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Use of Vibrational Spectroscopy and Proximate Compositions for Geographical Classification of Pearl Millets

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ABSTRACT

Pearl millet (*Pennisetum glucum*) is cherished among consumers for its high nutritional content and health benefits. The present study was focused on 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). All 41 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 applied on the spectral and chemical data to classify the pearl millet samples based on the geographical origin. Overall, this study revealed the efficiency of principal component analysis method in geographical classification of pearl millet samples on the basis of infrared spectral and chemically processed data.

Key words: Fourier transform infrared spectroscopy, near infrared spectroscopy, principal component analysis, proximate analysis, pearl millet

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. Pearl millet is recognized as sixth cereal crop in terms of the world agricultural production. As reported by Directorate of Millets Development during 2018-20 the annual production of pearl millet in India was 8.61 million tonnes and around 6.93 million hectares of land was covered for pearl millet production. It is mostly grown in Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana states of India. The nutritional value of pearl millet is comparable and even superior to the other cereal 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 (Rathore and Singh, 2018). Pearl millet is a rich source of minerals such as magnesium, phosphorus, zinc and proteins that are responsible for the potent health benefits such as anti-diabetic and anti-carcinogenic activity (Jukanti *et al.*, 2016). Pearl millet also contains 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. Pearl millet efficiently helps diabetic patients in maintaining a constant blood sugar level over a long period of time. Pearl millet is a type of grain that does 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 helping in weight loss, blood sugar control and other health issues (Hassan *et al.*, 2021).

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 approach employed for the exploratory data analysis. PCA transforms and represents large data sets into a new perspective that emphasizes the

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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 (Meenu *et al.*, 2016, 2022).

Literature reports that pearl millet contains significant amount of protein (13.84%), ash (2.03%), carbohydrates (79.92%) and moisture content (9.61%; Joshi *et al.*, 2015). The carbohydrates content of various varieties of pearl millet is reported to vary from 69.6 to 72.5%, moisture from 6.5 to 7.7%, protein content from 9.7 to 11.3, fat content 5.1 to 7.2%, ash content from 1.65 to 1.90% and fiber from 2.9 to 3.8% (Siroha *et al.*, 2016). Pearl millet varieties from Hisar, Haryana were 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%; Jandu and Kawatra, 2019). The flour of raw pearl millet was also reported to contain 4.5% moisture, 10.57% protein, 7.69% ash and 74.41% carbohydrates (Owheruo *et al.*, 2019).

Achten Elisabeth used attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) as a non-destructive technique to classify the 73 maize grain samples collected from Ukraine, USA and Peru (Achten *et al.*, 2019). In addition, PCA was employed for classification of adulterated milk and honey samples.

Previously, Fourier transform infrared (FTIR) and near infrared (NIR) spectroscopy combined with machine learning techniques was successfully employed to examine the variations among various cultivars of pearl millet grains collected from China using PCA (Kabir *et al.*, 2021).

Literature review presents that very little research had been carried out to characterize or classify pearl millet on the basis of its spectral and chemical information. Thus, the present study was conducted with an objective to classify pearl millet samples collected from various geographical locations of Punjab and Haryana regions of India based on their NIR and FTIR spectral data, proximate composition, by employing PCA.

MATERIALS AND METHODS

The pearl millet samples were collected from local farmers at 41 different locations of Haryana and Punjab states of India in the year 2021. Fig. 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. The Fourier transform infrared (FTIR) spectrum of pearl millet samples were collected by using Shimazu 8400S spectrophotometer using diamond attenuated total reflectance (ATR) and IRSOLUTION software (Shimazu, Japan). The spectra were collected in transmittance mode from 4000/cm to 500/cm at 100/cm interval using 10 scans for each sample. All spectra were recorded at room temperature and mean of 10 scans was further employed for analysis.

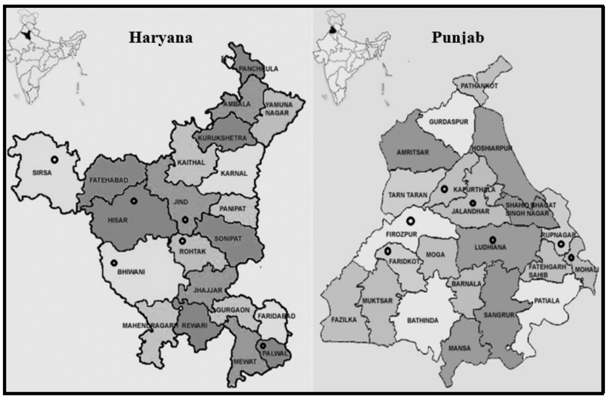

Fig. 1. Dotes showing the samples collected from districts of Haryana and Punjab in geographical maps of India.

