

Quality Assessment of Single Cottonseed Based on its Protein and Oil Parameters using NIR Spectrometer

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ABSTRACT

Measuring seed quality is important in the selection of seed lots for conditioning and in the quality control program of a seed industry. It is necessary to test the seed for giving good yield to the farmers as well to preserve with good quality for long term at the seed industry. The condition and management of bulk cottonseed in storage have a market effect on seed quality. Instead of bulk seed, the quality test for single cotton seed is also a need and to be a high priority. Comparing the conventional methods to test the quality of the individual seed is replacing by Near Infrared reflectance spectroscopy (NIRS) instrumentation by addressing the seeds' internal chemical compounds like protein and oil etc. A data set of 30 seed samples spectra were captured using XDS Optiprobe Analyzer, FOSS NIR spectrometer and analyzed the data set using PLS Tool box in Matlab environment and Unscrambler software to identify the wavelength band corresponding to the seed protein and oil in the range between 1100nm to 2499 nm. Also, this paper reports the roll of protein and oil in testing of single seed to ensure its quality.

KEY WORDS: Seed protein, Seed oil, NIR spectrometer, Matlab, PLS Tool box, Unscrambler.

1. INTRODUCTION

The cotton plant produces three important commodities, of which cotton fiber and other two both derived from cottonseed after it is ginned, are oil and meal. Cottonseed meal contains 30 percent protein on a dry matter basis and it is a common source of diets for animal. Protein extraction was performed on sprouted and un-sprouted pulses and identified the pulse which contains maximum protein content that can be readily consumed by substituting it in diet and prevents from the malnutrition, protein deficiency problem (Vivek Mumbarkar, 2013). Oil seeds play a great role in human and animal nutrition as they are used in feed compounds. Oil seed meals are high in protein and the products made mainly from vegetable oils and spreads, have a role of healthy balanced diet even though they are energy dense and contain a high proportion of fat (Muhammad, 2012). It also recommended that the oil seed crop, protein is a good source of nutrients than the oil content.

The quality of the cotton seed is ensured with the protein content, because the seed storage protein composition may be important factor influencing aflatoxin contamination in cottonseed. The other storage parameter tri-saccharide may serve as an accessible carbon source utilized by the fungus to produce aflatoxin in developing cottonseed under certain conditions (Mellon, 1997). Cottonseed contains 20-40% of Oil, 20-30% of protein and information of these factors influencing contents of oil and protein which have high value to breeders for formulation an appropriate breeding scheme. The content of seed oil and protein is associated with the seed size also and this has a considerable contribution to yield of the crop in cotton (Pahlavani, 2008). In any good quality cottonseed, the germination incorporates events that commence with the uptake of water by the quiescent dry seed, a chain of metabolic events is initiated that results in the emergence of the radical, thus completing germination. Therefore the major stored reserves with in a cottonseed like proteins, carbohydrates and oils were mobilized, providing nutrients to support early seedling growth (Derek Bewley, 2001). Another study made by Kaliyaperumal (2011) indicate that the conventional and molecular breeding methods are being important for developing variety or hybrids cottonseed based on its high oil and protein content.

Near-infrared Spectroscopy is a rapid, reliable and non-destructive alternative procedure for estimation of the cottonseed quality components. It provides a main advantage of simultaneous evaluation of multiple seed components like seed protein, oil, carbohydrate etc. Classical analytical methods are usually labor-intensive, time-consuming, and expensive, as well as causing undesired destruction of the test sample. The objective of this study was to investigate the feasibility of using NIR Spectroscopy to identify the wavelength band corresponding to the single cottonseed parameters protein, oil non-destructively.

2. MATERIALS AND METHODS

The NIR spectra of 30 individual cotton seeds were captured and obtained nearly 30 X 2799 data matrix for the analysis. Each seed was placed randomly in the seed holder (2) and irradiated by the NIR light source from the FOSS XDS Optiprobe Analyzer under reflection mode. The optical setup was made as shown in the Fig.1 in such way that the distance between the top surface of the seed (1) to the bottom level of optical fiber bundle (3) was 4mm. Then each seed was scanned by the spectrometer between the wavelength ranges 400nm – 2500nm at the resolution of 0.5nm. Finally the data were clipped between the range 1100nm – 2499nm for the identification of wavelength absorption for protein and oil parameter of the cotton seed.

