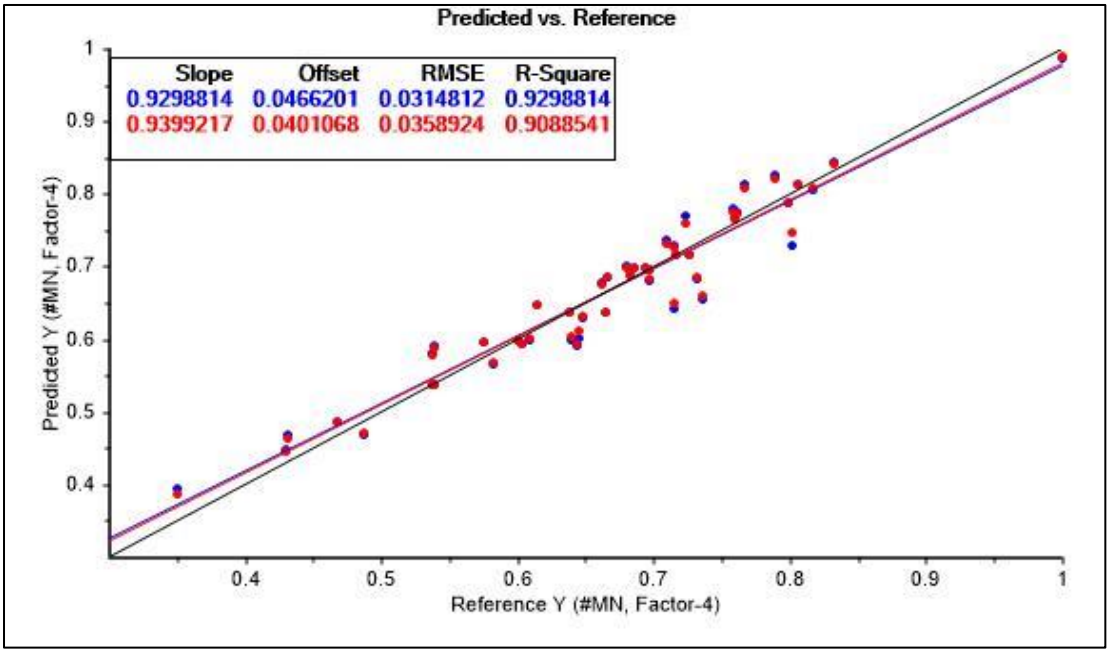


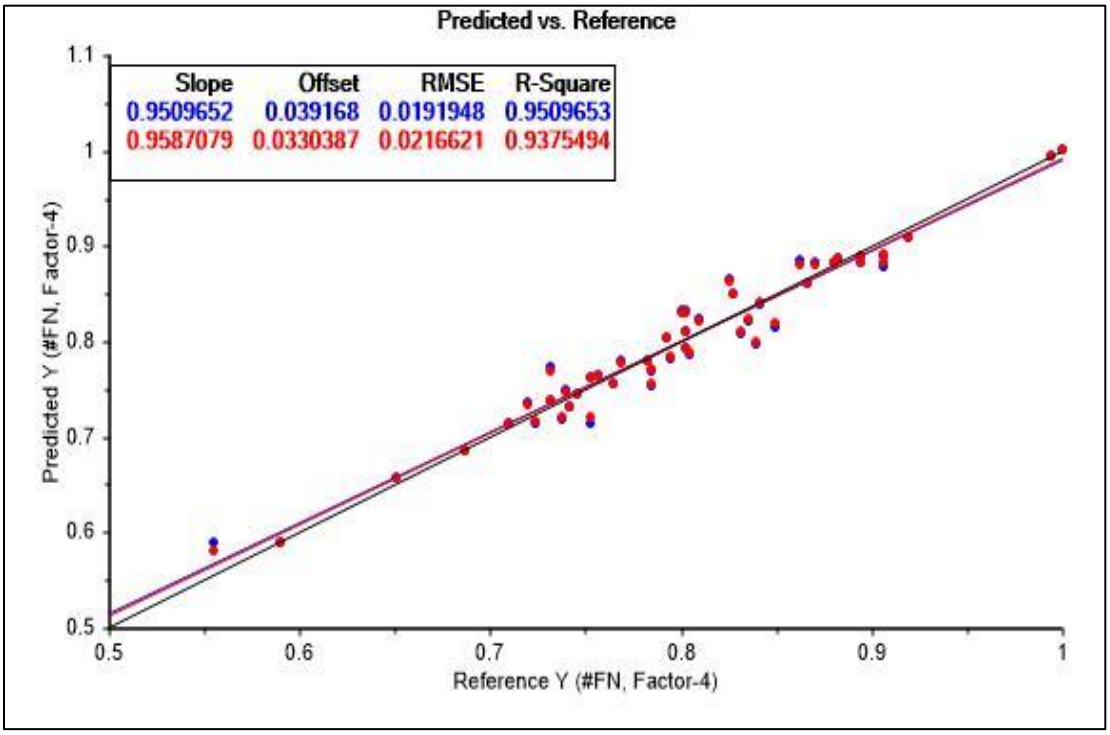

Predicted vs. Reference
Slope Offset RMSE R-Square
0.7636942 0.1284913 0.0675193 0.7636943
0.7876847 0.1155886 0.0733156 0.7213807
Predicted Y (#FB, Factor-3)
Reference Y (#FB, Factor-3)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1

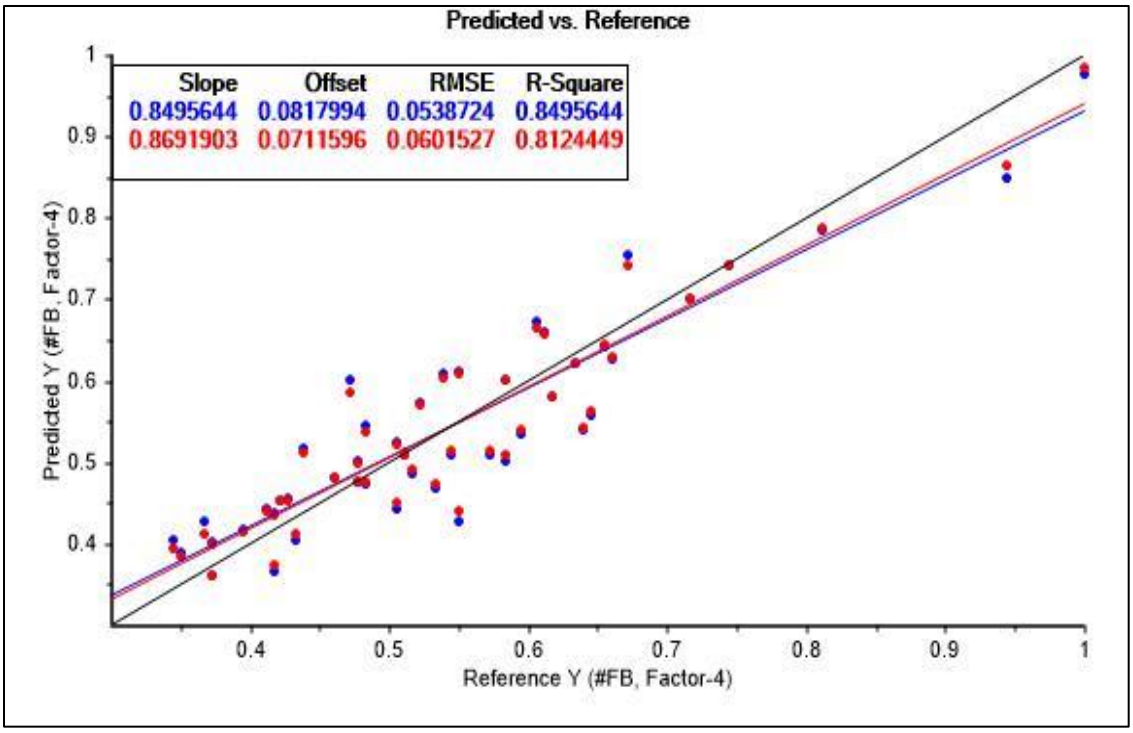

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8609833 0.0896132 0.0389378 0.8609833
0.8741723 0.0812838 0.0420115 0.8381693
Predicted Y (#PN, Factor-3)
Reference Y (#PN, Factor-3)
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9

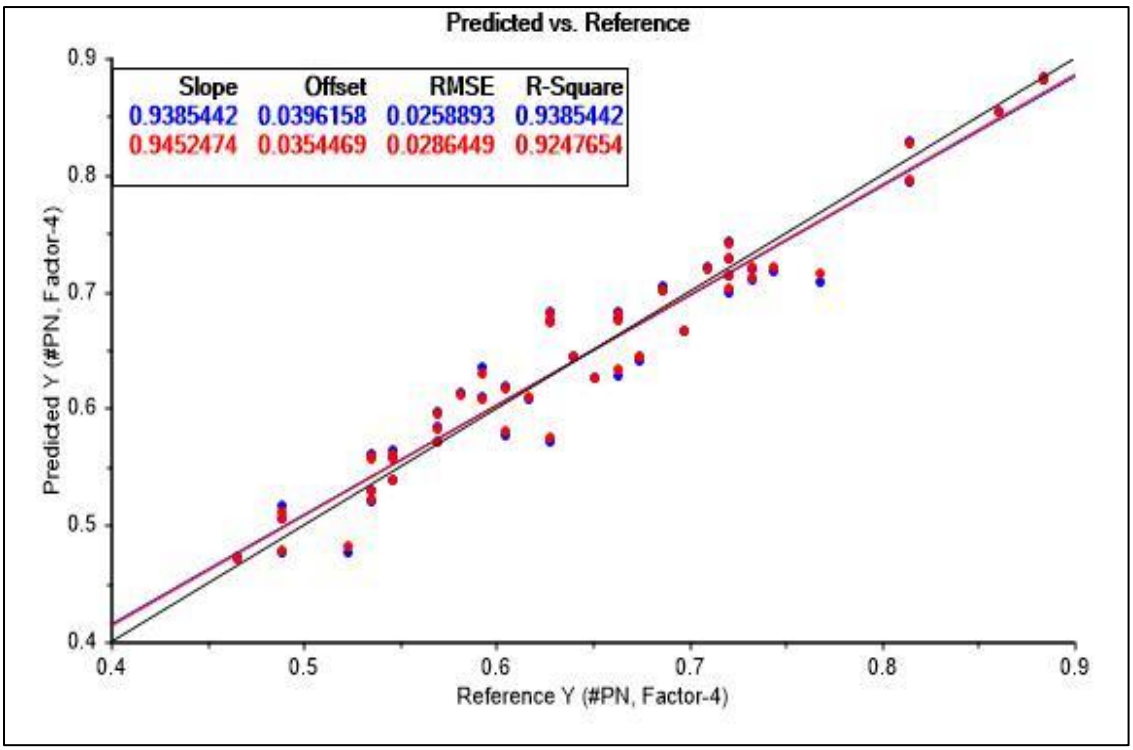

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8666242 0.1226039 0.0100023 0.8666243
0.8866892 0.1041693 0.011314 0.8293494
Predicted Y (#CB, Factor-3)
Reference Y (#CB, Factor-3)
1
0.9
0.8
0.8
0.9
1

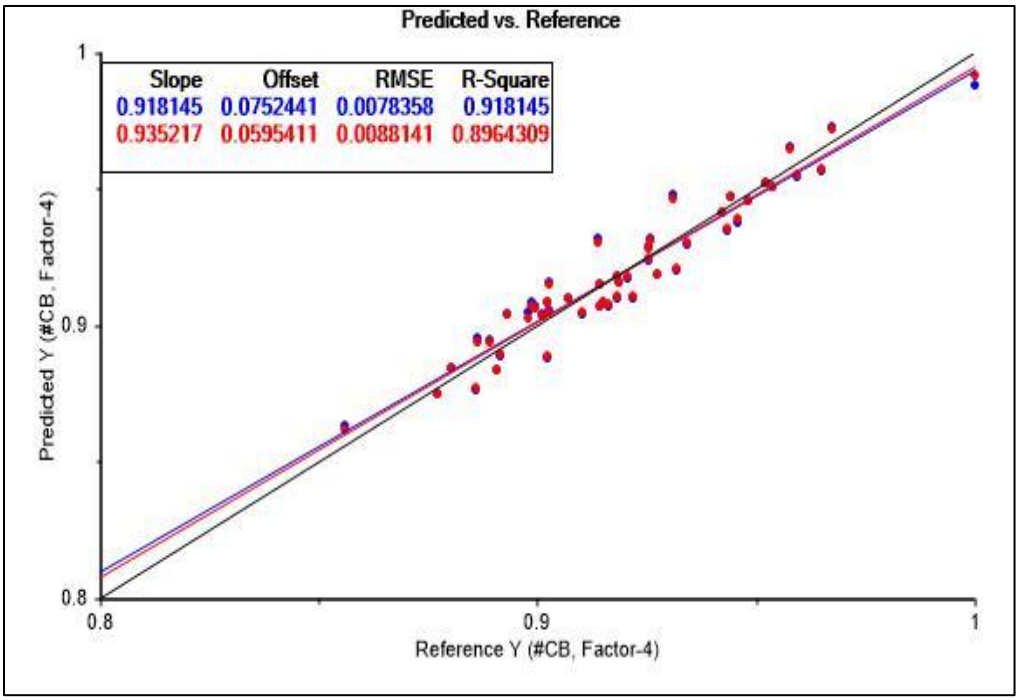
Appendix 4. Reference and predicted values of proximate compositions of pearl millet at Factor 4 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



