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Comparative Efficiency of Conventional and NIR Based Technique for Proximate Composition of Pigeon Pea, Soybean and Rice Cultivars

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ABSTRACT

Proximate composition of food crops is an essential and inevitable tool to identify their ability to suffice the nutritional security of society. Creating database for the key components of biochemical composition is also an essential step to categorized food crops on nutritional supplanting capacity. Conventionally, for the biochemical characterization was performed with tedious and time consuming proximate and wet methods which did not match with current analytical requirements viz., quick, easy cheap, effective rugged and accurate. Near Infrared (NIR) spectroscopy expected to fulfill the above mention characters. Therefore, a study was performed to determine the analytical efficiency of traditional as well as NIR spectroscopic methods to determine Protein, fiber and oil contentfrom31, 25 and 17 commonly available cultivars of soybean, rice and pigeon pea, respectively. A NIR spectrophotometer (Instalab7200) was standardized with different varieties of above crops as per the protocol. The analytical results obtained with NIR spectroscopic technique was significantly correlated with those from conventional method with high degree of repeatability (% RSD≈10) in results, cost effectiveness and speed of analysis. The outcome of this work indicates that NIR spectroscopy has potential to serve as an accurate and rapid alternative method for quantifying the common biochemical components of different cultivars of soybean, rice and pigeon pea with acceptable accuracy, precision and reproducibility.

Introduction

In the food industry, food safety and quality are still performed as an important issue all over the world, which are directly related to people's health and social progress. Consumers are gradually looking for quality seals and trust marks on food products, and expect manufacturers and retailers to provide products of high quality. All of these factors have underlined the need for reliable techniques to evaluate the food quality (Haiyan and Yong, 2007). Protein, Fiber and fat content are the routine biochemical food quality parameters which are employed world-wide to determine the quality of any food matrices. Traditional analytical methods *viz.* Folin-Lowry (Protein), Gravimetric (fiber) and Soxhlet method (oil content) are time tested but are tedious and time consuming. These methods are suitable for laboratory level analysis where representative samples can be analyzed. But at industrial level, these methods are not fitted in the scheme and could not serve the purpose of screening or monitoring of quality parameters of each product. Near infra-red spectroscopy (NIRS) provides an alternative, non-destructive technology for measuring constituents of biological materials with little sample preparation and is able to provide reliable and accurate results of larger range of samples of multiple properties at one time (Stuth *et al.*, 2003).

NIRS is widely used for the quantitative determination of quality attributes such as moisture, protein, fat, and kernel hardness in agriculture and food products (Williams and Norris, 2001).NIRS is broadly accepted in quality assessment of foods, beverages and various other matrices in contemporary scientific fraternity.

NIRS is an accepted method to predict forage fiber traits of barley straw (Mathison *et al.*, 1999), rice (Kong *et al.*, 2005; Jin, 2007), green cereal crops (Bruno-Soares *et al.*, 1998), leguminous shrubs (Garcia *et al.*, 2004), and oat hulls (Redaelli, 2007).

The objective of this study was to determine the analytical efficiency of Near Infrared spectroscopy over traditional analytical methods for estimation of biochemical quality parameters such as protein, fiber and oil content of rice, soybean and pigeon pea.

Materials and Methods

Sampling

Different cultivars of soybean, rice and pigeon pea were taken for comparative study for their biochemical analysis. Total 31, 25 and 17 commonly available cultivars of soybean, rice and pigeon pea were collected respectively (Table 1).

Conventional analysis

For analysis of sample using conventional method, samples were grounded in fine powder. Protein was estimated by the method of Folin-Lowry *et al.*, (1951). Fat content was estimated by Soxhelt extraction method (Sadasivam *et al.*, 1992). Fiber content was estimated by Gravimetric method (AOAC, 1990).

NIR analysis

For NIR analysis sample were grounded and passed through 0.5 mm sieve to prepare fine powder. Powder was dried in oven at 50 °C for 6 hrs to remove moisture. Protein, fiber and oil content of samples were analyzed using NIR Product Analyzer (Instalab® 700, DICKEY-John Corporation). Throughout the experiment instrument was operated at a constant temperature (50±10^{o}C) with 40–50%relative humidity.