The NIR spectra of pearl millet samples of flour was 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. 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.

The analytical grade sulfuric acid (98%), sodium hydroxide solution (45%), boric acid and petroleum ether (60-80°C) were purchased from Loba Chemie for the reference analysis. The pearl millet samples were ground using Aero mixer grinder (500 watt) and sieved through 1.0 mm sieve with 18 mesh to achieve fine powder with uniform particle size. The proximate composition of pear millet sample was determined by Association of Official Analytical Chemists (AOAC) standard methods. Briefly, moisture content was determined by oven method, ash content was determined by furnace method, fat content was accessed by SOCUS PLUS apparatus (Pelican Equipments, Chennai, India), crude protein content was determined by KEL PLUS apparatus (Pelican Equipments, Chennai, India), whereas FIBRA PLUS apparatus (Pelican Equipments, Chennai, India) was used to determine crude fiber content of pearl millet samples. The carbohydrates content was calculated by employing following formula:

Carbohydrates% = 100 – (moisture% – ash% – protein% – crude fat% – crude fiber%).

The proximate and all other analyses of pearl millet samples were carried out in triplicate and data were represented as mean of three independent values.

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 was also investigated using maximum normalized data from samples.

The principal component analysis was carried out using a web application framework ClustVis, (*http://biit.cs.ut.ee/clustvis/*). Principal components (PCs) were computed by employing two default methods that were Singular Value Decomposition (SVD) and non-linear iterative partial least square (NIPALS). In this study, NIPALS (PCA) method was chosen for further analysis because while calculating inner products, it iteratively found the components by leaving out missing values. The group of similar objects in the data was obtained by using score plot in PCA (Metsalu and Vilo, 2015). The data were inserted in the matrix form for classification of pearl millet samples from different locations.

RESULTS AND DISCUSSION

Descriptive statistics of chemical parameters in the pearl millet samples are shown in Table 1. The minimum value of moisture content was 5.19% and maximum was 12.35% (Table 1). The ash content was in between 1.49-1.88% and crude fat was 3.64-6.56%. Similarly the crude fiber was observed in between 1.68-1.50% and crude protein was in between 5.56-11.95%, whereas carbohydrate content remained in between 66.53 to 79.46%. It was observed that the proximate composition like moisture, ash, crude fat, crude protein and carbohydrates showed the maximum value such as 12.35, 1.88, 6.56, 11.95 and 79.46%, respectively, in the pearl millet samples collected from Palwal, Adampur (Sirsa), Dhotar (Sirsa) of Haryana region. Nehra *et al.* (2021) reported the pearl millet of Haryana region contained 7.44% moisture, 2.11% ash, 5.53% crude fat, 9.81% crude protein, 3.41% crude fiber and 71.70% carbohydrates. Similarly, According to the literature various varieties of pearl millet samples originated in Haryana contained moisture, ash, fat, protein, fiber and carbohydrate content ranging from 6.5-7.7,

Table 1. Descriptive statistical analysis of chemically processed pearl millet samples

Statistical parameters	Moisture (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	Carbohydrates (%)
Mean	9.72	1.71	5.22	2.60	7.62	73.13
Standard error	0.27	0.02	0.09	0.10	0.22	0.36
Median	10.13	1.72	5.27	2.45	7.50	72.97
Standard deviation	1.70	0.10	0.57	0.65	1.39	2.34
Range	7.17	0.39	2.92	2.82	6.39	12.93
Minimum	5.19	1.49	3.64	1.68	5.56	66.53
Maximum	12.35	1.88	6.56	4.50	11.95	79.46
Count	41	41	41	41	41	41

1.65-1.90, 5.1-7.2, 9.7-11.3, 2.9-3.8 and 69.6-72.5%, respectively (Siroha *et al.*, 2016). The proximate composition of pearl millet samples collected from Haryana was comparable with proximate composition of pearl millet samples explored previously.

All the samples obtained from Sirsa, Haryana, India, as well as the parameters of the present study provided equivalent results. However, the samples collected from Punjab region presented lowest values for crude fat, crude protein and crude fiber. Overall, the pearl millet samples collected from Haryana were rich in basic nutrients compared to the samples collected from Punjab region of India. Pearl millet is good source of protein, carbohydrates and fiber as compared to other cereal grains.