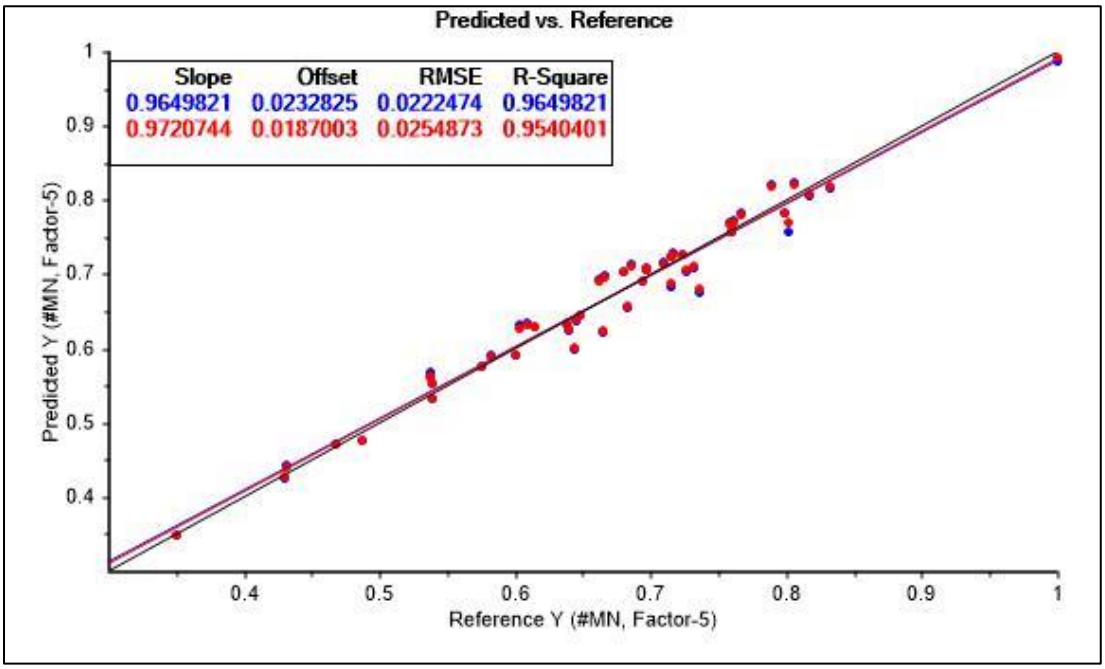


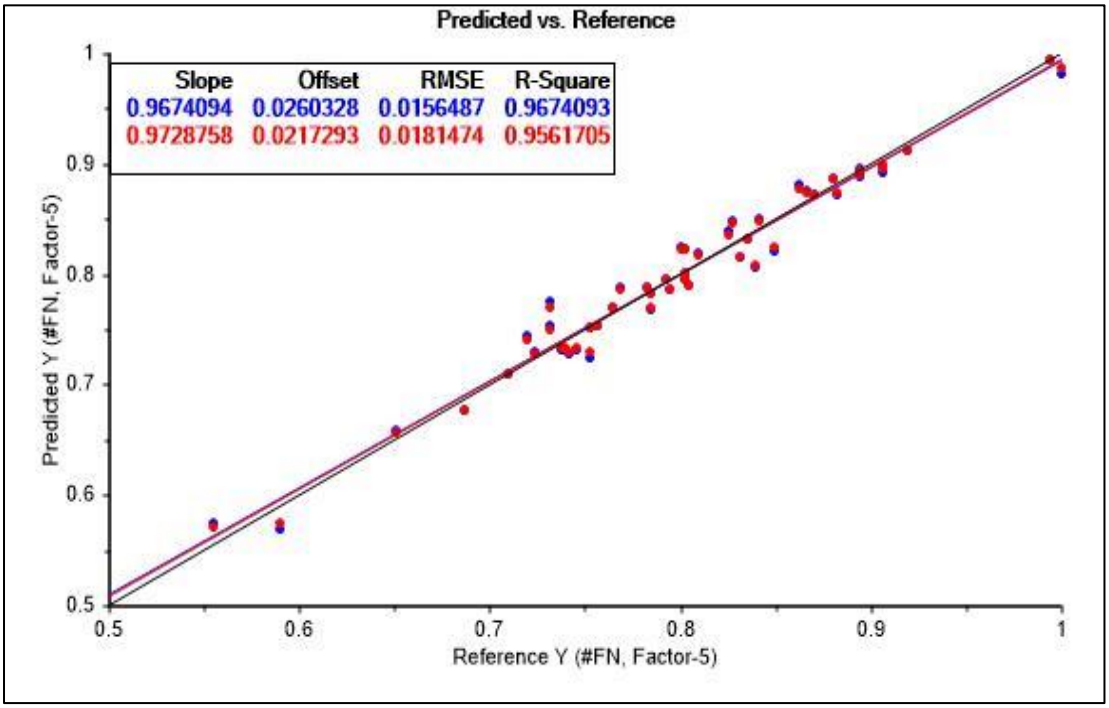

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8495644 0.0817994 0.0538724 0.8495644
0.8691903 0.0711596 0.0601527 0.8124449
Predicted Y (#FB, Factor-4)
Reference Y (#FB, Factor-4)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1

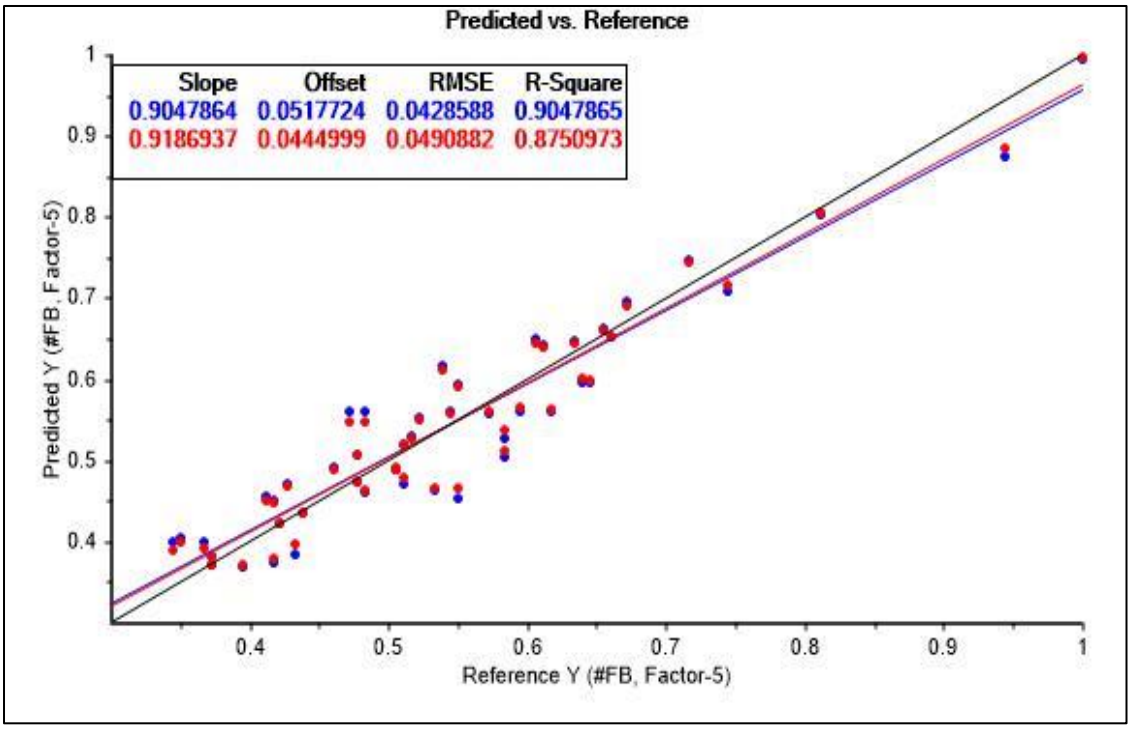

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9385442 0.0396158 0.0258893 0.9385442
0.9452474 0.0354469 0.0286449 0.9247654
Predicted Y (#PN, Factor-4)
Reference Y (#PN, Factor-4)
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9

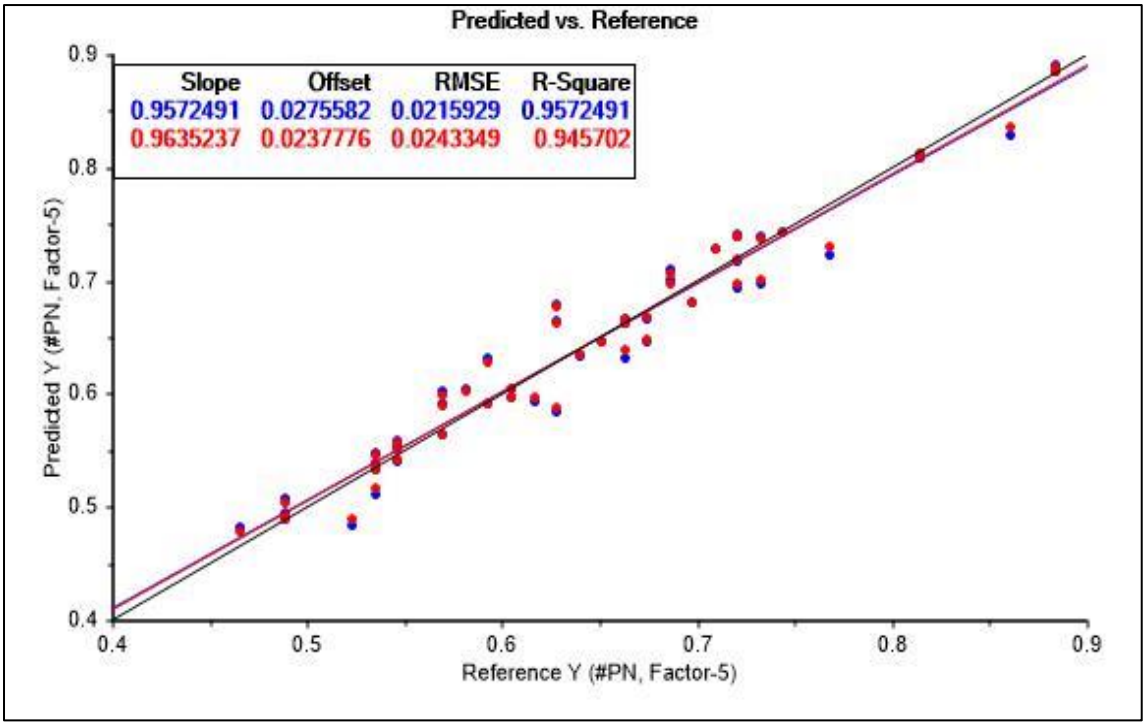

Predicted vs. Reference
Slope Offset RMSE R-Square
0.918145 0.0752441 0.0078358 0.918145
0.935217 0.0595411 0.0088141 0.8964309
Predicted Y (#CB, Factor-4)
Reference Y (#CB, Factor-4)
1
0.9
0.8
0.8
0.9
1

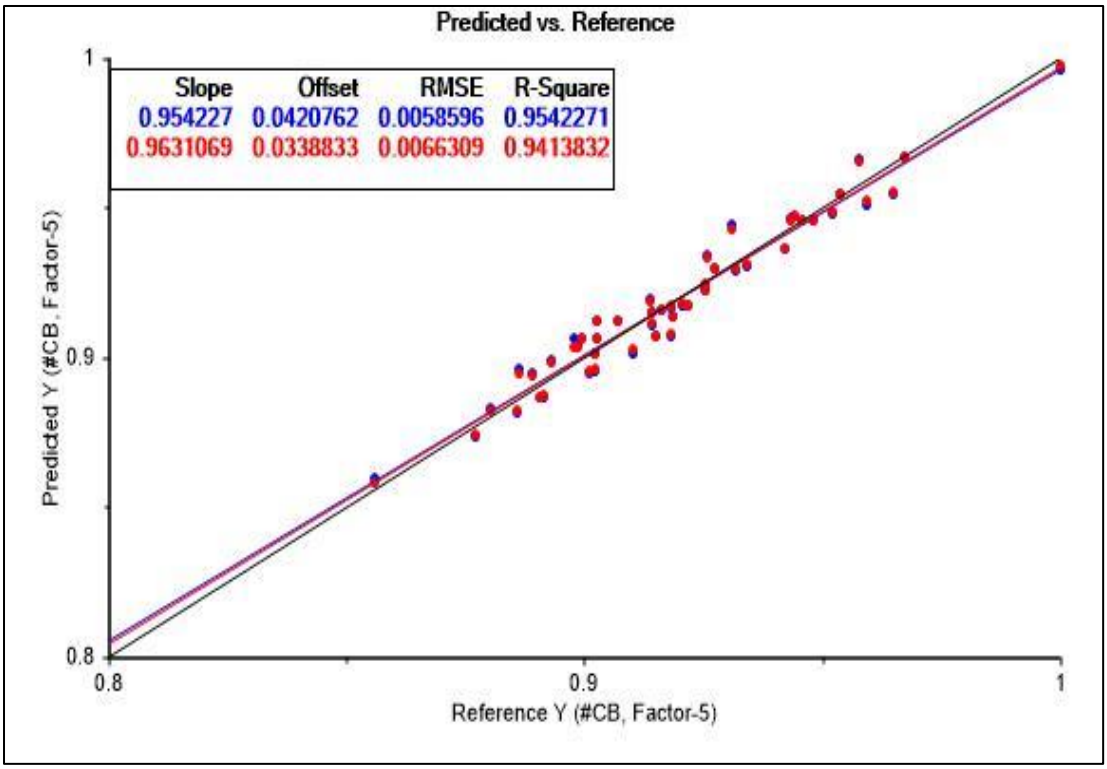
Appendix 5. Reference and predicted values of proximate compositions of pearl millet at Factor 5 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