Calibration of NIR product analyzer for protein, fiber and oil

Calibrations were validated by analyzing an additional 25 samples each of soy bean, rice and pigeon pea. Bias and standard error of prediction (SEP) were calculated. Before NIR analysis, the samples were kept at room temperature (25 ^{o}C) for 6 h to balance the moisture and temperature as these factors can affect the reflectance and absorbance of NIR wave. A small cup was used for scanning of the sample with full spectrum (400–2500 nm) taking about 15 g of each sample. The reflectance spectra (log1/R) from 400 to 2500 nm were recorded at 10 nm intervals. After incorporating the laboratory value in spectra file, the regression equation was developed and simultaneously, various trial and error methods of mathematics under modified partial least square (mPLS) were also developed to find out a best regression equation for prediction of different parameters. The calibration were carried out at five different wave lengths viz., 2310, 2230, 2180, 2100, 1940, 1680 nm to find out the best reflectance for determining the oil, fiber and protein content from of soybean, rice and pigeon pea.

Across the near-infrared spectrum, there are wavelengths typically unaffected by composition. Their main source of variation is from particle size differences. Filter 5 (1680 nm) is such a wavelength. A wavelength associated with oil (2230 nm), and a wavelength associated with protein (2310 nm) were identified on the basis of calibration). For analysis 20 to 30 g fine dried powder was placed in sample cup and scanned at 400 – 2500 nm for analysis. The NIR ray scan through the sample as it rotates within it conferment and immediately, the result was displayed on the NIR product analyzer screen in less than 1 to 2 min (Chukwu *et al.*, 2014). Each analysis was carried out in triplicates.

Statistical analysis

Data obtained in this research work were statistically analyzed to determine the level of significance in the parameters evaluated when the two methods were applied. Proximate compositions analysis was replicated (n = 3) in both methods. Results presented are mean values of each determination + standard error mean (SEM). Completely Randomized Block Design (CRD) was used to study the variation in protein, fiber and oil content in different genotypes of rice, soybean and pigeon pea due to different analytical techniques.

Results and Discussion

Analytical efficiency

The results obtained in study of protein, fiber and oil content in 31 soybean genotypes is given in table 1. The protein, oil and fiber content recorded in the study were found in the range from 26.0 to 37.9%, 13.4-19.9% and3.5 to 5.7% respectively when analyzed either with traditional or NIR product analyzer. The CV% which is an indicator of variation in repetitive analysis was found lesser in NIR product analyzer with respect to respective conventional analytical techniques adopted to determine protein, oil and fiber content in soybean. The analytical results obtained from both techniques were highly correlated at 95% and 99% confidence interval in soybean.

Similar trend was also observed in rice and pigeon pea varieties which are given in table 2 and 3 respectively. The analytical results of 25 rice verities by both the methods shows that protein content was in 7.6 to 9.3% range, fiber content varied from 0.45 to 0.80%, whereas, oil content showed 2.5 to 3.77% range. High correlation (significant at 1%) (r = 0.86) between Folin-Lawry and NIRS Protein values were observed in result. Likewise, observing the result of 17 varieties of pigeon pea, it showed that the protein content ranged from 22.1 to 28.4%, oil content ranged from 1.4 to 2.4%, whereas, the fiber content varied from 4.2 to 6.4%. High correlation (significant at 1%) (r = 0.61) between Soxhlet and NIRS oil values were observed.

A higher repeatability was observed in the results obtained with NIR over other techniques. The maximum %RSD of different routine methods of protein, oil and fiber was in the range of 0.2 to 10.2. Here, the values of % RSD and CV% of precision study for NIR method for protein, oil and fiber analysis were within the acceptable limits (<10% for RSD and <5% for CV % in majority of cases) (Table 1 to 3). The measured value of protein, oil and fiber in NIR were significantly correlated with respective measured value of protein, oil and fiber in routine method (Table

4). Several scientists working on myriad of crops e.g. Soybean (Lee *et al.*, 2011; Hymawitz *et al.*, 1974; Rinne *et al.*, 1975), Brown Rice (Bagchi *et al.*, 2015), straw berry (Jin, 1994) had already proven that NIR reflectance technique can successfully be adopted over conventional methods for various biochemical quality parameters. Rosenthal (1973) mentioned in his report an instrument for the determination of moisture, oil, and protein content rapidly and accurately ingrain and grain products by means of the NIR technique. The findings of our study further strengthen this statement and found that NIRS can be used for the analysis of protein oil and fiber content in soybean, rice and pigeon pea with acceptable analytical criteria.