The arithmetic mean and standard deviation in the descriptive statistics express the central tendency and variation of data. The low value of standard deviation indicated that the data were more symmetric and higher value showed the variation in data. The standard deviation was highest for carbohydrates and lowest for ash content.

The maximum normalized data for all the chemical parameters were employed for the correlation and regression analysis. The correlation analysis was employed to perceive the relation among the variables (Table 2). It was observed that the carbohydrate content of pearl millet was negatively correlated with the moisture content. The protein and carbohydrates content was also negatively correlated with each other.

Table 2. Correlation matrix of chemical parameters

	MN	AN	FBN	PN	CN	FN
MN	1.00					
AN	0.03	1.00				
FBN	-0.16	0.46	1.00			
PN	0.00	-0.09	-0.10	1.00		
CN	-0.69	-0.14	-0.13	-0.61	1.00	
FN	-0.12	-0.04	-0.01	-0.11	-0.09	1.00

Where, MN–Moisture normalized, AN–Ash normalized, FBN–Fiber normalized, FN–Fat normalized, PN–Protein normalized and CN-Carbohydrates normalized.

Regression analysis was further performed to elucidate the relation among moisture, carbohydrates and protein content as :

$$C = -0.1674\,M + 1.0265 \quad ...(1)$$
$$P = -2.7056\,C + 3.1294 \quad ...(2)$$

Where, C represents carbohydrates and M denotes for moisture and P represents protein. It was observed that the equations 1 and 2 had negative slope with different intercepts. Consequently, it was concluded that if any one of these parameters was determined by chemical approach then the other parameters could be predicted by using these equations.

PCA is a powerful technique employed as mathematical tool for analyzing, classifying and dimensionality reduction of numerical data sets in multivariate problems (Fowler *et al.*, 2020). PCA based upon the decomposition of data matrix X into two matrices V and U and these matrices were 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 analysis under study.

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

$PC_k = PC_1, PC_2, PC_3$.., representing the principal components. In this case, the first principal component PC_1 had greatest possible variance in the all possible linear functions and second principal component PC_2 accounted for next highest possible variance being mutually uncorrelated to each other (Dunn, 2019). The main features 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. The PCA applied to the proximate composition of pear millet samples revealed the negative correlation between carbohydrates and moisture content (Fig. 2).

In addition, protein and carbohydrates content also presented negative correlation. PCA was applied to a specific chemical and spectral data set that in turn, explained the classification of pearl millet samples according to their region. Based on the FTIR spectral data, the PC1 and PC2 accounted for 82.9 and 13.3% of the total variance, respectively (Fig. 3). Based on the NIR spectral data, PC1 explained 82.7% and PC2 explainsed 13.5% of total variance (Fig. 4). It is important to note that the PCA applied on chemical data of various samples presented least variance compared to the spectral data (Fig. 5). It was observed that samples from Punjab region exhibited PC1 values from

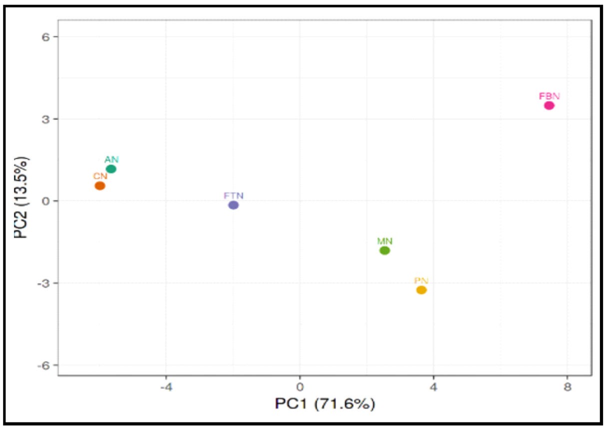

Fig. 2. Correlation analysis between chemical properties named as maximum normalized moisture (MN), ash (AN), protein (PN), fibre (FBN), fat (FN) and carbohydrates normalized (CN) using Principal Component Analysis (PCA). MN : moisture normalized, AN : ash normalized, FBN : fiber normalized, FN : fat normalized, PN : protein normalized and CN : carbohydrates normalized.