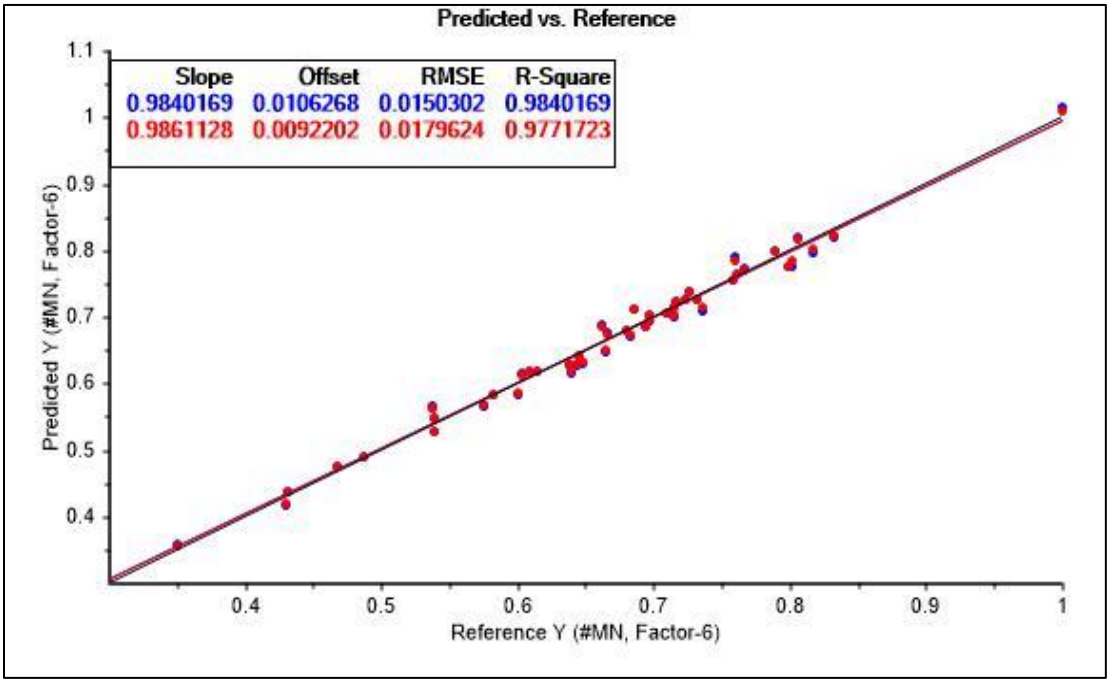


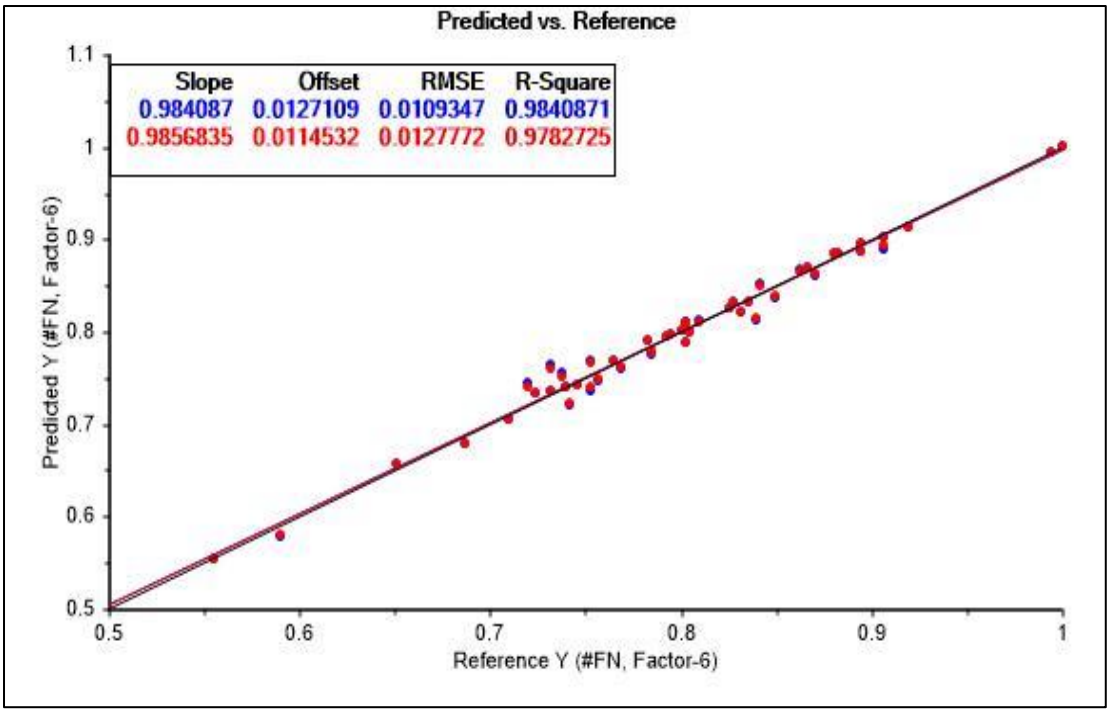

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9047864 0.0517724 0.0428588 0.9047865
0.9186937 0.0444999 0.0490882 0.8750973
Predicted Y (#FB, Factor-5)
Reference Y (#FB, Factor-5)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1

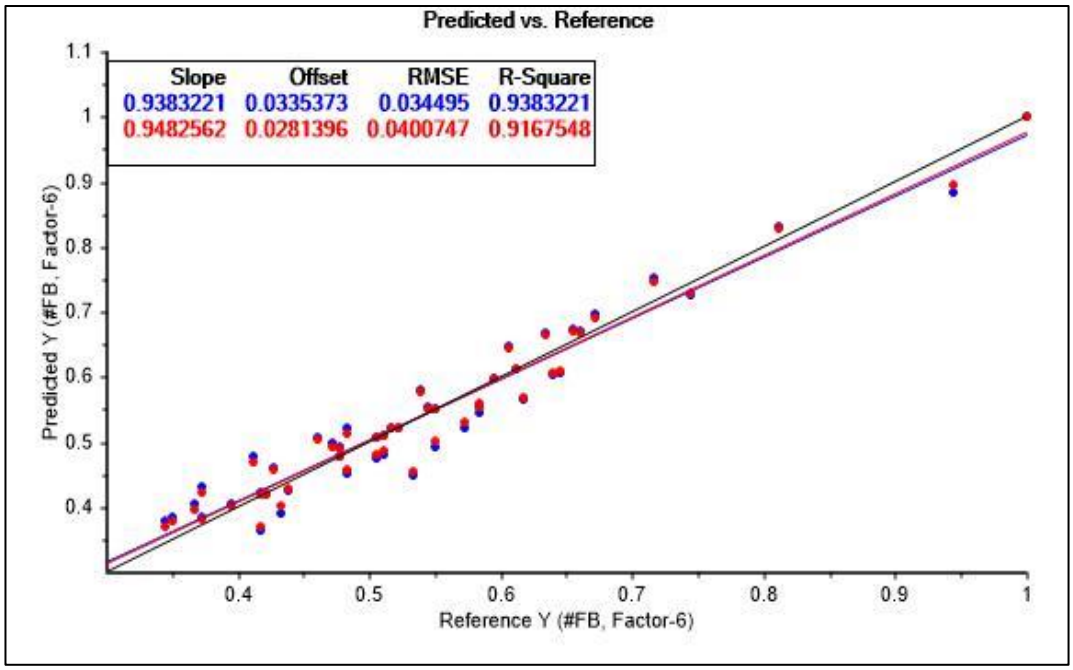

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9572491 0.0275582 0.0215929 0.9572491
0.9635237 0.0237776 0.0243349 0.945702
Predicted Y (#PN, Factor-5)
Reference Y (#PN, Factor-5)
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9

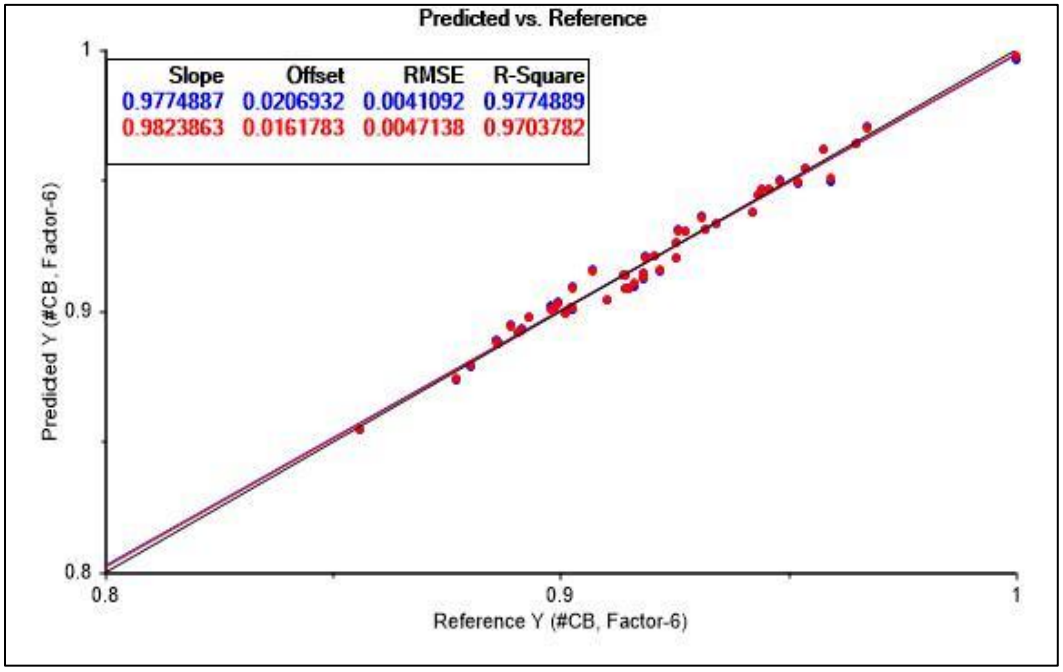

Predicted vs. Reference
Slope Offset RMSE R-Square
0.954227 0.0420762 0.0058596 0.9542271
0.9631069 0.0338833 0.0066309 0.9413832
Predicted Y (#CB, Factor-5)
Reference Y (#CB, Factor-5)
1
0.9
0.8
0.8
0.9
1