Table.1 Details of experimental materials

Crop	Cultivars	Sample site	Total no. of samples	Sample Size
Soybean	JS-84-1615, DS-112, JS-75-10, PK-805, JS-79-190, JS-81-607, PK-805, AGS-51, DS-86-75, AMR-SEL-KH-06, EC-93601, PK-820, DS-71-1-29, J-563, JS-79-4-11, SL-20, JS-335, Gujarat soybean-1, Gujarat soybean-2, Gujarat soybean-3, JS-793, PK-472, MACS-450, JS-93-05, BRAG, KB-85, AGS-46, AMS-25, AMS-48, AGS-51, MACS-1252	KVK, Amreli, JAU and Niger Research Centre, N.A.U., Vanarasi.	31	100g
Rice	NAUR -1, GNR-2, GNR-3, GNR-4, IET-22084, IET-22224, IET-22598, IET-22569, IET-22565, PR-113, MTW-1010, GR-104, IR-64, LG/GT, SGSYGSREE, IET-21515, IET-22095, GURJARI, MASURI, GAR-13, GAR-1, GAR-103, GR-6, GR-11, GR-10	Rice research station, Navsari	25	100g
Pigeon pea	Vaishali, GT-102, GT-103, GT-101, GT-100, GT-1, AGT-2, BDN-2, P-992, ICPL-87119, ICPL-87, GTH-1, C-11, UPAS-120, 288-B, 2199-B, 2188-B	Pulse Research station, Navsari	17	100g

Table.2 Protein, oil and fibre content in different cultivars of Soybean

Varieties	Protein (%)		Oil (%)		Fibre (%)	
	Folin-Lowry*	NIR	Soxhlet*	NIR	Gravimetric*	NIR
JS-84-1615	32.4	31.9	14.9	16.3	5.1	4.5
DS-112	32.7	33.7	16.7	17.0	4.4	4.1
JS-75-10	31.3	24.7	15.6	18.2	3.9	4.8
PK-805	28.5	28.1	17.4	18.1	4.9	4.7
JS-79-190	28.3	28.9	15.8	17.6	4.1	4.5
JS-81-607	32.1	31.2	16.4	17.7	4.3	4.0
PK-805	29.7	32.1	13.4	16.6	4.1	4.5
AGS-51	32.6	31.1	13.9	17.4	4.3	4.6
DS-86-75	27.9	29.0	16.9	19.4	4.6	4.1
AMR-SEL-KH-06	26.0	30.3	16.6	16.7	4.0	4.3
EC-93601	28.3	30.4	16.5	18.5	3.9	3.8
PK-820	34.0	32.6	15.6	16.8	4.7	4.5
DS-71-1-29	32.2	30.4	17.5	18.5	3.7	3.9
J-563	28.1	31.7	13.6	17.6	4.1	4.1
JS-79-4-11	32.3	29.8	14.6	17.7	3.7	4.0
SL-20	29.3	33.9	15.9	16.5	3.7	4.4
JS-335	35.2	29.8	16.8	17.1	3.9	4.4
Gujarat soybean-1	28.1	27.6	17.0	17.2	4.2	4.0
Gujarat soybean-2	36.9	37.3	19.4	15.9	4.0	4.4
Gujarat soybean-3	30.9	37.2	18.4	17.2	4.9	4.2
JS-793	30.3	28.9	16.8	18.1	5.7	4.5
PK-472	32.9	30.9	17.4	18.1	4.1	4.1
MACS-450	33.2	31.8	17.3	17.5	3.5	4.1
JS-93-05	27.0	25.8	19.9	19.5	3.6	4.5
BRAG	32.9	25.0	19.3	19.0	3.8	4.2
KB-85	26.0	27.2	19.0	19.5	3.6	4.2
AGS-46	35.2	30.9	19.4	18.1	4.7	4.7
AMS-25	33.3	32.5	19.2	18.6	3.8	3.6
AMS-48	35.0	31.5	19.2	18.0	3.7	3.6
AGS-51	34.5	29.8	15.2	17.2	4.3	4.9
MACS-1252	27.1	31.2	17.4	18.2	4.3	4.4
Mean	**31.1**	**30.5**	**16.9**	**17.7**	**4.2**	**4.3**
Minimum	**26.0**	**24.7**	**13.4**	**15.9**	**3.5**	**3.6**
Maximum	**36.9**	**37.3**	**19.9**	**19.5**	**5.7**	**4.9**
S.Em±	**1.10**	**0.03**	**0.49**	**0.05**	**0.15**	**0.03**
CD	**3.11**	**0.09**	**1.38**	**0.14**	**0.44**	**0.09**
CV%	**6.12**	**0.18**	**5.00**	**0.48**	**6.36**	**1.22**