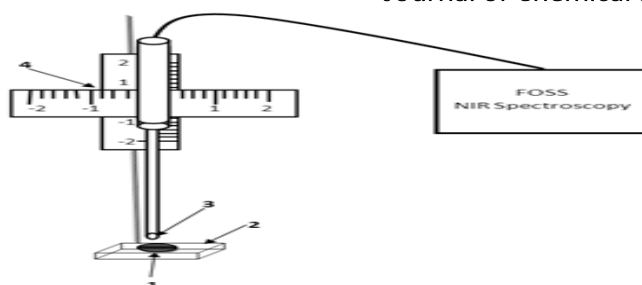


Figure.1. Setup to place the seed

Near-Infrared Spectroscopy: The absorptions observed in the Near-infra red region (900nm – 2500nm) are overtones or combinations bands of the fundamental frequencies. Overtones has frequencies corresponding approximately to two, three time that of the fundamental, while combination bands arise by interaction of two or more vibration taking place simultaneously and the frequency of a combination band is the sum of the multiples of the relevant fundamental frequencies. Vibration involving C-H, O-H, N-H and possible S-H and C=O bonds are responsible for the majority of the observed absorption bands in the NIR region

NIR absorption band for seed protein content: The protein content in the cotton seed will have positive correlation with the C=O & N-H group frequencies. The band assignment for protein is difficult in the NIR region due to numerous and overlapping bands. In the combination band region 2000nm to 2500nm bands characteristic of the protein amide groups appear 2055nm and 2060nm due to N-H stretch/bend molecule (Hari Prasad Reddy, 2011). The broad 2180nm band has commonly been ascribed to N-H bend, C-H stretch/C=O stretch and amide II molecule vibration related to the protein identification. Agnieszka also presents the principal absorption band of protein, shown in the table 1. Another class of important bonds in the range 1560nm – 1670nm and 2080nm – 2220nm ascribed N-H deformation combination referred to the protein absorption (Digvir, 2009).

NIR absorption band for seed oil content: The oil content in the cotton seed mainly consist of fatty acids, the absorption observed in NIR spectrum are primarily due to vibrational modes from C-H functional group. This C-H group frequencies can be attributed to three main functional groups –CH₂ methylene, –CH₃ methyl and –CH=CH- ethenyl which, in turn, can be assigned in different regions of NIR as shown in the following Table.2. Agnieszka, also presents the principal absorption band of oil, shown in the Table.3.

Table.1. Principal NIR Absorption Bands For Protein

Region	Walvelength (nm)	Molecule	Vibration
1.	1208	-CH Stretching	2 nd Overtone
2.	1465	N-H & O-H Stretching	1 st Overtone
3.	1734	-CH Stretching	1 st Overtone
4.	1932	N-H & O-H Stretching	Combinations
5.	2058		
6.	2180		
7.	2302	C-H Stretching	Combinations
8.	2342	C-H Stretching	Combinations
9.	2470	C-N-C Stretch	First overtone

Table.2. Assignment of Major NIR Absorption Bands For Oil

Region	Wavelength (nm)	Molecule	Vibration
1.	1090 – 1180	-CH ₂	2 nd Overtone
	1100 – 1200	-CH ₃	
	1150 – 1260	-CH=CH-	
2.	1350 – 1430	-CH ₂	Combination
	1360 – 1420	-CH ₃	
3.	1650 – 1850	-CH ₂	1 st Overtone
		-CH ₃	
		-CH=CH-	
4.	2010 – 2020	-CH=CH-	Combination
5.	2100 – 2200		
6.	2240 – 2360	-CH ₃	Combination
7.	2290 – 2470	-CH ₂	Combination

Table.3. Principal NIR Absorption Bands for Oil

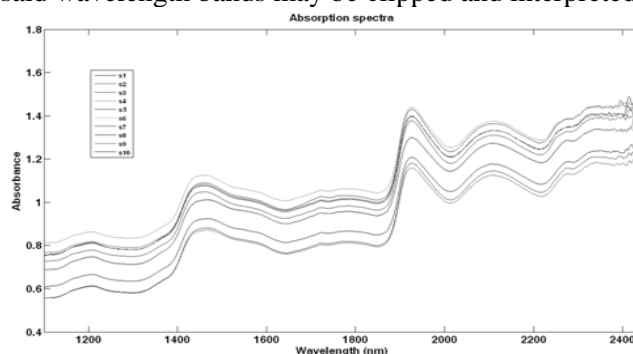
Region	Wavelength (nm)	Molecule	Vibration
1.	1210	-CH Stretching	2 nd Overtone
2.	1406	N-H & O-H Stretching	1 st Overtone
3.	1718	-CH Stretching	1 st Overtone
4.	1760	-CH Stretching	1 st Overtone
5.	2114	N-H & O-H Stretching	Combinations
6.	2308	C-H Stretching	Combinations
7.	2346	C-H Stretching	Combinations

Multivariate analysis: A data matrix of 30 X 2799 spectral data, then pre-processed by the Unscrambler ver.10 and PLS Toolbox (Eigen vector Research) on Matlab environment in order to correct the baseline drift, smoothing the spectra followed by standard normal variate (SNV) which were due to unwanted systematic variation emanating from light scattering, instrumental drift and path length differences. Such systematic noise should be removed from the raw spectral signal to prevent them from dominating over the chemical signals.