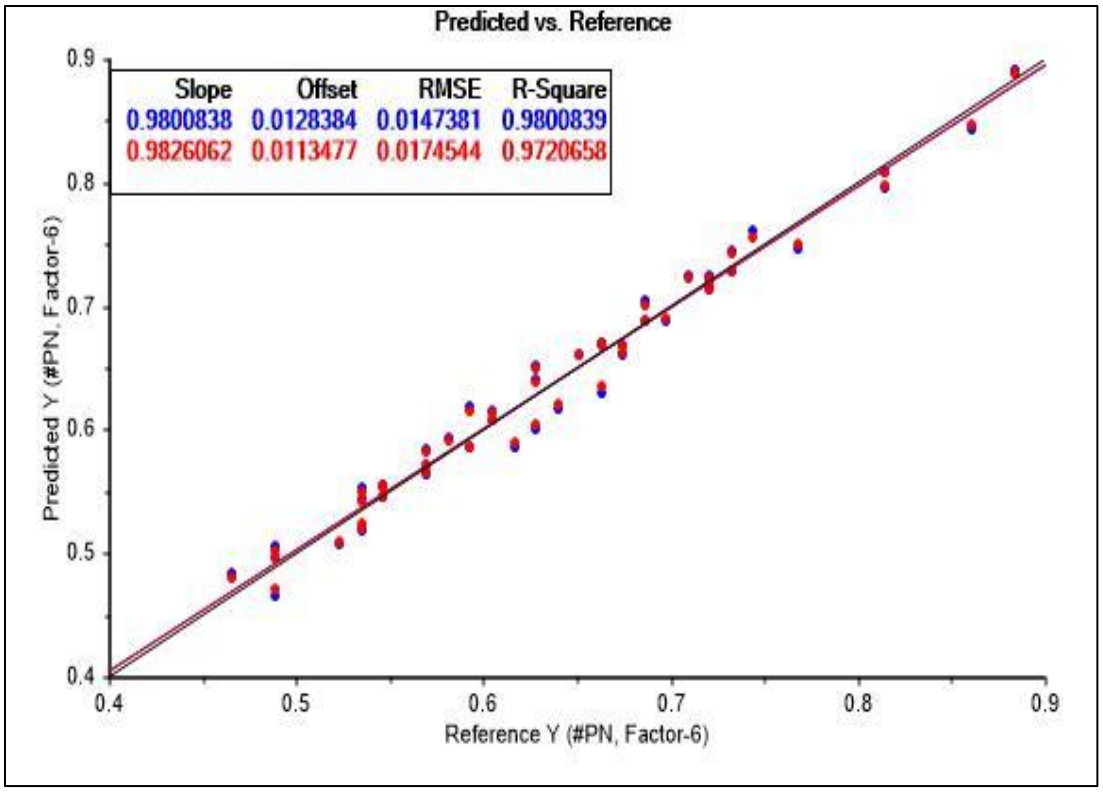
Appendix 6. Reference and predicted values of proximate compositions of pearl millet at Factor 6 using NIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



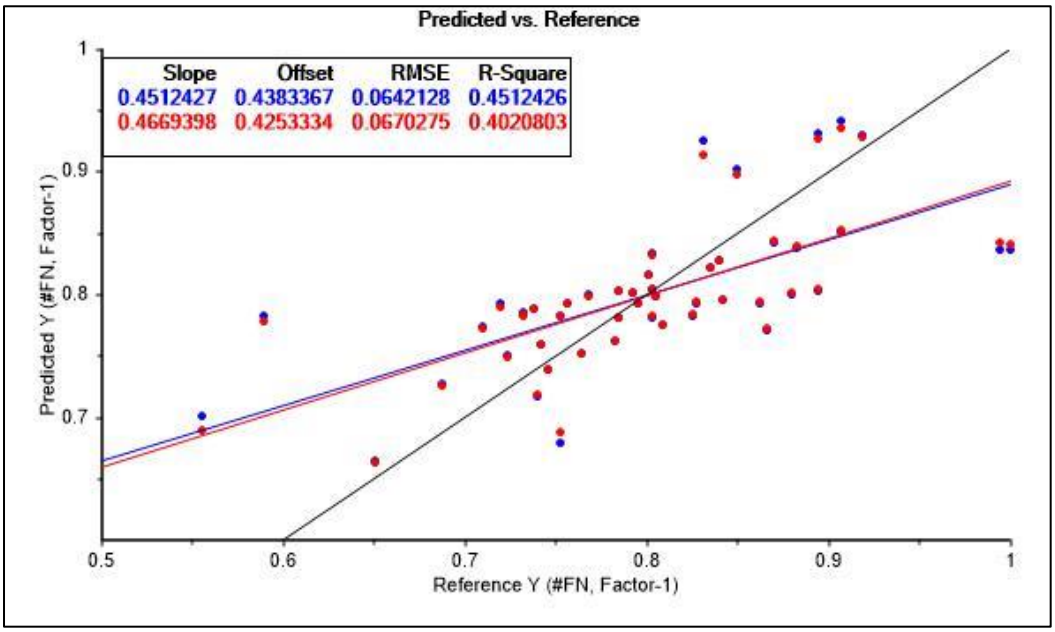


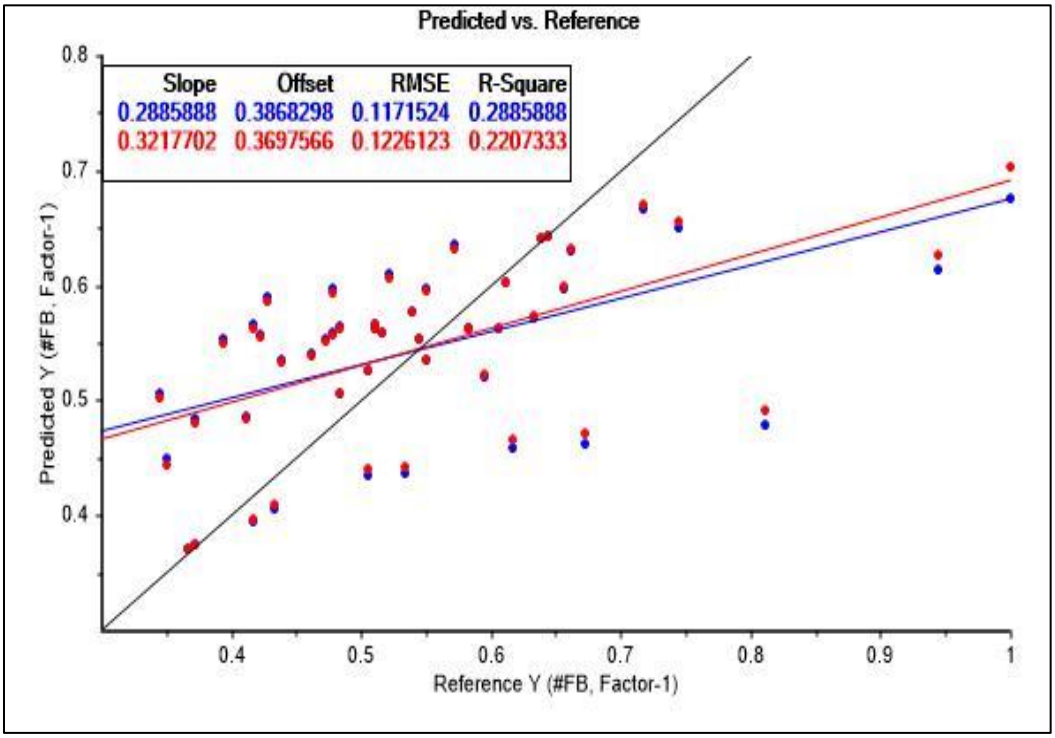

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9383221 0.0335373 0.034495 0.9383221
0.9482562 0.0281396 0.0400747 0.9167548
Predicted Y (#FB, Factor-6)
Reference Y (#FB, Factor-6)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1

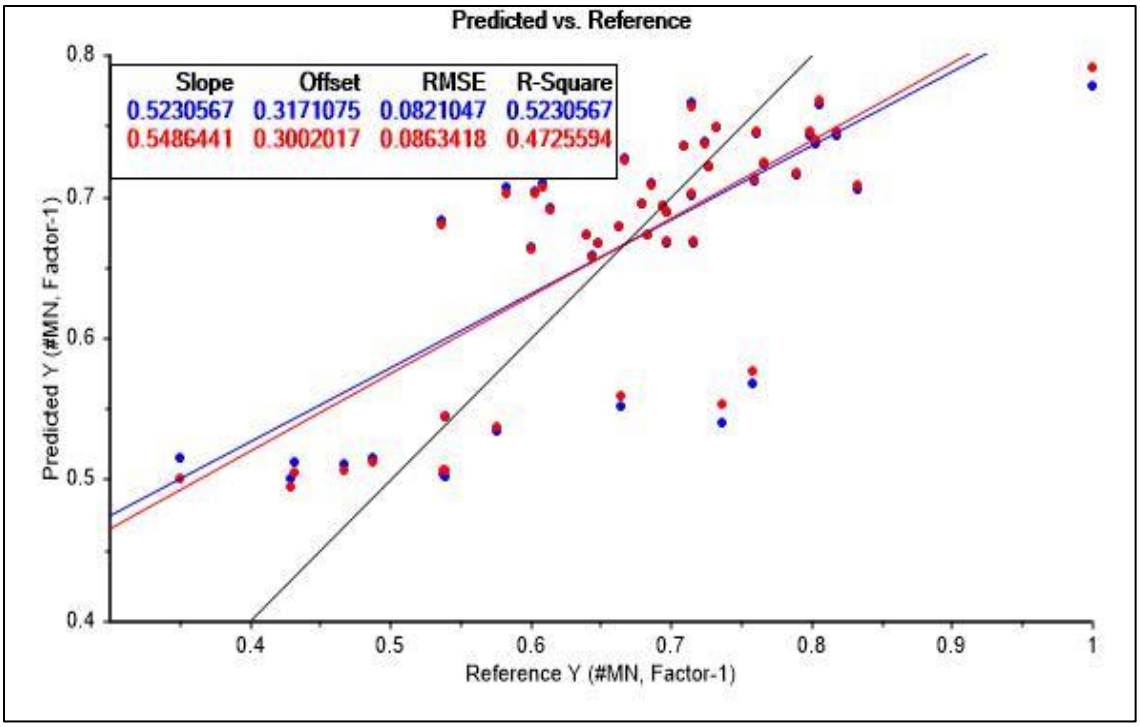

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9774887 0.0206932 0.0041092 0.9774889
0.9823863 0.0161783 0.0047138 0.9703782
Predicted Y (#CB, Factor-6)
Reference Y (#CB, Factor-6)
1
0.9
0.8
0.8 0.9 1

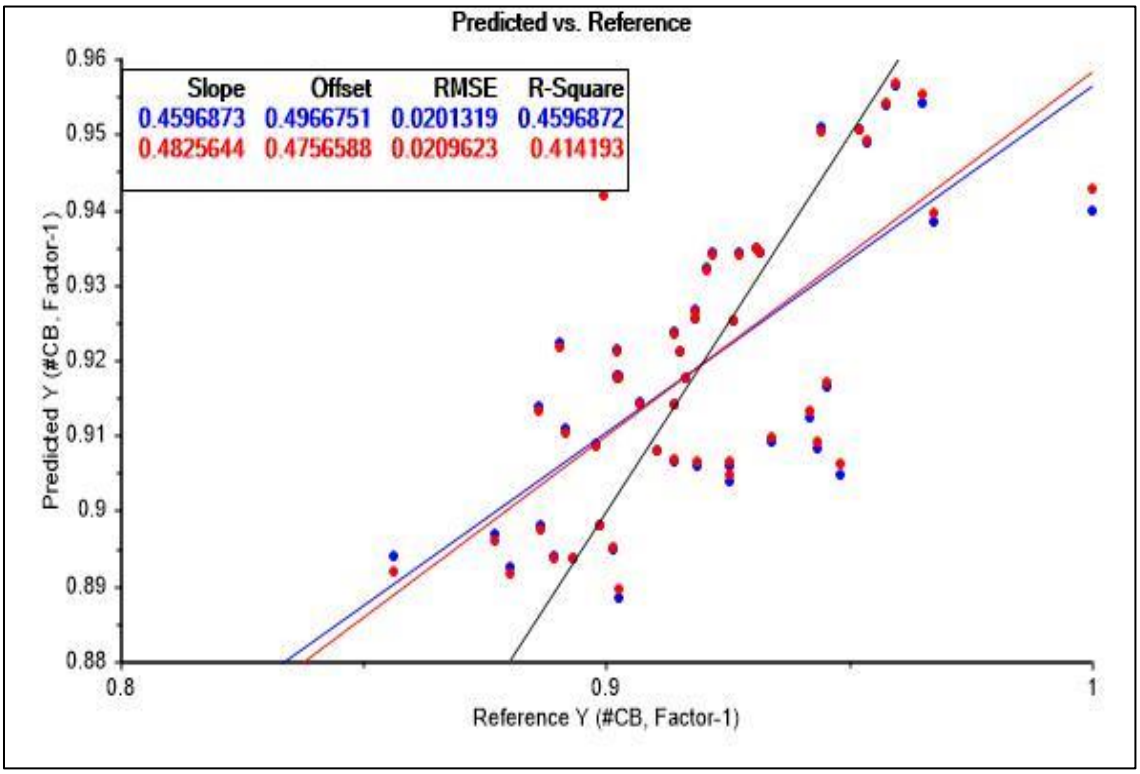

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9800838 0.0128384 0.0147381 0.9800839
0.9826062 0.0113477 0.0174544 0.9720658
Predicted Y (#PN, Factor-6)
Reference Y (#PN, Factor-6)
0.4
0.5
0.6
0.7
0.8
0.9