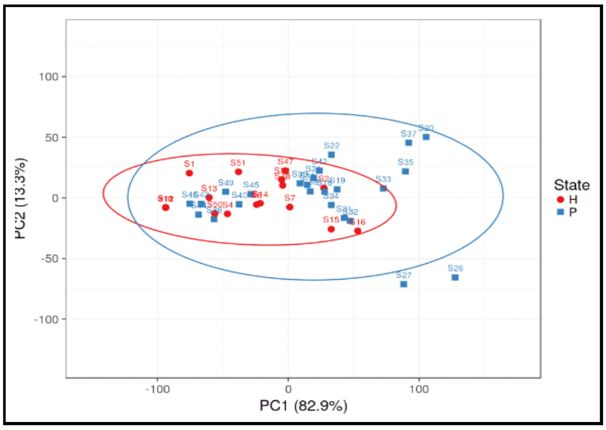

Fig. 3. Score plot of Principal Component Analysis (PCA) to classify the samples using Fourier Transform Infrared (FTIR) spectral data on the basis of region Haryana (H) and Punjab (P).

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 clustered near the origin. The samples from Haryana region presented negative values of PC1, whereas, the samples from Punjab region exhibited variation from negative to positive values in case of PCA based on FTIR spectra (Fig. 3). PCA revealed that samples were least 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

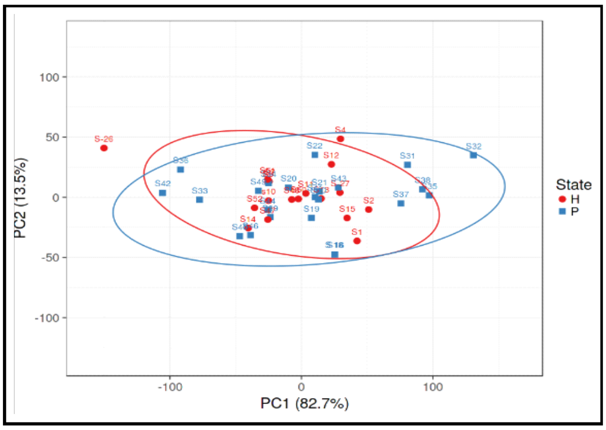

Fig. 4. Score plot of PCs illustrating the differentiation of pearl millet samples from regions using of near infrared (NIR) spectral data.

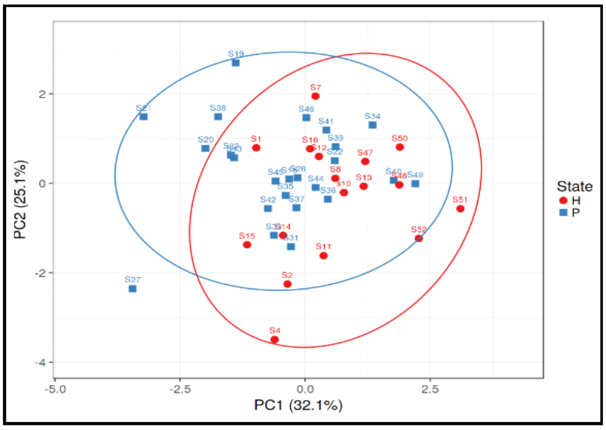

Fig. 5. Principal component analysis (PCA) on chemical based data to classify the pearl millet samples according to their regions.

of Haryana. This may be due to the reason that these samples from Punjab and Haryana region presented 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 using near infrared spectroscopy (NIR). It was observed that the samples from Punjab region lied on the top left, whereas the samples from Haryana region lied on bottom right (Fig. 5). Thus, it was observed that the samples on the basis of properties were roughly able to classified using principal component analysis (PCA) due to effect of environmental conditions of Haryana and Punjab on the nutritional parameters. Thus, as a result, the total PCs accounted as 96.2% of variance for IR spectral data and 57.2% for the chemical data. It stated that the complete information of IR spectral data from various regions was 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% (Kabir *et al.*, 2021).

CONCLUSION

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 48 different locations of Punjab and Haryana states of India were analyzed for proximate composition by employing AOAC methods. The correlation analysis of proximate composition revealed the negative correlations between carbohydrate and moisture, as well as carbohydrates and protein content. These results were compared to PCA technique and an acceptable rate of variance was obtained. It was concluded that the PCs accounted for 96.2% classification rate of the total variation in case of IR spectral data and 57.2% in case of chemical data. It appeared that the almost complete spectral information of IR spectral data from various regions was 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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