Table.3 Protein, oil and fibre content of different cultivars of rice

Varieties	Protein (%)		Oil (%)		Fibre (%)	
	Folin-Lowry*	NIR	Soxhlet*	NIR	Gravimetric*	NIR
NAUR -1	8.0	7.9	2.8	3.3	0.5	0.6
GNR-2	7.8	8.3	3.3	3.4	0.5	0.6
GNR-3	8.3	8.0	3.1	3.5	0.7	0.6
GNR-4	8.4	8.4	3.3	3.7	0.8	0.7
IET-22084	8.5	8.4	2.6	3.1	0.7	0.6
IET-22224	7.8	8.3	2.8	3.2	0.7	0.6
IET-22598	7.5	8.2	2.7	3.2	0.6	0.6
IET-22569	8.0	8.1	3.2	3.5	0.6	0.6
IET-22565	7.8	7.9	3.3	3.6	0.6	0.6
PR-113	8.0	7.9	3.7	3.6	0.6	0.7
MTW-1010	8.6	8.3	3.1	3.5	0.5	0.6
GR-104	7.2	7.7	3.3	3.7	0.7	0.7
IR-64	9.4	9.2	3.0	3.2	0.5	0.6
LG/GT	7.8	8.0	3.2	3.5	0.7	0.7
SGSYGSREE	8.1	8.3	3.3	3.5	0.6	0.7
IET-21515	7.8	8.3	2.9	3.2	0.5	0.6
IET-22095	7.9	8.1	3.2	3.2	0.6	0.6
GURJARI	8.5	8.6	3.1	3.4	0.5	0.7
MASURI	9.1	8.9	3.2	3.4	0.6	0.7
GAR-13	8.6	8.7	3.4	3.8	0.5	0.6
GAR-1	8.9	8.8	3.5	3.4	0.5	0.6
GAR-103	9.1	8.8	3.7	3.6	0.5	0.6
GR-6	7.9	8.1	3.3	3.3	0.5	0.6
GR-11	8.2	8.7	3.8	3.7	0.7	0.7
GR-10	8.4	8.5	3.4	3.7	0.7	0.7
Mean	**8.23**	**8.34**	**3.21**	**3.41**	**0.60**	**0.63**
Minimum	**7.15**	**7.67**	**2.57**	**3.07**	**0.45**	**0.57**
Maximum	**9.39**	**9.17**	**3.77**	**3.77**	**0.80**	**0.70**
S.Em.	**0.18**	**0.07**	**0.14**	**0.03**	**0.04**	**0.02**
CD	**0.52**	**0.20**	**0.39**	**0.10**	**0.12**	**0.05**
CV%	**3.82**	**1.46**	**7.42**	**1.69**	**11.97**	**5.16**