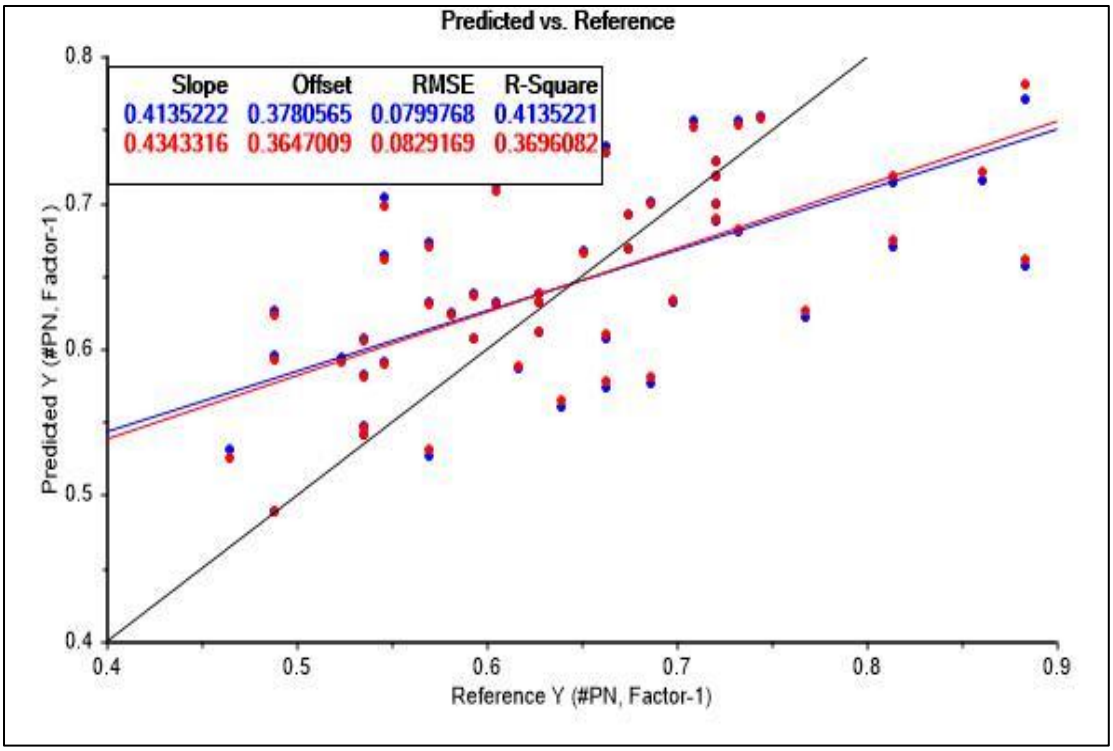
Appendix 7. Reference and predicted values of proximate compositions of pearl millet at Factor 1 using FTIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



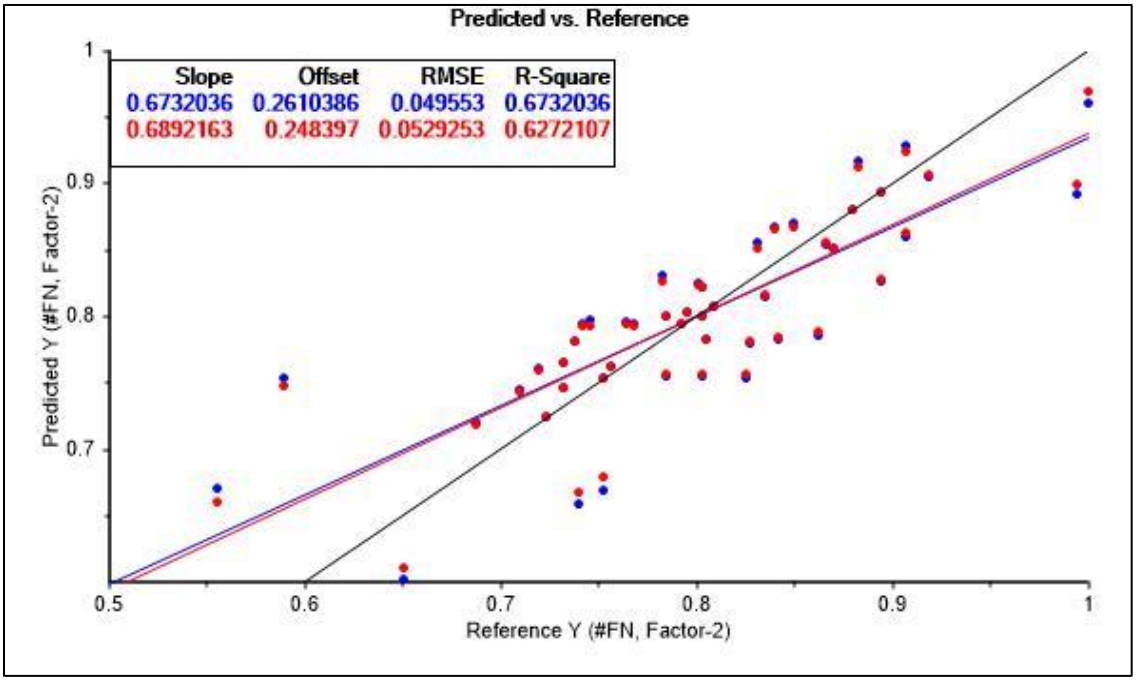


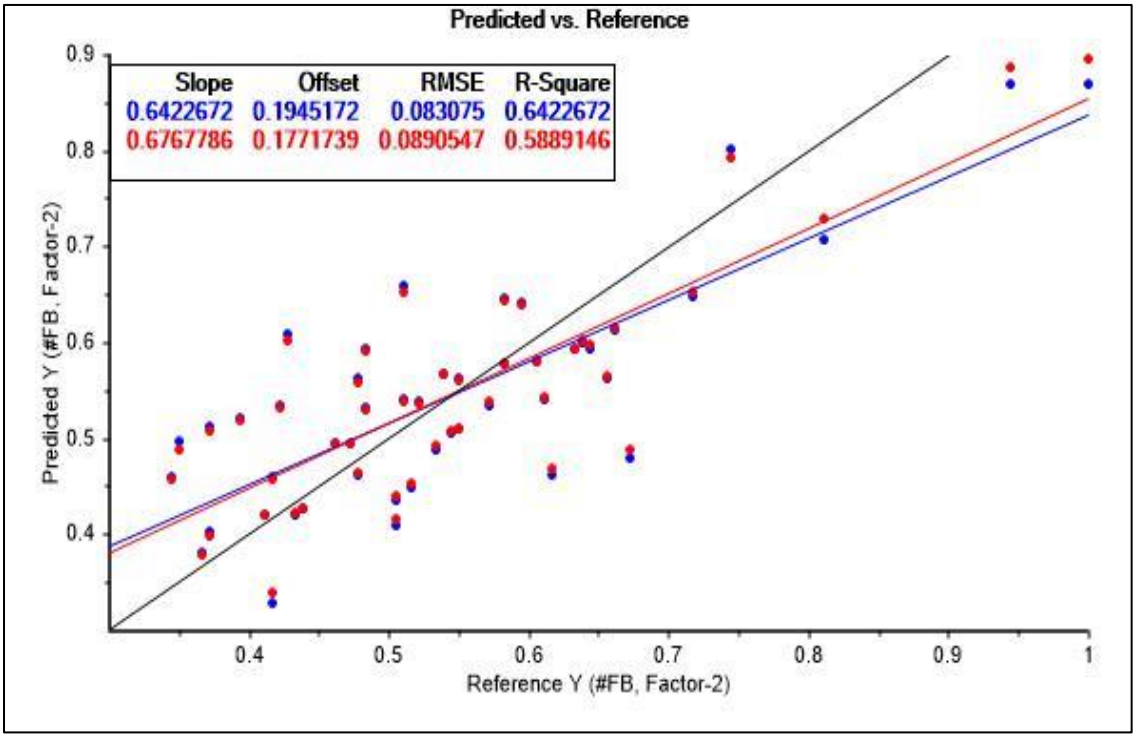

Predicted vs. Reference
Slope Offset RMSE R-Square
0.5230567 0.3171075 0.0821047 0.5230567
0.5486441 0.3002017 0.0863418 0.4725594
Predicted Y (#MN, Factor-1)
Reference Y (#MN, Factor-1)
0.8
0.7
0.6
0.5
0.4
0.4
0.5
0.6
0.7
0.8
0.9
1

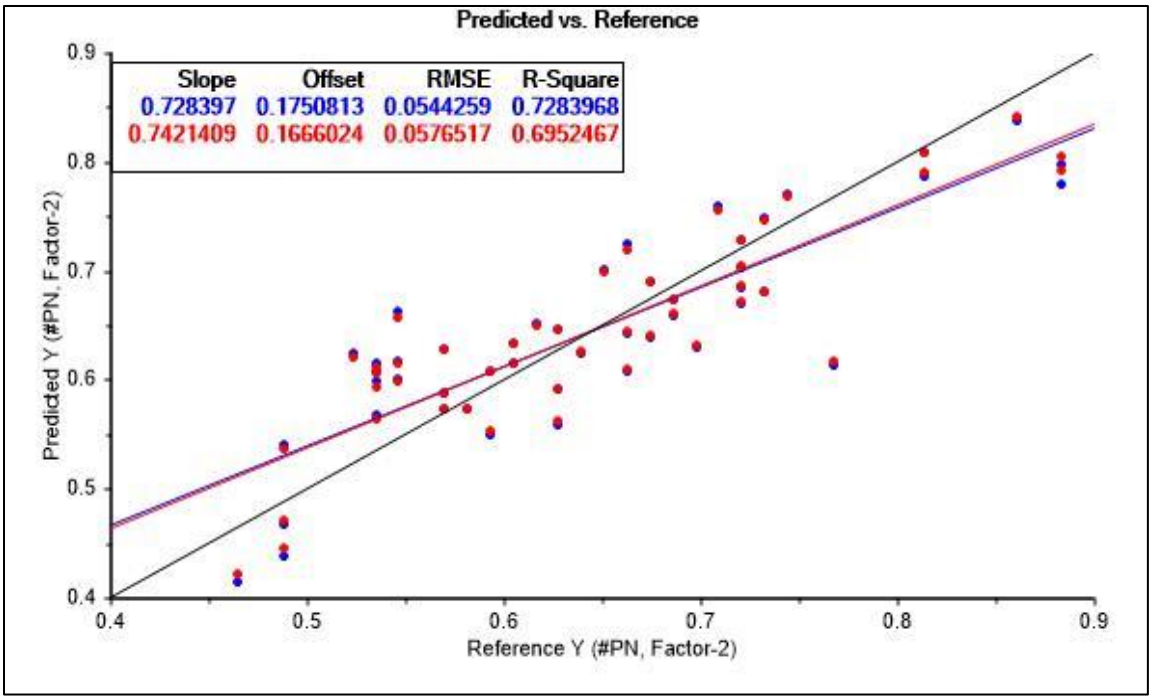

Predicted vs. Reference
Slope Offset RMSE R-Square
0.4596873 0.4966751 0.0201319 0.4596872
0.4825644 0.4756588 0.0209623 0.414193
Predicted Y (#CB, Factor-1)
Reference Y (#CB, Factor-1)
0.96
0.95
0.94
0.93
0.92
0.91
0.9
0.89
0.88
0.8
0.9
1

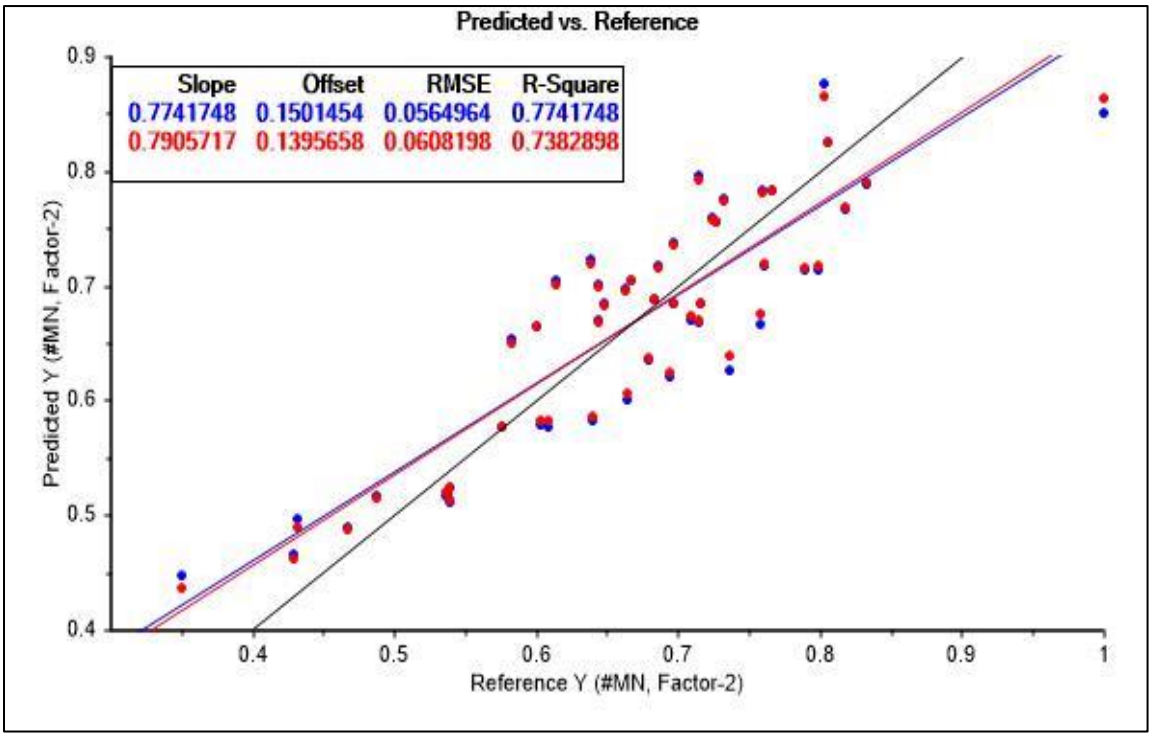

Predicted vs. Reference
Slope Offset RMSE R-Square
0.4135222 0.3780565 0.0799768 0.4135221
0.4343316 0.3647009 0.0829169 0.3696082
Predicted Y (#PN, Factor-1)
Reference Y (#PN, Factor-1)
0.8
0.7
0.6
0.5
0.4
0.4
0.5
0.6
0.7
0.8
0.9