Roll of seed oil and Protein: In maximizing the yield in terms of quality and quantity of cottonseed is in terms of its nutritional values of oil and protein and it is necessary to identify the constraints to the cotton breeders and producers. Accordingly the cotton breeder and producer have traditionally been guided by consideration of seed parameters. Cottonseed oil is an important source of fat and the meal is classed as a protein supplement in the feed trade as it is almost considered as in soybean meal. These seed parameters are highly important to estimate the amount of macro-elements like Potassium, Zinc and Phosphorus requirement by the breeders to improve the cottonseed yield and seed protein, oil contents. Potassium and Zinc will response to increase the seed protein content (Zakaria, 2006). Also, the seed weight and seed parameters have a strong linear relationship. Some artificially aged seeds were easily identified by the seed oil analysis, because the oil analysis reveals gradual increase in free fatty acid and lipid peroxidation. Once the peroxide value of artificially ages seeds showed a gradual increase but the saponification value, iodine value and ester value showed decreasing trend with increasing the period of seed ageing (Nadia Iqbal, 2002). The cultivar of the cottonseed is also affected in its size, oil and protein. Therefore, it is necessary to test the cottonseed to ensure its quality in terms of its oil and protein parameters.

3. RESULTS AND DISCUSSION

A data matrix of 30 X 2799 spectral data of 30 individual cottonseed were analyzed using Unscrambler ver 10.0 The major reserve components absorption in the cottonseed would be interpreted in three bands between 1100nm – 1300nm (band-A), 1300nm – 1600nm (band-B) and 2000nm – 2499nm (band-C) in order to address the seed oil and protein components. The raw spectra of cottonseed between the wavelength region 1100nm and 2499nm shown in the Fig.2 and other said wavelength bands may be clipped and interpreted further.

**Figure.2. Raw spectra of cottonseed**

It is more evident in the wavelengths band-A & B, the contributed wavelength for the variables seed oil and protein are as shown in the Fig.3 and Fig.4. In the band-A, a single absorption peak centered near 1206nm which associate for the oil content due the second overtone of C-H stretching vibration of various functional group $-CH_2$, $-CH_3$, $-CH=CH-$ and in band-B, two peaks around 1394nm and 1510nm which corresponds to oil parameter due to C-H combination and protein due to first overtone of N-H stretching vibration by CH_2 group. Also the other weak crest centered near 1392 and 1414 that contained little useful information for oil and fat classification. In the band-C, the plot shows between the Principal Component (PC) -1 loading vector for the data clipped in the band-C region and wavelength, a peak centered on 2470nm, which corresponds to protein parameter due to symmetric C-N-C stretch first overtone, a shown in the Fig.5.

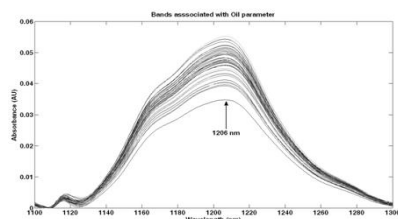


Figure.3. Wavelength band associated with Oil parameter

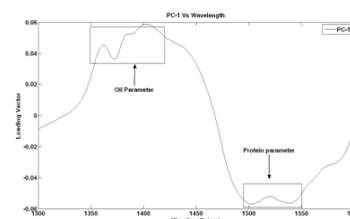


Figure.4. Wavelength band associated with Oil and Protein parameter

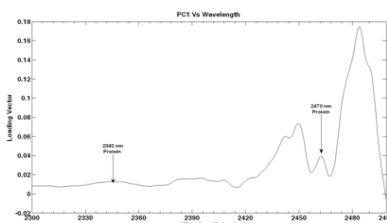


Figure.5. Wavelength band associated with Protein parameter

4. CONCLUSION

For the chosen cottonseed, its oil and protein parameter contents evaluated non-destructively using NIR spectrometer. In the NIR wavelength bands, the oil content is evaluated in the band-A at 1206nm and band-B at 1394nm respectively. The protein content is evaluated in the band-B at 1510nm and band-C at 2470nm. The parameters contributed for the above said wavelengths would further ensure the seeds' nutritional values, ageing value and the required amount of Potassium, Zinc and Phosphorus as macro elements to the seed breeder to improve the cottonseed yield.

5. ACKNOWLEDGMENTS

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