Table.4 Protein, oil and fibre content of different cultivars of Pigeon pea

Varieties	Protein (%)		Oil (%)		Fibre (%)	
	Folin-Lowry*	NIR	Soxhlet*	NIR	Gravimetric*	NIR
Vaishali	22.9	23.2	2.1	2.3	5.8	5.6
GT-102	25.7	26.4	1.8	1.9	4.8	4.5
GT-103	28.5	23.8	1.6	1.9	5.3	5.4
GT-101	26.2	26.0	1.7	1.8	5.0	4.8
GT-100	26.5	25.2	2.0	2.0	4.8	5.5
GT-1	25.5	25.8	1.8	2.1	5.0	5.3
AGT-2	23.4	24.9	1.6	1.8	5.8	5.2
BDN-2	25.5	25.7	2.4	2.1	4.8	5.3
P-992	22.7	24.4	1.8	2.0	5.1	5.6
ICPL-87119	24.7	25.7	1.8	1.9	6.1	4.9
ICPL-87	23.0	22.9	1.6	1.4	6.0	6.2
GTH-1	23.5	23.3	2.2	2.2	6.2	5.7
C-11	22.1	24.3	1.7	1.6	6.0	5.8
UPAS-120	22.3	23.2	1.8	1.8	5.7	6.5
288-B	24.6	25.9	2.2	2.0	4.2	4.2
2199-B	24.7	24.8	2.2	2.1	6.0	5.2
2188-B	25.6	26.0	2.1	1.6	5.6	5.3
Mean	**24.6**	**24.8**	**1.9**	**1.9**	**5.4**	**5.3**
Minimum	**22.1**	**22.9**	**1.6**	**1.4**	**4.2**	**4.2**
Maximum	**28.46**	**26.38**	**2.40**	**2.33**	**6.17**	**6.47**
S.Em.	**0.44**	**0.40**	**0.11**	**0.04**	**0.08**	**0.03**
CD	**1.27**	**1.15**	**0.32**	**0.11**	**0.23**	**0.10**
CV%	**3.12**	**2.78**	**10.20**	**3.55**	**2.54**	**1.08**

Table.5 Correlation between routine and NIR method

Parameter	"r"		
	Soybean	Rice	Pigeon pea
Protein	0.37*	0.86**	0.50*
Oil	0.45*	0.76**	0.61**
Fibre	0.42*	0.40*	0.57*

* Significant at 5%, ** Significant at 1%

Table.6 Operating cost (INR) and speed of different methods

Technique	Chemicals/ sample	Instrumental cost	Total Cost/Analyte	Cost/Sample	Time/Analytes	Time/ Samples
Spectrophotometer (Protein)	8	0.3	9	92	3.5 hrs	6 hrs
Soxhlet (oil)	40	8	48		3.0 hrs	
Gravimetric method (fibre)	28	7	35		3.5 hrs	
NIR* (all above)	-	2	2	2	5 min	5 min

*No chemical required.

NIRS gave a similar accuracy to conventional HPLC techniques, but with the advantage of near-instantaneous, non-destructive and chemical free analysis. The prediction of crude protein in grain remains the most common application of NIRS in agricultural industries but plant nutrients and carbohydrate fractions have been successfully predicted in arrange of different shrub and tree leaves as well (Meuret *et al.*, 1993; Martin and Aber 1994).The result obtained in our study about the economics and speed of analysis is in agreement with NIRS superiority for rapidity and cost effectiveness over traditional method for protein, oil and fiber analysis from soy bean, pigeon pea and rice genotypes.

Economic efficiency and speed of analysis

Routine methods are costlier as compared to NIR method. As the analysis cost per sample is much higher in case of routine methods as compared to NIR method (Table 6). Traditional methods are very time consuming, which require whole day for generating the results. In case of NIR, sample preparation time is less than 2 min as well as many analytes can be determined from a single sample at a time, so NIR is as fast as compared to routine methods (Table 5).

The results of this study shows that non-destructive method (NIR product analyzer) could be used to determine proximate compositions (protein, oil and fiber content) of cereals, grains and legumes over the destruction (conventional)method as the analytical results obtained from both techniques were significantly correlated for these parameters. Cost effectiveness and rapidness of NIR analyzer over traditional method entail this technique for further application in online product analysis in food industry. Considering the cost and time of analysis and safety, the laboratory analysts are suggested to use Near Infra-Red analyzer for the accurate and rapid estimation of protein, oil and fiber content from rice, soybean and pigeon pea over routine methods when the samples are homogenous in nature.

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