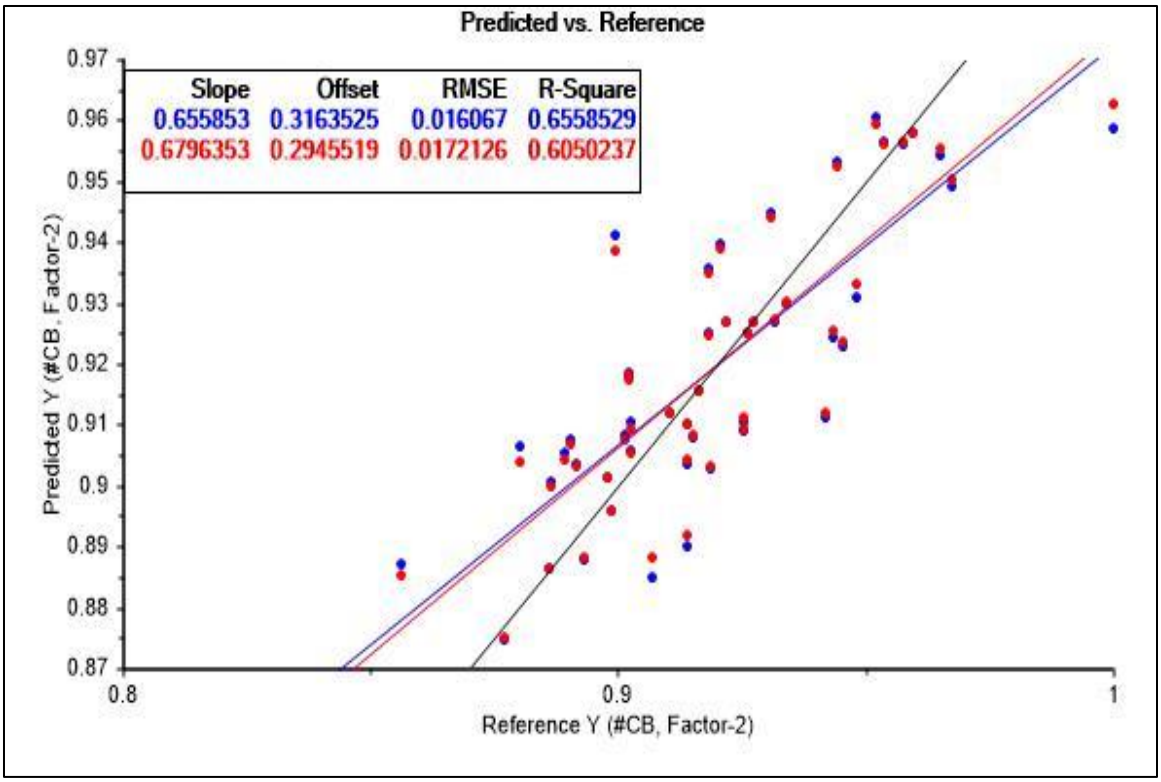
Appendix 8. Reference and predicted values of proximate compositions of pearl millet at Factor 2 using FTIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



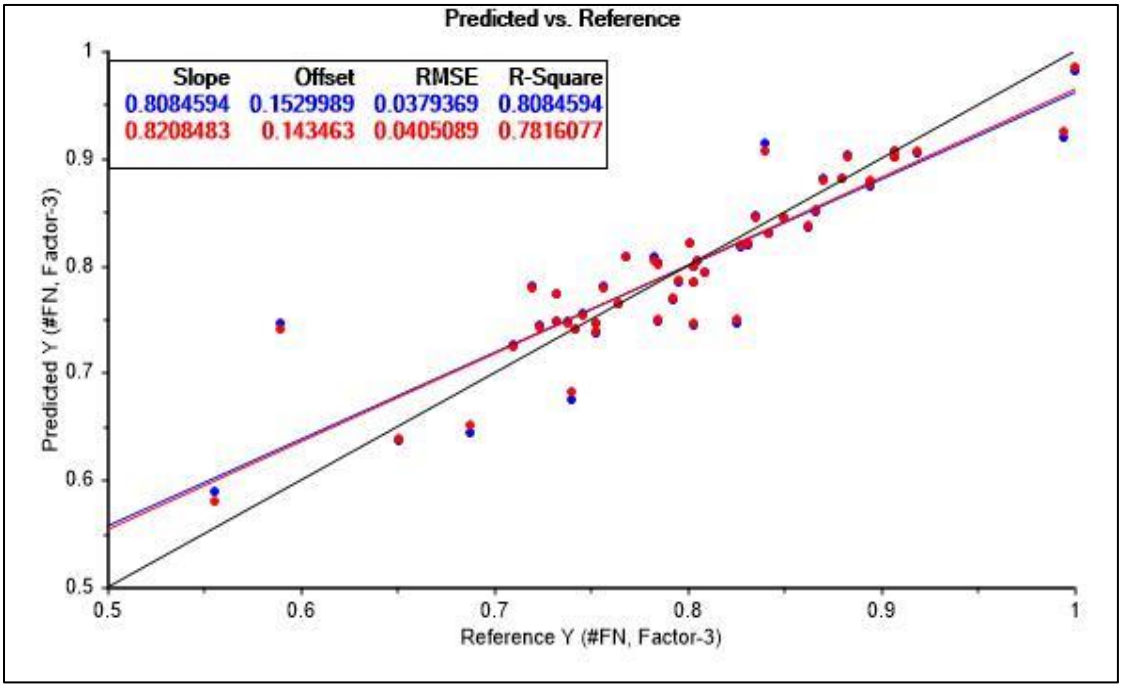


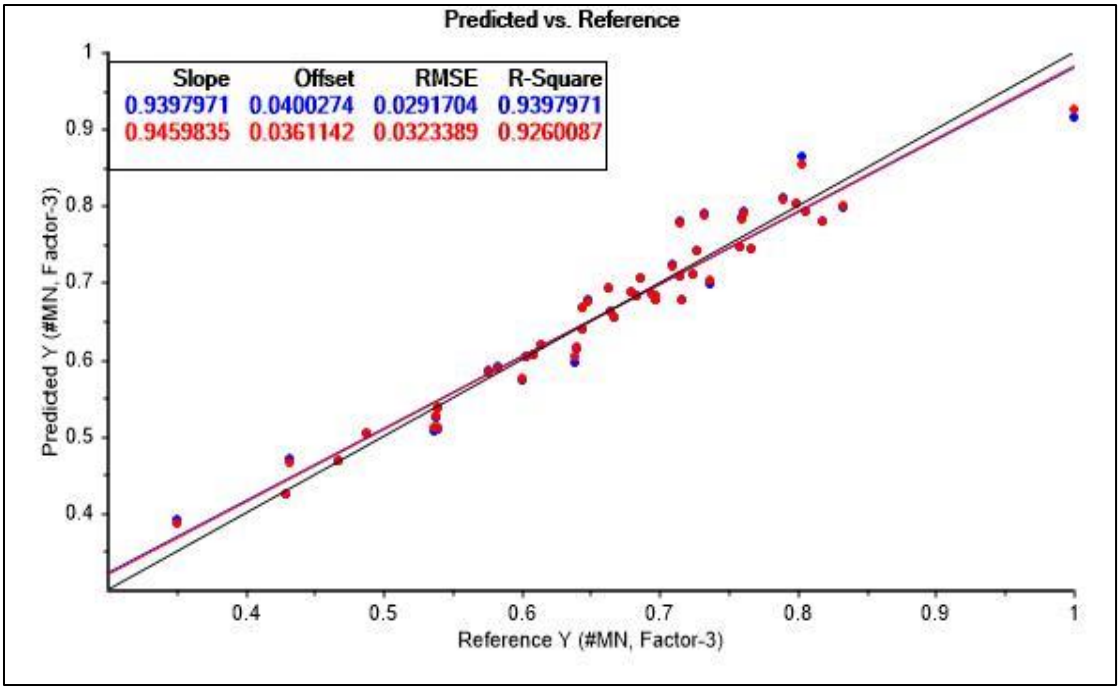

Predicted vs. Reference
Slope Offset RMSE R-Square
0.728397 0.1750813 0.0544259 0.7283968
0.7421409 0.1666024 0.0576517 0.6952467
Predicted Y (#PN, Factor-2)
Reference Y (#PN, Factor-2)
0.4
0.5
0.6
0.7
0.8
0.9

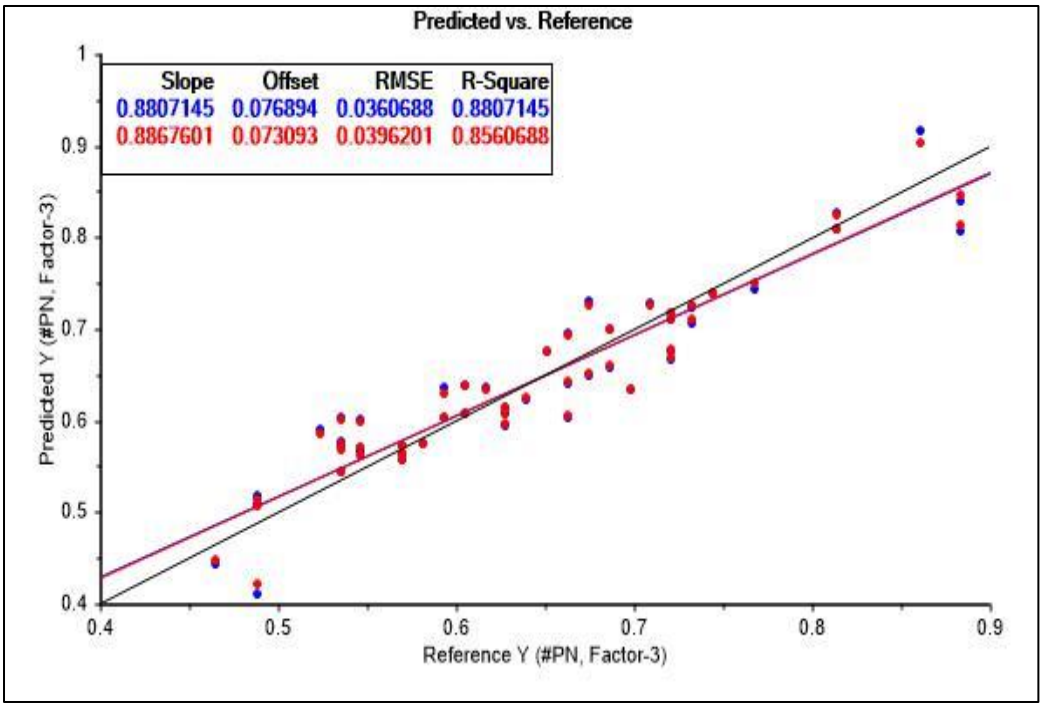

Predicted vs. Reference
Slope Offset RMSE R-Square
0.7741748 0.1501454 0.0564964 0.7741748
0.7905717 0.1395658 0.0608198 0.7382898
Predicted Y (#MN, Factor-2)
Reference Y (#MN, Factor-2)
0.4
0.5
0.6
0.7
0.8
0.9
1

