and ASD may have a natural closure before the age of five years. Further, we performed study in a school where it is expected that patients with severe CHD may not attend. Community-based study might have given more information and higher prevalence.

CHD leads to high morbidity and mortality among infants and children. In developing countries like India, a substantial number of infants or children with CHD remains undetected leading to high morbidity and mortality. The present study highlights the burden among school-going children that needs to be properly investigated to identify the risk factors and measures to alleviate the same.

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PRASANTA K. BORAH[1]
UTPALA DEVI[1]
HEM CH KALITA[2]
SUMAN K. PAINE[1]
MEENAKSHI SHARMA[3]
JAGADISH MAHANTA[1,*]

[1]*Regional Medical Research Centre, NE Region (ICMR), Dibrugarh 786 001, India*
[2]*Assam Medical College, Dibrugarh 786 002, India*
[3]*Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029, India*
**For correspondence.*
e-mail: jmahanta@hotmail.com

Comparative evaluation of protein content in groundnut samples by near infrared reflectance spectroscopy and Skalar colorimetric methods

A lot of research has been done in developing groundnut cultivars with high-quality oil. As a result, methods for routinely determining oil content and quality have been developed and utilized[1]. However, groundnut is also a source of protein, and obviously, there is a need to develop a rapid, accurate and economic method that can be routinely used for screening a large number of groundnut cultivars for protein content. At the ICRISAT analytical service laboratory, protein (total N) in various crops is routinely determined by colorimetric method using Skalar autoanalyser. However, near infrared reflectance spectroscopy (NIRS) also provides an opportunity to determine protein content in groundnut samples; and the method seems attractive as it is low cost, simple and rapid. The NIRS based method provides an automated measurement and has the potential to become a valuable tool for providing analytical support for agricultural research[2,3]. The objectives of this study were to estimate and compare the relative efficacy of the NIRS method, with that of a conventional colorimetric method, following digestion of ground samples, using Skalar autoanalyser for determining protein in groundnut samples.

In this study, a total of 928 groundnut samples were selected. The samples were kept in an oven at 60°C for 48 h for drying, and then ground (<0.5 mm) using porcelain mortar and pestle.

Powdered groundnut (20–30 g) was loaded into sample cell and scanned by NIRS (Foss XDS rapid content analyser) to develop calibrations for protein analysis. Each sample was scanned thrice, and nearly 60 sec was required for each scan. The absorbance bands of N–H bonds in protein at 2 nm wavelength intervals within the range 400 to 2498 nm were recorded, using Win ISI software (version 4.5.0). Other chemical bonds like C–H, C–O, H–O, etc. in groundnut were not considered in the analysis, though they may appear in the overtone bands in NIR region. Brilliant reflectance spectra was generated in the solid, with high scatter coefficients[4].

The modified partial least square regression (MPLS) method was used to develop the calibration equation. Before scanning, all calibration samples were analysed by Skalar method, reference method and the predicted protein values determined by NIRS were also validated with Skalar data by regression parameter.

Finely ground groundnut samples (0.3 g) were digested with 2.5 ml of concentrated sulphuric acid-selenium (Se) mixture (sulphuric acid containing 0.4% Se, v/w, was heated to dissolve Se)[5], and total N (%) in the digests was analysed using Skalar autoanalyser. All samples were analysed in three replications, and the results presented are the means of three independent analyses. Nitrogen (%) was converted into protein by multiplying with a factor of 5.46.

The data were statistically analysed, and the significance of the results by the two methods was tested for protein analysis. Correlation between the values of protein in groundnut samples by NIRS and Skalar methods was determined, and regression equations were developed to predict protein content.

The precision obtained in determining protein content in groundnut samples by NIRS and Skalar methods was

Table 1. Precision on protein estimation of groundnut samples and relationship between the results obtained by NIRS and Skalar methods

Parameter	NIRS-protein (%)	Skalar-protein (%)	Correlation coefficient (r)	p-value
Range	15.30–30.81	16.30–32.48	0.94	<0.00001
Mean	25.55	26.24		
SD	2.69	2.76		
CV (%)	10.55	10.54		

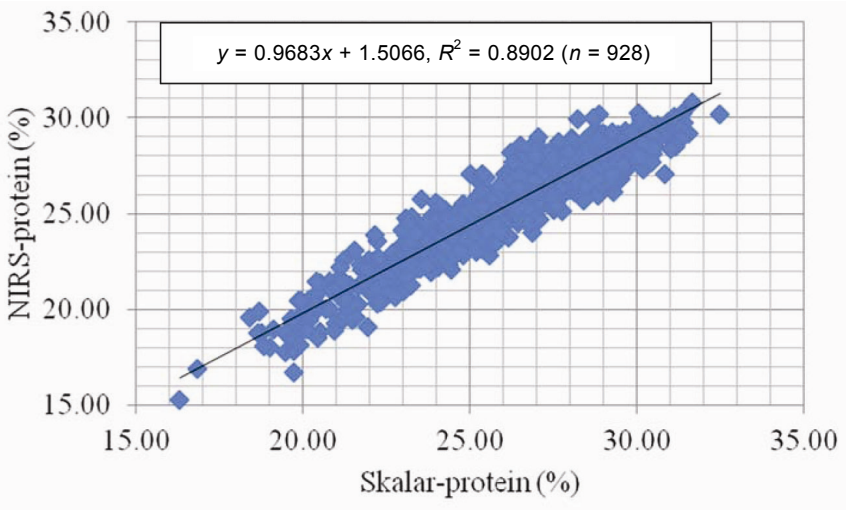

Figure 1. Relationship between Skalar-protein and NIRS-protein in 928 groundnut samples.

comparable, as judged by statistical analysis. The range, mean, standard deviation and CV(%) data are presented in Table 1. There was a positive correlation between protein data determined by the NIRS method and the Skalar colorimetric method; the combined correlation coefficient (r) for the 928 groundnut samples was 0.94.

The relationship between NIRS-protein and Skalar-protein was significant ($P < 0.00001$) and positively correlated ($R^2 = 0.8902$) for groundnut samples (Figure 1); and the relationship represented by the following regression equation

$$\text{NIRS-protein (\%)} = 1.5066 + 0.9683 \times \text{Skalar-protein (\%)},$$

$$R^2 = 0.8902 \ (n = 928).$$

The results on the analysis of protein content in groundnut samples by NIRS method suggest that it could be a useful tool to analyse proteins in groundnuts.

NIRS is an ideal method for quantitative estimation of protein in groundnut. The method based on NIRS provides an alternative cost-effective analytical tool for simple, accurate and rapid determination of protein with small sample size compared to the standard analytical procedures.

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SWATI CHAUDHURY[1,*]
KANWAR L. SAHRAWAT[1,†]
K. SRINIVASU[1]
SUHAS P. WANI[1]
NAVEEN PUPPALA[2]

[1]*International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India*
[2]*New Mexico State University, Agricultural Science Center at Clovis, 2346 SR 288, Clovis, NM-88101, USA*
**For correspondence.*
e-mail: schaudhury9@gmail.com
[†]*Deceased.*

Advertisement calls of Amboli leaping frog *Indirana chiravasi* (Anura: Ranixalidae) from northern Western Ghats, India

The anuran amphibians are one of the most actively vocalizing animal groups. Acoustic characteristics of anurans are species-specific and can be used for their identification, description of new species, understanding phylogenetic relationships among species, resolving cryptic speciation and in the conservation of species[1–3]. Although the Western Ghats of India is rich in anuran diversity, with new species continuously being described, limited information is available on calls of endemic and threatened species. Because call structures could help in designing non-invasive methods for identification