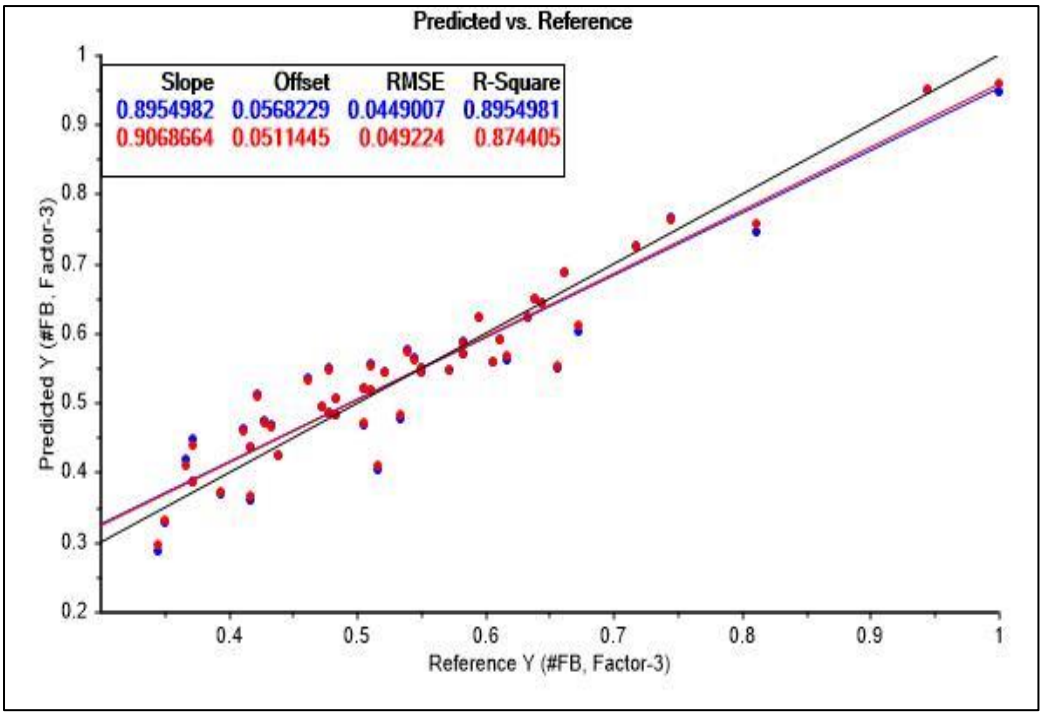

Predicted vs. Reference
Slope Offset RMSE R-Square
0.655853 0.3163525 0.016067 0.6558529
0.6796353 0.2945519 0.0172126 0.6050237
Predicted Y (#CB, Factor-2)
Reference Y (#CB, Factor-2)
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.9
0.89
0.88
0.87
0.8
0.9
1

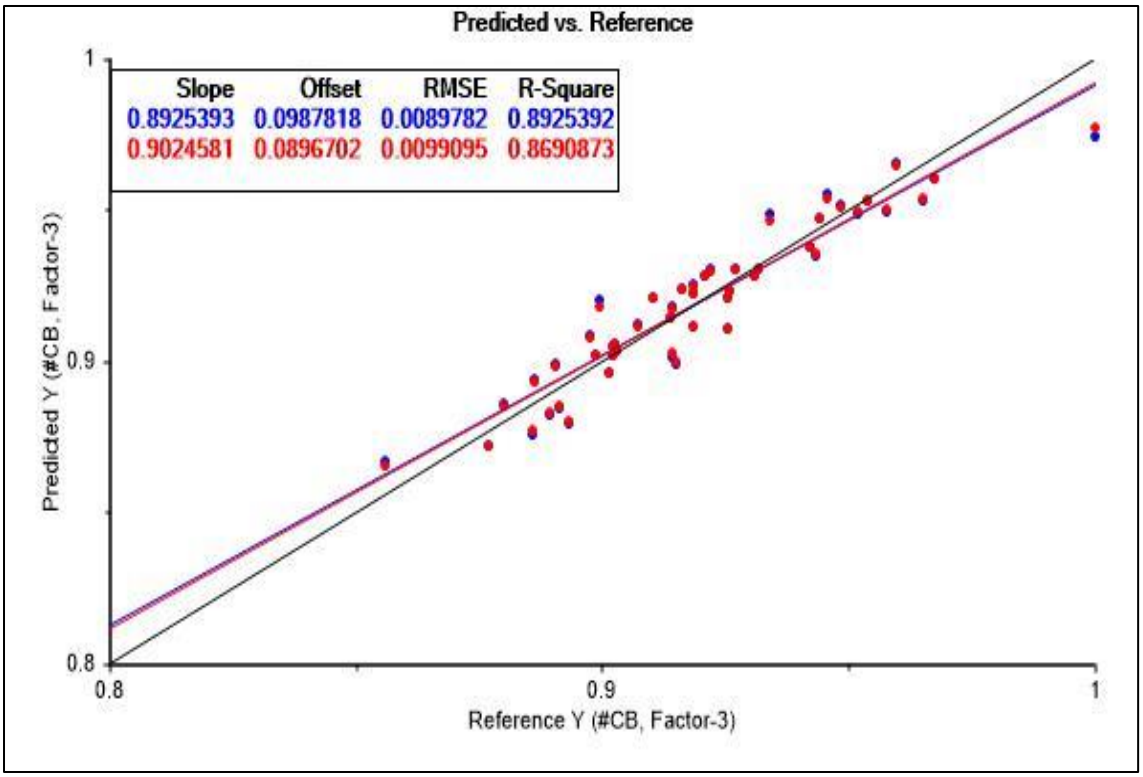
Appendix 9. Reference and predicted values of proximate compositions of pearl millet at Factor 3 using FTIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



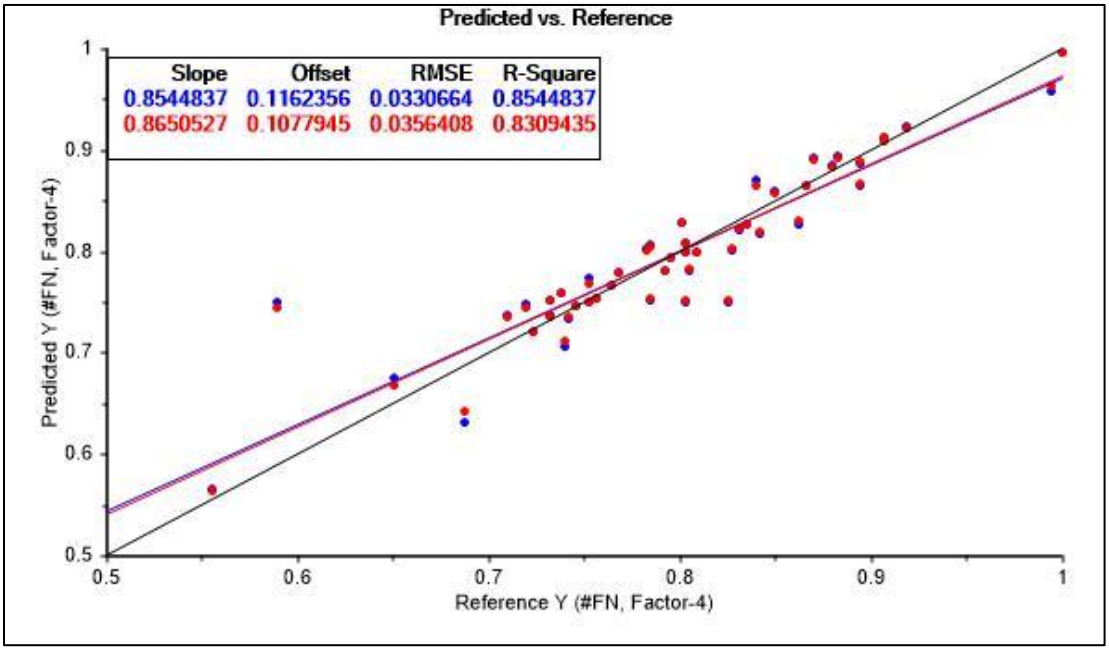


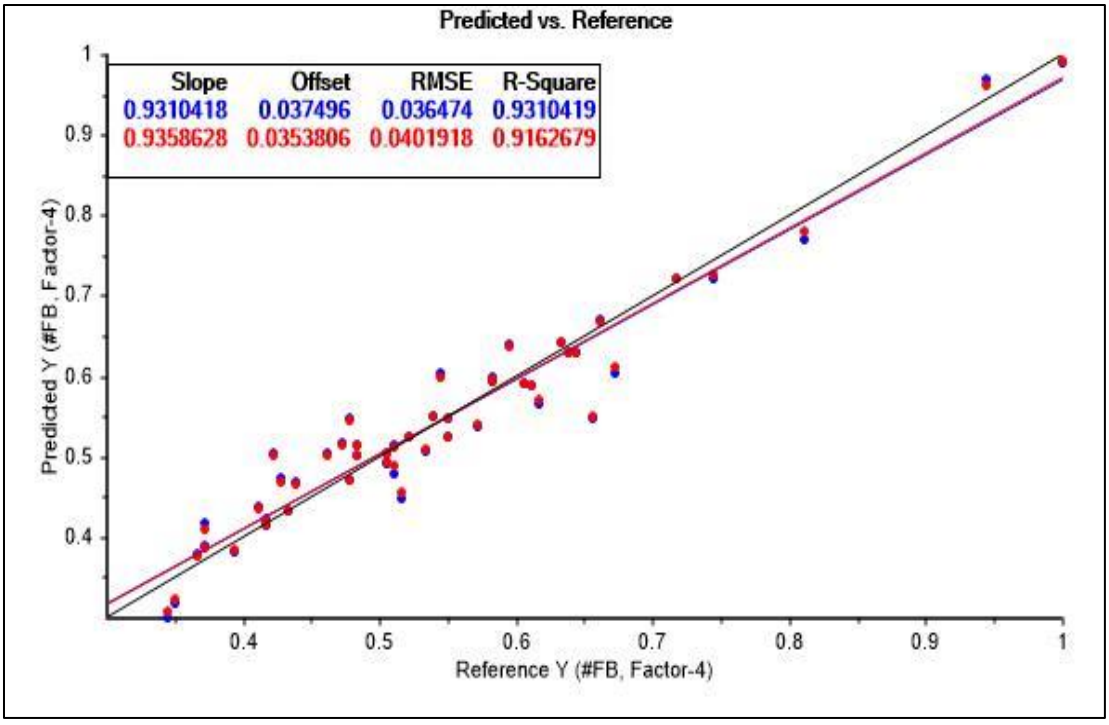

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8807145 0.076894 0.0360688 0.8807145
0.8867601 0.073093 0.0396201 0.8560688
Predicted Y (#PN, Factor-3)
Reference Y (#PN, Factor-3)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9

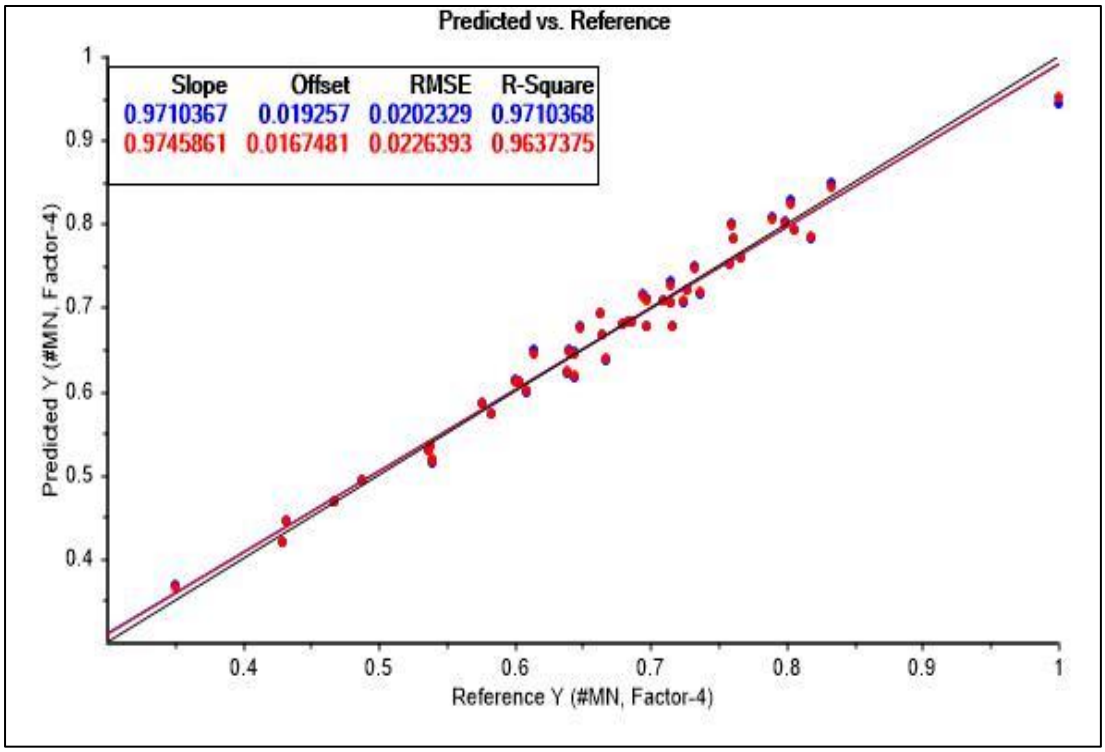

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8954982 0.0568229 0.0449007 0.8954981
0.9068664 0.0511445 0.049224 0.874405
Predicted Y (#FB, Factor-3)
Reference Y (#FB, Factor-3)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.4 0.5 0.6 0.7 0.8 0.9 1

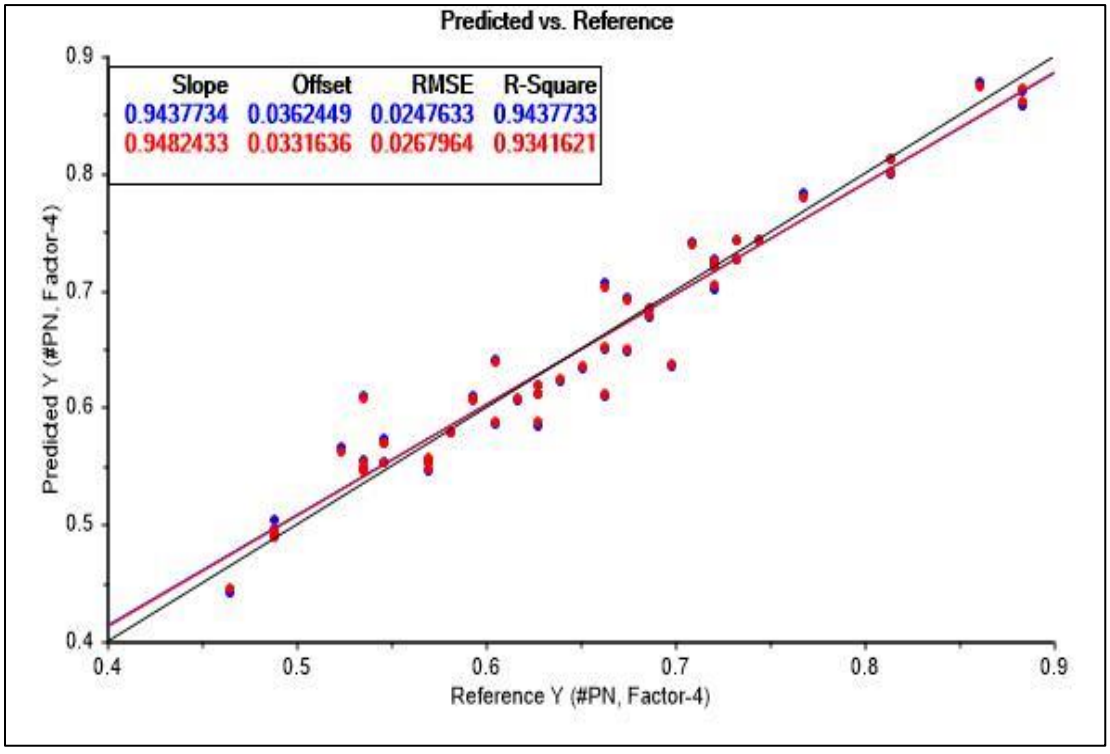

Predicted vs. Reference
Slope Offset RMSE R-Square
0.8925393 0.0987818 0.0089782 0.8925392
0.9024581 0.0896702 0.0099095 0.8690873
Predicted Y (#CB, Factor-3)
Reference Y (#CB, Factor-3)
1
0.9
0.8
0.8
0.9
1

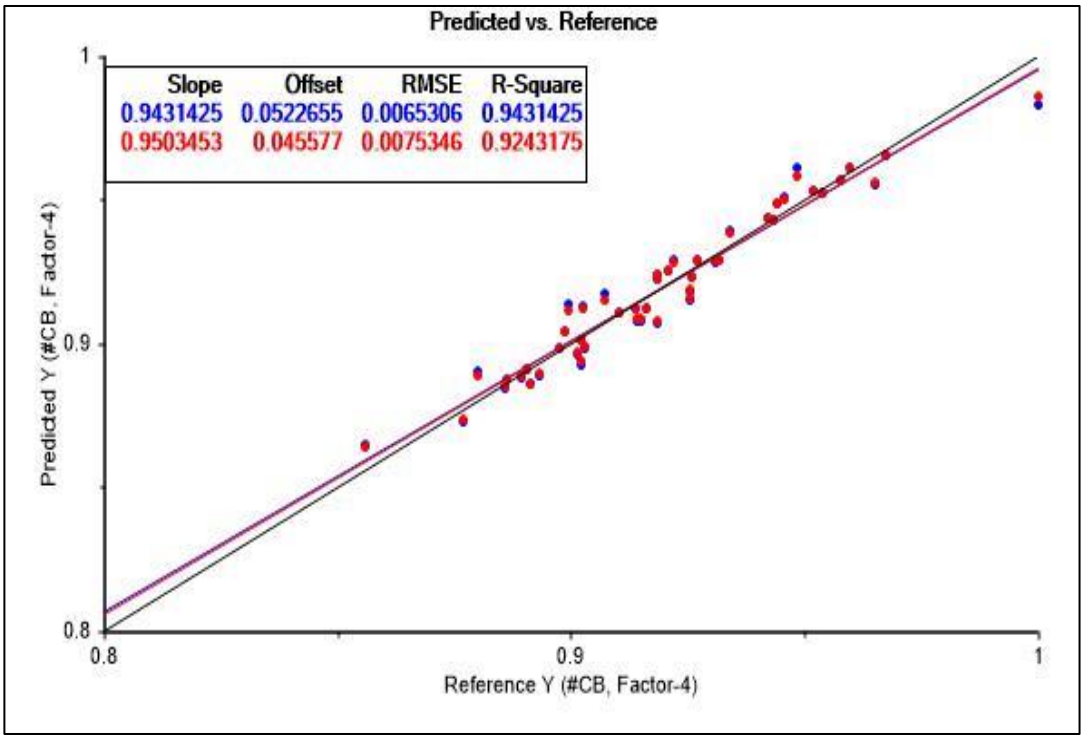
Appendix 10. Reference and predicted values of proximate compositions of pearl millet at Factor 4 using FTIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)



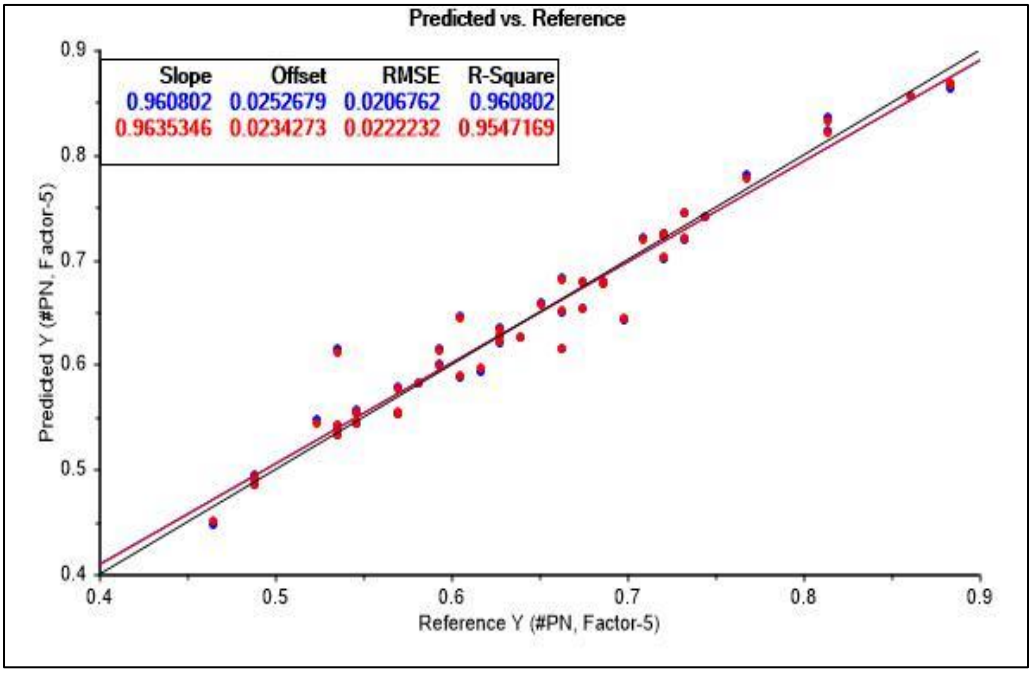


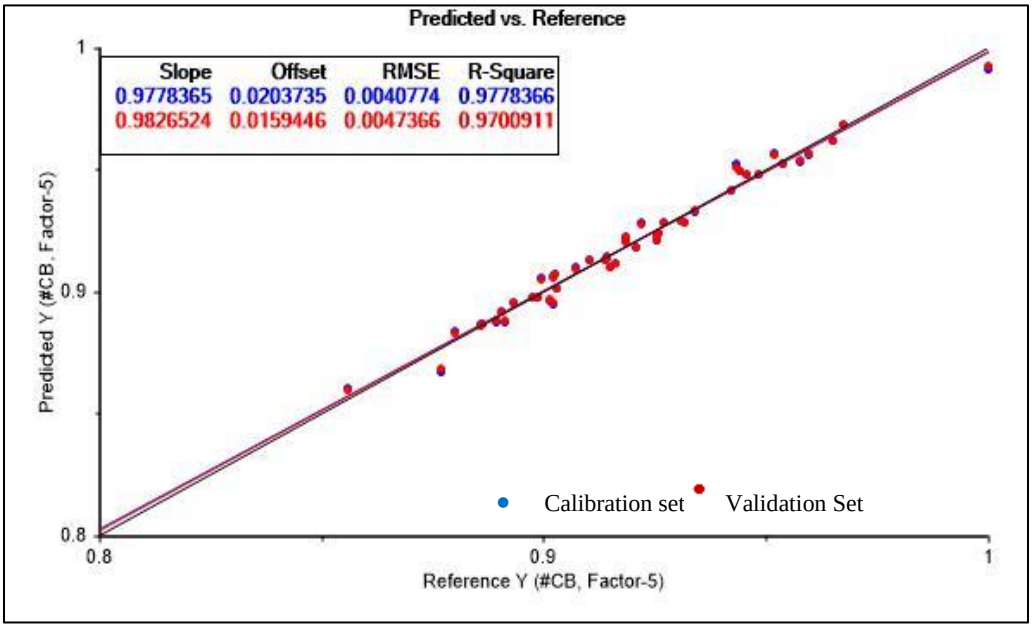

Predicted vs. Reference
Slope Offset RMSE R-Square
0.9710367 0.019257 0.0202329 0.9710368
0.9745861 0.0167481 0.0226393 0.9637375
Predicted Y (#MN, Factor-4)
Reference Y (#MN, Factor-4)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9 1


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9437734 0.0362449 0.0247633 0.9437733
0.9482433 0.0331636 0.0267964 0.9341621
Predicted Y (#PN, Factor-4)
Reference Y (#PN, Factor-4)
0.9
0.8
0.7
0.6
0.5
0.4
0.4 0.5 0.6 0.7 0.8 0.9


Predicted vs. Reference
Slope Offset RMSE R-Square
0.9431425 0.0522655 0.0065306 0.9431425
0.9503453 0.045577 0.0075346 0.9243175
Predicted Y (#CB, Factor-4)
Reference Y (#CB, Factor-4)
1
0.9
0.8
0.8
0.9
1

Appendix 11. Reference and predicted values of proximate compositions of +pearl millet at Factor 5 using FTIR spectroscopy (#MN- Moisture, #FN- Crude Fat, #PN- Crude Protein, #FB- Crude Fiber, #CB- Carbohydrates)





List of Publications

1. Pavas, Neha Munjal, Maninder menu, and Uma Kamboj. “Use of Vibrational Spectroscopy and Proximate Compositions for Geographical Classification of Pearl Millets” Annals of Biology (Accepted manuscript with MS No.- 2023/34)
2. Pavas, Suman, Agnibha das Majumdar, Neha Munjal, and Uma Kamboj. "Characterization of Pennisetum Glaucum (Pearl Millet)." Journal of Physics: Conference Series. Vol. 2267. No. 1. IOP Publishing, 2022.
3. Pavas, N. Munjal, and U. Kamboj. “Study of Physico-chemical properties and spectral data of Pearl millet” AIP conference proceeding (accepted)

List of Conferences

1. Integrated Approaches in Sciences & Technology for Sustainable Future (IASTSF-2022) (28th Feb- 1st March) at J.C Boss University of Science and Technology, YMCA, Faridabad.
2. “5th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES (ICAATAS 2022) on June 4-5, 2022 (IN HYBRID MODE)” being organized by the Society of Agriculture Research and Social Development, MS Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha and Association of Rice Research Workers (ARRW), NRRI.
3. “3rd International Conference on Functional Materials, Manufacturing and Performances (ICFMMP-2022)” held on July29-30th, 2022, organized by Division of Research and Development, Lovely Professional University, Punjab.
4. International Conference on Materials and Emerging Technologies (ICMET- 2021) on Feb 18-19, 2022 organized by Division of Research and Development, Lovely Professional University, Punjab.

5. International conference on “Recent Advances in Fundamental and Applied Science (RAFAS-2021)” (June 25-26, 2021) at Lovely Professional University
6. International Multidisciplinary Conference on Current Research Trends-2020 (September 19-20, 2020).
7. Recent Advances in Fundamental and Applied Science (RAFAS-2019) (November 5-6, 2019) at Lovely Professional University
8. 13th Chandigarh Science Congress (CSC-2019) (March 13-15, 2019) at Punjab university Chandigarh
9. 106th Indian Science congress (ISC-2019) (Jan 3-7, 2019) at Lovely Professional University

List of Workshops

1. weeks National Level workshop on SPSS Statistical Software – (10 Three May - 1 June 2021)
2. One day workshop on “Precision Farming Techniques & Technologies for Sustainable Agriculture” (5th March 2020) CSIR-CSIO Chandigarh.